Anthelmintic Treatment and Digestive Organ Morphology of Captive-Reared Kakï (*Himantopus novaezelandiae*) Released to the Wild

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I will conclude my description of the natural history of these islands [the Galapagos], by giving an account of the extreme tameness of the birds.

This disposition is common to all the terrestrial species; namely, to the mocking-thrushes, the finches, wrens, tyrant-flycatchers, the dove, and carrion-buzzard. All of them often approached sufficiently near to be killed with a switch, and sometimes, as I myself tried, with a cap or hat. A gun is here almost superfluous; for with the muzzle I pushed a hawk off the branch of a tree. One day, whilst lying down, a mocking-thrush alighted on the edge of a pitcher, made of the shell of a tortoise, which I held in my hand, and began very quietly to sip the water; it allowed me to lift it from the ground whilst seated on the vessel: I often tried, and very nearly succeeded, in catching these birds by their legs. Formerly the birds appear to have been even tamer than at present. Cowley (in the year 1684) says that the 'Turtle-doves were so tame, that they would often alight upon our hats and arms, so as that we could take them alive: they not fearing man, until such time as some of our company did fire at them, whereby they were rendered more shy.' Dampier also, in the same year, says that a man in a morning's walk might kill six or seven dozen of these doves. At present, although certainly very tame, they do not alight on people's arms, nor do they suffer themselves to be killed in such large numbers. It is surprising that they have not become wilder; for these islands during the last hundred and fifty years have been frequently visited by buccaneers and whalers; and the sailors, wandering through the woods in search of tortoises, always take cruel delight in knocking down the little birds.

These birds, although now still more persecuted, do not readily become wild: in Charles Island, which had then been colonized about six years, I saw a boy sitting by a well with a switch in his hand, with which he killed the doves and finches as they came to drink. He had already procured a little heap of them for his dinner; and he said that he had constantly been in the habit of waiting by this well for the same purpose. It would appear that the birds in this archipelago, not having as yet learnt that man is a more dangerous animal than the tortoise or the *Amblyrhynchus* disregard him, in the same manner as in England shy birds, such as magpies, disregard the cows and horses grazing in our fields.

Source: Excerpt from *Journal of Researches into the Geology and Natural History of the Various Countries Visited by H.M.S. Beagle* by Charles Darwin (London: H. Colburn, 1839).

Abstract

The continued existence of New Zealand's critically endangered and endemic black stilt or kakï (*Himantopus novaezelandiae*) relies on an intensive captive management programme. While this is successful at rearing large numbers of birds for release to the wild, poor survivability of these birds is limiting significant increases in the wild population. Predation and starvation are suspected to be the most common causes of death in released birds, but underlying contributing factors to these mortalities have not been fully evaluated. This research investigates the possible contribution of gastrointestinal (GI) helminth burdens and suboptimal digestive organ development to the high mortality rates of released kakï. Emphasis is placed on evaluating the methods used to assess the importance of these factors and to make informed recommendations for future management.

The efficacy of the anthelmintic regime used for kakï was assessed by dosing half of the 80 captive birds with praziquantel (PZQ) prior to release in 2007. Faecal samples were collected before and after anthelmintic treatment, and before and after release to the wild. *Post mortem* worm counts were conducted on 11 birds that died following release and historical worm count records dating back to 1997 were accessed. The main findings were: PZQ had high efficacy against trematodes; treatment did not prevent re-infection; birds were exposed to helminths at release site; and there was no advantage of treatment for survival. Overall, the results suggest that anthelmintic treatment is an unnecessary cost. Consequently, recommendations were made to cease anthelmintic treatment or reduce its intensity, continue health screening, and incorporate annual efficacy testing to monitor the emergence of anthelmintic resistance.

The reliability of faecal screening for GI helminths was evaluated. Faecal egg counts (FECs) were found to be poor indicators of worm burden. The two modified sedimentation methods used to detect trematodes provided relatively low egg recovery rates. Trematode egg shedding varied between days but not by hour of the day or temperature. The collection and analysis of pooled faecal samples proved to be more cost and time-effective than samples from individual birds and the larger masses collected resulted in greater sensitivity. In conclusion, faecal analysis of pooled samples is a useful qualitative indicator of helminth presence or absence but is quantitatively unreliable.

In order to assess the importance of digestive organ development to captive-reared and released kakï, the digestive organs of healthy and emaciated captive, released and wild *Himantopus* sp. were compared. Captive and released kakï had generally smaller digestive organs than wild birds, released birds did not increase digestive organ size to match the high fibre wild diet, and emaciated birds did not have atrophied organs. However, the greatest mortality rates occur soon after release, while the birds were still being supplementary fed. These results suggest that reduced digestive efficiency is probably not contributing significantly to mortality rates and the direct impacts of the translocation are probably greater risk factors. The continued provision of supplementary food to released birds and an increased focus on habitat enhancement and predator control at release sites were recommended. The reliability of comparing fresh and formalin fixed *Himantopus* sp. gut specimens was evaluated. Significant differences in fresh and formalin fixed organ dry masses and variation in preserved organ lengths indicate that this variation should be considered in future studies.

In conclusion, current management practices appear to be successful in ensuring that GI helminths and reduced digestive efficiency do not significantly lower the survivorship of captive-reared and released kakï. There is a need for further research into developing a more direct physiological assessment of the impacts of GI helminths and gut morphology as well as considering the role that starvation may have on poor survivability.

Acknowledgements

My biggest thanks go to my supervisors: Associate Professor Maurice Alley, Dr. Brett Gartrell and Dr. Isabel Castro. Thank you all for believing in me; for making me laugh when things didn't go to plan; for filling my head with lots of weird and wonderful ideas; for increasing my level of enthusiasm whenever it started to wane; and for bringing me back down to earth when I had too many ideas floating around in my head! Thanks also to Nicolas Lopez-Villalobos for being a great statistics advisor.

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During the course of my research, I spent 8 weeks in Twizel collecting bird droppings. I would like to thank Twizel DOC for letting me stay at the kakï aviary house during that time. I wouldn't have survived without the company of Jo Hiscock and Emma Stephen who also lived at the house. Thanks to Lana Hastie and Ivan Andrews for their companionship in Twizel also. Thank you Dave Murray and Marcia Fairhall for taking me out to the study sites and for teaching me so much about kakï and the MacKenzie Basin. Mum, thank you for making the trip down to visit me. You were also very good at scrubbing the faecal collection sheets!

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I also acknowledge the financial support that I received from the Association of Avian Veterinarians, the IVABS research and travel funds, the Phyllis Irene Grey Fellowship in Veterinary Science, the Manawatu Branch of the Federation of Graduate Women and the Golden Plover Award (from the NZ Wetland Trust).

As anyone who has written a thesis before will know; it can be quite an eventful / stressful time! I am so grateful for the support I had from my fellow Ecology post-grads during this time. Thanks so much to my amazing flat mate Nicki Atkinson and also to Esta Chappell, Lorraine Cook and Danielle Middleton; in a weird way I will miss all the times we spent complaining about the state of our theses. And last, but not least, Laura Donaldson: Yay, we finally finished!

There are a few others who deserve special thanks for their support throughout my research and they are my partner Cameron Linton, my best friend Rhiannon “Boomz” O’Leary, Mum, Dad, Nan, my workmates at Sue Fordyce and my numerous pet rats (who sadly didn’t survive to see the day I handed my thesis in).

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List of Abbreviations

ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
BIA	Biological Impedance Analysis
DH	Definitive Vertebrate Host
DOC	New Zealand Department of Conservation
EPG	Eggs per Gram
FEC	Faecal Egg Count
FECR	Faecal Egg Count Reduction
FIH	First Intermediate Host
GI	Gastrointestinal
IUCN	International Union for the Conservation of Nature and Natural Resources
IVABS	Institute of Veterinary, Animal and Biomedical Sciences
MANOVA	Multivariate Analysis of Variance
NIWA	National Institute of Water and Atmospheric Research
PRR	Project River Recovery
PZQ	Praziquantel
SAS	Statistical Analysis System
SIH	Second Intermediate Host
TOBEC	Total Body Electrical Conductivity
TWC	Total Worm Count

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Appendix 2: Supplement to Chapter 2: Spatial variation in the distribution of gastrointestinal helminths along the alimentary tract of *Himantopus* sp. in New Zealand.

Preface

This thesis is formatted as a general introduction, three distinct research papers and a general discussion. Because each research chapter represents an individual paper, there is some repetition between chapters and the general introduction. The format and language (American vs. New Zealand English) of the research chapters also differs depending on the specific requirements of each journal.

All the work presented in this thesis is my own and the multiple authorships that will be ascribed to each research chapter will acknowledge the contributions made by my supervisors and other collaborators.

Approval from the Massey University Animal Ethics Committee was not required for this research as data collected from live birds was carried out in conjunction with normal Department of Conservation release protocols. Kaki are taonga of the Ngāi Tahu tribe and permission was granted by them to utilise specimens from dead kakï.

Both the European and Māori names for the black stilt are used throughout the thesis. For the purposes of publication, the name ‘black stilt’ is used in the research chapters. For ease of writing, the name ‘kakï’ is used in the main abstract, general introduction and general discussion.

Chapter 1

General Introduction

Newly hatched kakï chick

(Photo by J. Hiscock)

1. Black stilt, kakï (*Himantopus novaezelandiae*)

i. Biology and ecology

Black stilt (*Himantopus novaezelandiae*) are New Zealand endemic waders in the family Recurvirostridae (stilts and avocets). They are commonly known by their Māori name, kakï, meaning neck or throat. This refers to the mottled hind neck of juvenile birds; a characteristic that distinguishes them from the closely related pied stilt (*Himantopus h. leucocephalus)*. Kakï are considered to be the world's rarest wading bird (Gaze 1994) with an estimated wild population size in August 2008 of just 80 adults (Cleland 2008). Kakï are classified as critically endangered by the International Union for the Conservation of Nature and Natural Resources (IUCN) (BirdLife International 2000) and the New Zealand Department of Conservation (DOC) (Maloney & Murray 2002). High country braided rivers, glacial lakes, tarns and wetlands are the dominant habitats of kakï. They feed in these waterways by filtering and scything with their long bills and consume a wide variety of prey items including insects, molluscs and small fish (Pierce 1986). Kakï breeding pairs are highly territorial in the reproductive season and show high levels of parental investment. Young birds stay with their parents for about 9 months after hatching. After this time subadults form colonial flocks, pairing up to breed in their second or third year (Marchant & Higgins 1993).

ii. Reasons for decline and current threats

The former range of kakï during the nineteenth century extended over much of the South Island and from Wellington to at least Rotorua in the North Island. Small flocks were also known to winter as far north as Northland (Figure 1.) (Pierce 1986). Breeding kakï are now completely confined to the Upper Waitaki Basin in central Canterbury in the South Island (Pierce 1984). The rapid decline of kakï has been attributed primarily to predation and habitat loss and degradation. Introduced mammalian predators such as feral cats (*Felis catus*), ferrets (*Mustela putorius furo*), stoats (*Mustela erminea*), Norway rats (*Rattus norvegicus*) and hedgehogs (*Erinaceus europaeus*) were important predators of kakï during initial declines and still are today. Harriers (*Circus approximans*) and black-backed gulls (*Larus dominicanus*) are significant avian predators of kakï and are artificially abundant due to the presence of rabbits (*Oryctolagus cuniculus*) and rubbish dumps in the Waitaki basin. Habitat loss and degradation was due to: agricultural practices; wetland drainage; introduction of rabbits, invasion of wetlands and braided rivers by exotic weeds;

and water extraction for irrigation and hydro-electric power development. These habitat changes were major causes of declines and still threaten the remnant kakï population. Less significant causes of population loss include nest mortalities due to flooding and anthropogenic factors such as wire strike by stock fences and power lines (Maloney & Murray 2002, Moore & Battley 2003).

Some inherent behaviours of kakï make them especially prone to the above threats. These include: (1) nesting on river banks leaving them susceptible to flooding and directly exposing them to passageways of terrestrial predators; (2) lack of colonial nesting behaviours to facilitate defences against predators; (3) unsuccessful distraction displays; (4) a very long fledgling period; and (5) lack of cryptic plumage (Pierce 1986, Reed & Murray 1993).

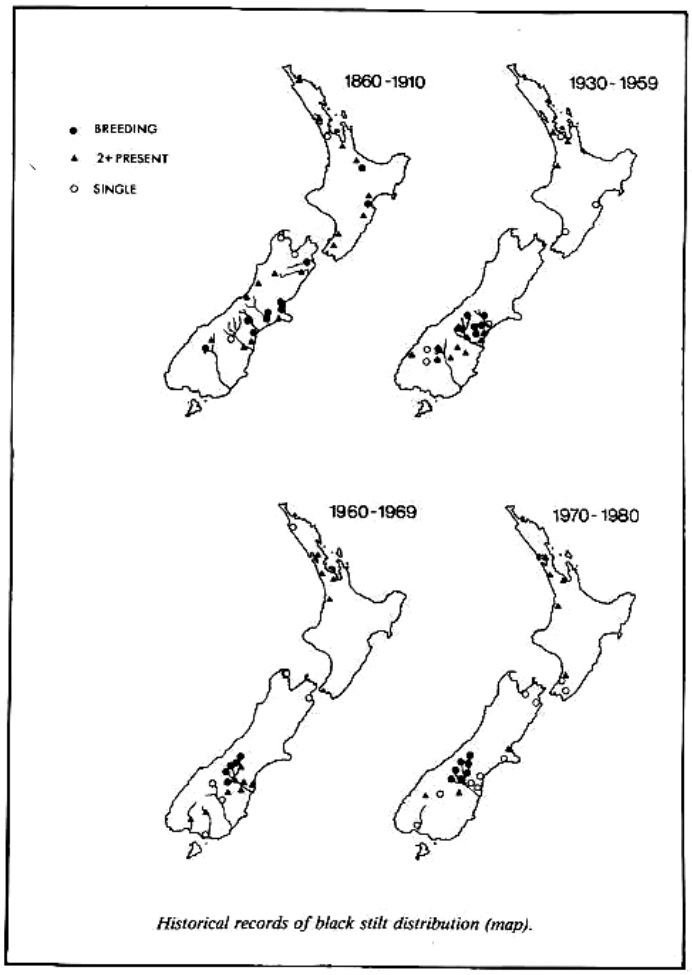

Figure 1. Changes in the distribution of black stilt / kakï (*Himantopus novaezelandiae*) in New Zealand from 1860 to 1980 (taken from Pierce, 1986).

iii. How the population is responding to these threats

The threats discussed above are intensified by the extremely small size of the remnant kakï population. In the past there was a strong bias towards males in the population; males outnumbered females 2.4:1. This was problematic due to the dispersed nature of kakï along a linear river system which limits mate availability and reduces the chance for pair formations. Although kakï show strong positive assortative mating towards their own species, excess or isolated male kakï will pair with female pied stilt or hybrid kakï in the absence of pure female kakï. Mitochondrial DNA analysis suggests that kakï and pied stilt are genetically distinct (Wallis 1999) and the resultant

offspring are considered to be undesirable hybrids. Genetic studies suggest that a level of 5% gene flow with pied stilt is the maximum that will preserve the integrity of the kakï lineage. At present, gene flow is about 15% but hybrid offspring do have reduced vigour (survival is only half that of pure kakï) which probably acts to reduce the effects of this gene flow. Captive breeding efforts have now evened the sex ratio but dispersion of kakï along the linear river system still allows hybridisation to occur. Mortality in adult kakï is high (15 – 25%) and adults have a short life expectancy in the wild (6 years) compared to that in captivity (up to 20 yrs) (Maloney & Murray 2002, Maloney et al. 2006). Therefore the recruitment rate needs to be relatively high to compensate for the high adult losses. Recruitment in wild raised kakï is estimated to be only 4% but is higher (29 – 34%) in captive-reared kakï (Maloney et al. 2006). On average, about 100 captive-reared juveniles and subadults are released to the wild annually but high adult and released kakï mortality means that the population is only able to increase at a slow rate.

iv. Conservation action

Detailed studies of kakï ecology and conservation did not begin until the mid 1970s when populations had already become confined to the Upper Waitaki basin. Kakï were first brought into captivity in 1979 to Mt Bruce National Wildlife Centre as a safeguard against extinction. Intensive management was implemented in 1981 (when only 23 adults remained in the wild) by the then New Zealand Wildlife Service (now the Department of Conservation) (Maloney & Murray 2002, Reed & Murray 1993). Since then, management has been wide and varied.

- *Predator control*

A predator control operation was initiated in 1981 and involved a combination of trap-lines along river valleys and rings of traps around individual nests. Predator control was only implemented in 14 of the 20 years between 1981 and 2000 and the degree and methods of control varied from year to year. There is some evidence to suggest that predator trapping does increase the survival rate of kakï but the variation in trapping effort among years renders this difficult to assess (Keedwell et al. 2002). Keedwell et al. (2002) recommended an adaptive approach to predator control whereby the effectiveness of trapping is assessed with intense monitoring of kakï survival. A predator control regime was set up in 2005 in the Tasman river valley as a 5 year experiment to test the effectiveness of control on kakï and other riverbed species (including reptiles and invertebrates).

Predator control in the Upper Ahuriri River Valley to protect breeding and released kakï is also underway (Leseberg et al. 2006).

- *Habitat restoration*

On a large scale, restoration of habitat is being achieved mainly through Project River Recovery (PRR). This project is fully funded by Meridian Energy and run by the Twizel DOC office. PRR staff have set up artificial managed wetlands and conduct weed and predator control as well as research and species surveys. On a smaller scale, two electric fenced artificial wetlands were set up through the kakï recovery programme in the 1980s. In the past, these wetlands were useful release sites but in recent years, management has ceased as their maintenance has not been seen as cost-effective (E. Sancha 2006, personal communication).

- *Captive management*

At the commencement of captive management in 1981, the primary focus was to increase the breeding success of wild pairs. Eggs were taken from wild pairs (to protect them from flooding and predation) then incubated in captivity and cross-fostered to wild hybrid or pied stilt. Kakï will re-clutch if their nest is destroyed. The second clutches of eggs were also removed from the nest, replaced by dummy eggs then incubated in captivity and returned to the nest shortly before hatching (Keedwell et al. 2002, Reed & Murray 1993, Sancha et al. 2004). This method encouraged kakï parents to multi-clutch so more chicks could be produced each year. However, the survival of these chicks was very poor, probably because of the vulnerability of eggs and young chicks to predation (Keedwell et al. 2002). Furthermore, a high proportion of kakï chicks fostered to hybrid and pied stilt parents followed the migration route of their foster parents and did not return to breed with other kakï. There were also concerns that cross-fostered kakï could be sexually imprinted on their foster parents and would not breed with other kakï anyway. For these reasons cross-fostering was discontinued in 1987 (Reed et al. 1993).

From 1987 onwards, a new emphasis was placed on rearing chicks in captivity instead of in their natural habitat. The practise of collecting eggs from wild pairs and incubating them in captivity was continued but instead of returning chicks to the wild, they were reared in captivity. However,

this approach was initially unsuccessful. Few captive-reared juveniles were released to the wild from 1987 to 1990 and those that were, suffered a high mortality rate (Reed & Murray 1993).

In order to set out specific goals for the future management of kakï based on lessons from the past, the first kakï recovery plan was written in 1993 (Reed & Murray 1993). This plan placed high emphasis on the importance of increasing the productivity of the captive kakï population to provide larger numbers for release to the wild. Even with the improvements that were made to captive management with the advent of this plan, survival of released kakï was initially low. Starvation and goitre were implicated as contributing to this in 1998 (Sancha et al. 2004). To overcome these problems, kakï are now supplementary fed at the release site for up to 8 weeks and iodine is added to the standard diet of all captive birds to avoid goitre (Cleland et al. 2006). Since 1997, *all* wild laid eggs have been incubated in captivity and *all* chicks have been raised in captivity (Sancha et al. 2004), either being released as juveniles (ca. 2 months) or subadults (ca. 9 months). As a result of this, an estimated 93% of all wild adult kakï have been captive reared (Maloney et al. 2006).

iv. Current issues

The latest kakï recovery plan (2001 to 2011) has the short term goal of increasing the number of kakï in the wild through captive breeding. The long term goal is to move away from captive-rearing as the primary management tool and to manage the population in the wild. Specifically, the kakï recovery plan aims to improve the status of kakï from critically endangered by increasing the wild population to 250 breeding adults with the capability of being self sustaining and naturally increasing by 2011. The current high mortality rates of released kakï is a major factor limiting the success of these goals (Maloney & Murray 2002, Maloney et al. 2006).

Recent releases of captive-reared kakï to the wild demonstrate that survival can be highly variable. Initial survival in the 2006/07 season was poor. Eleven of 31 (35%) subadults released to the wild died within 3 weeks of release, while they were still being supplementary fed (Johnston et al. 2007). Previous releases have had high survival rates soon after release. In the 2005/06 season, survival averaged 84% 3 to 7 weeks post-release (Cleland et al., 2006). However, these high initial survival rates did not persist. One year after the 2005/06 release, 69% of juveniles and 64% of subadults were known to be deceased (Johnston et al. 2007). The kakï recovery plan (2001 to

2011) identifies the need to research the causes of post-release failure and mortality in kakï and the 2005/06 kakï recovery programme annual report (Maloney et al. 2006) makes special mention of the need for this to be conducted as soon as possible. To start this research off, a large scale transmitter operation began in 2006 with many released kakï being fitted with backpack transmitters with 18 months of battery life. Already, the recovery rate of carcases has increased and so has the chance of being able to determine cause of death in kakï. So far, causes of death in released kaki have been identified as predation, trauma, accidental drowning, starvation and disease (mainly aspergillosis).

The following two sections of the General Introduction review the significance of gastrointestinal helminths and digestive organ morphology to captive-reared and released birds.

2. Gastrointestinal (GI) helminth burdens

i. How parasites can affect host populations

Parasites and disease are often considered important drivers of population and community dynamics (de Castro & Bolker 2005, Smith et al. 2006, Valera et al. 2006). They are a threat to population viability and contribute to extinction in endangered species. Simple disease theory however, predicts that a parasite or disease will always become extinct before its host and cannot therefore drive populations to extinction alone (de Castro & Bolker 2005). Nevertheless there are specific circumstances under which disease and parasites can drive a population to extinction. Parasites and disease can drive populations to low numbers leaving them vulnerable to extinction due to demographic and environmental stochasticity and allee affects. This is especially true when the host population is small before infection (de Castro & Bolker 2005, Deredec & Courchamp 2006). In circumstances were parasites and diseases have the ability to survive and amplify in the biotic (via reservoir hosts) or abiotic environment they can overcome host density thresholds and drive a species to extinction. Pathogenic agents can also cause extinction indirectly by inducing extinction or declines in one species which has a cascading effect on other species in the community (de Castro & Bolker 2005).

Gastrointestinal (GI) helminths are parasites that inhabit the gastrointestinal tracts of vertebrates. In contrast to some other diseases and pathogens, GI helminths are often sublethal. However, they may also have the capability of increasing the mortality rates of their hosts. Subclinical helminth burdens can reside inside an animal for many years, providing a small but constant demand on the host. They pose a cost to an animal through the maintenance of immune defences and constant physical repair. They can also alter an animal's activity and behaviour. Subsequently, GI helminth infections can hinder an animal's ability to cope with physiological stresses (such as nutritional demands and cold exposure) as well as ecological pressures like translocations, predation and habitat loss (Hatcher *et al.*, 2006; Kristan & Hammond, 2000; Mathews *et al.*, 2006; McCallum & Dobson, 1995; McCallum & Dobson, 2002). GI helminth infections can induce morphological and physiological changes such as reduced body condition, increased organ size and changes in enzyme production (Hatcher et al. 2006, Kristan & Hammond 2000, Mathews et al. 2006,

McCallum & Dobson 1995, McCallum & Dobson 2002). Kristan and Hammond (2000) demonstrated an increased response to stress in mice parasitised by GI helminths. Infected mice were observed to be more adversely affected by cold exposure than non-parasitised mice. Internal and external stresses can sometimes induce clinical signs from GI helminth infections by reducing immune defences (McCallum & Dobson 2002). In these cases, animals often die as a result of infection or the increased demand of infection may leave them more susceptible to stresses such as predation, starvation and cold exposure.

i. Implications for captive management and translocations

Clearly, GI helminth infections have many potential consequences for endangered species management. Helminth infections are especially relevant to captive breeding and translocation operations where there is a risk of translocation induced immuno-incompetence and transmission from the captive to the wild population (and vice versa). Mathews et al. (2006) put forward the view that routine health screening of captive individuals is of utmost importance. The acquisition of baseline disease information is invaluable in protecting the wild population from new pathogens, increasing the awareness of stress induced risks and establishing the risks to the captive population from the wild population post-release. Baseline data also allows changes in disease frequency to be detected and the identification of diseases to be monitored in the future (Mathews et al. 2006).

ii. Conservation of parasites

In contrast to the adverse effects that parasites can have on endangered species, the rare status of hosts can threaten the existence of their parasitic microfauna (Perez et al. 2006). This is especially true if the parasites are endemic to their endangered hosts. Conservation of parasites is not often considered in management programmes but it must be remembered that these organisms have their own evolutionary value and should not be regarded simply as agents of disease (Rozsa 1992). As a paradox to this classical view that parasites are always undesirable, Hudson et al. (2006) proposed that a healthy ecosystem is one that is rich in parasites. The reasoning behind this theory is that parasites help form host population dynamics, manipulate energy flow (Hudson et al. 2006), modify interspecific competition (Hatcher et al. 2006, Hudson et al. 2006) and provide selective pressures to drive biodiversity (Hudson et al. 2006).

The helminth fauna of kakï has not been fully described but the preliminary work of McDonald (1998) suggests that these birds share there parasite fauna with the more common pied stilt. With this in mind, parasite conservation may not be required for kakï. However, the kakï population has suffered a severe bottleneck and the original helminth species may have already been lost.

iv. Ecology of GI helminths, helminth screening and anthelmintic treatment in kakï

- *GI helminths found in kakï*

The most important GI helminths in kakï are thought to be trematodes (flukes), cestodes (tapeworms) and *Capillaria* spp. (nematodes [roundworms]) (Jakob-Hoff 2001). The symptoms that these three helminth groups cause range from no symptoms at all to lethargy, diarrhoea, anaemia, loss of appetite, weight loss, ill thrift and death. All three types of parasites are thought to affect the survival of translocated kakï when present in high numbers (Jakob-Hoff 2001) but this presumption has not be quantitatively evaluated. Each helminth type differs in their physiology, pathogenicity and development and transmission strategies as outlined below:

(a) Trematodes:

These usually dorso-ventrally flattened parasites attach to their hosts with oral and ventral suckers. Feeding is usually by mechanical browsing on the gut epithelium. Some species also secrete lytic substances to help break down the intestinal wall (Erasmus 1972) and others absorb nutrients directly across the body surface (Crompton 1973).

Clinical disease associated with adult trematodes is considered to be rare in natural populations but artificial conditions (such as farming and captive-rearing) can facilitate unnaturally high parasite numbers within hosts, leading to significant pathology (Erasmus 1972).

Transmission of trematodes to the definitive vertebrate host (DH) is indirect, and requires the presence of one or more molluscan intermediate hosts (Figure 2). Eggs released from the adult worm hatch into free-swimming miracidia which detect and penetrate the first intermediate host (FIH). Alternatively, the FIH is infected with miracidia through the ingestion of hatching eggs. Within the FIH (usually in the digestive glands), multiplication occurs with the miracidia

developing into sporocysts or redia (depending on the species). The sporocysts then develop into cercaria, which infect the secondary intermediate hosts (SIH). Within the SIH, cercaria develop into metacercaria which are usually encysted and very resistant. When the SIH is ingested by the DH the metacercaria develop into adult trematodes and produce a large number of eggs (Erasmus 1972, Poulin & Cribb 2002). While this is the classic trematode life cycle, this is somewhat truncated in many species. Life cycles can vary in the number of intermediate hosts used and some species do not require a definitive vertebrate host (Poulin & Cribb 2002).

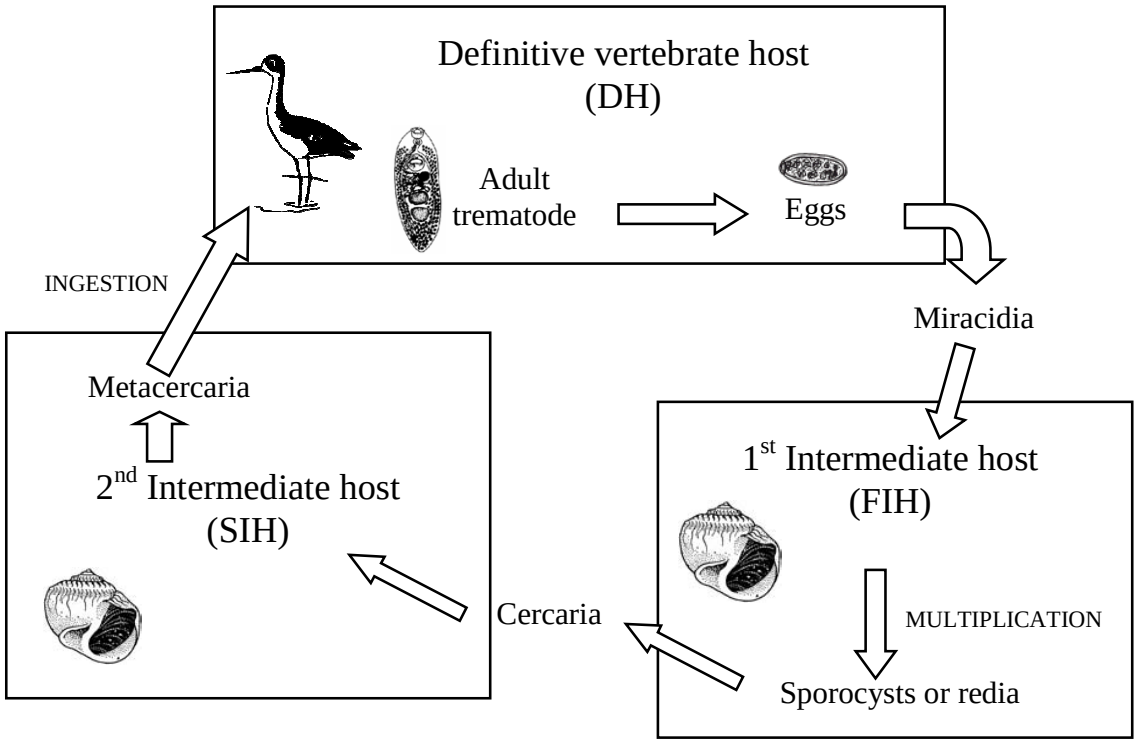

Figure 2. Typical life cycle of the trematode or fluke.

(b) Cestodes:

These parasites are usually ribbon-shaped; the body consisting of three distinct regions, the scolex (attachment apparatus), neck and strobila (which is comprised of maturing proglottids). Cestodes do not have a gut and absorb nutrients from their hosts' intestines directly through their external surfaces (Crompton 1973).

Cestodes are considered to have low pathogenicity to their hosts (Morand et al. 1995, Rees 1976) because the size and number of cestodes within a host is limited the 'crowding effect', a form of density-dependence. This effect probably occurs as a result of a combination of competition amongst individuals for carbohydrates, inflammatory reactions in the hosts gut, and compounds secreted by the worms that inhibit the growth of others (Roberts 2000).

Transmission of cestodes is indirect, requiring the presence of one or more arthropod or mammalian intermediate hosts (Figure 3.). In general, eggs are released from the adult worm singularly or within body segments (proglottids). The eggs are ingested by the FIH and embryonate to develop oncospheres. Usually, the eggs hatch within the FIH, the develop into the infective juvenile (or metacestode) stage which is passed to the DH through ingestion of the tissues of the FIH (see 2. below). Alternatively, the eggs may be ingested by the DH before hatching. In this case, the cestode develops into a metacestode and finally an adult tapeworm within the intestinal tract of its final host (see 1. below) (Crompton & Joyner 1980, Wardle & McLeod 1952).

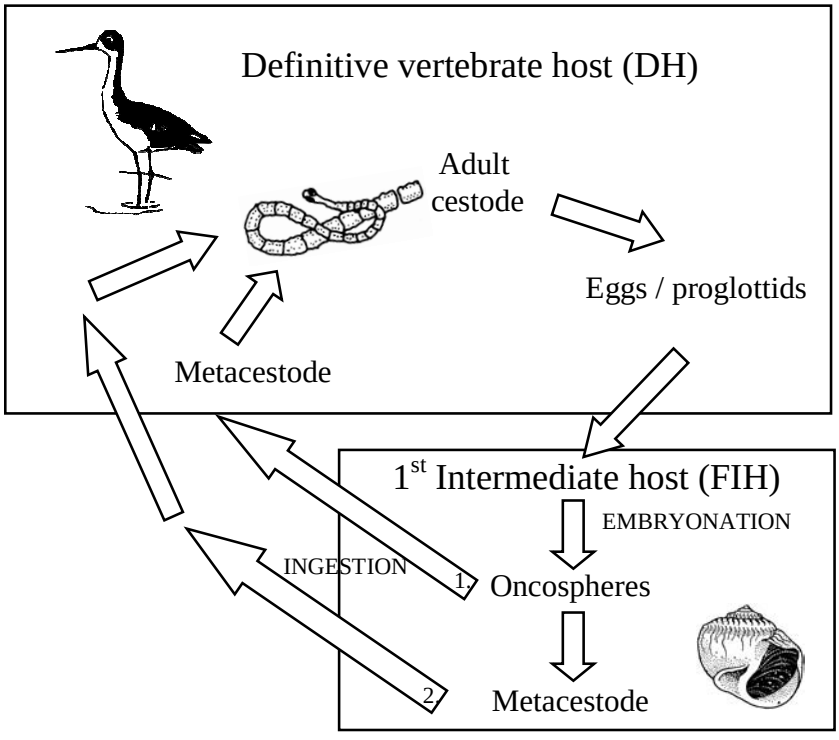

Figure 3. Typical life cycle of the cestode or tapeworm.

(c) *Capillaria* spp.:

Capillaria spp. are parasitic nematodes. Nematodes are long, thin worms and are the only helminths to possess an alimentary tract (Crompton 1973). Because of the well developed gut, nematodes exhibit a wide range of feeding behaviours which include: tissue and mucosa browsing; blood sucking; puncturing vessels with a stylet; secretion of histolytic substances; and direct ingestion of the hosts ingesta (Crompton 1973). *Capillaria* spp. are distinguished from other nematodes by the structure of their oesophagus (Anderson 2000)

Disease caused by *Capillaria* spp. can be severe, especially when high intensities infect young and immunosuppressed individuals (Lloyd 2003, Rickard & Pohl 1969).

These parasites can be transmitted to the vertebrate host either through the direct ingestion of eggs (there is no intermediate host) or indirectly (using earthworms as the intermediate host) (Figure 4.). Depending on the species, eggs are shed from the adult worm living inside the vertebrate host in faeces, urine, mucous or viscera. Eggs are usually deposited from the adult unembryonated and become infective after a period of growth and when specific humidity and temperature conditions have been met (Anderson 2000).

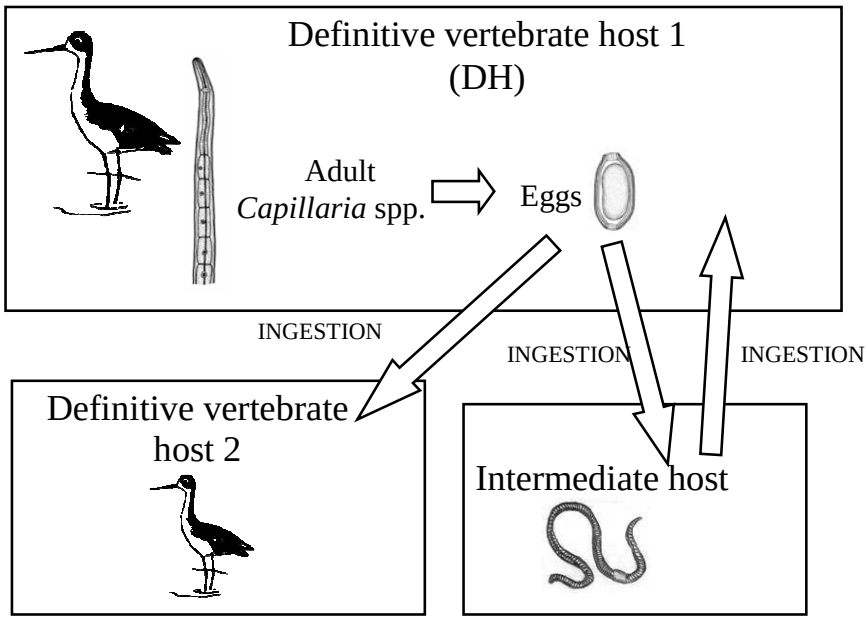

Figure 4. Typical life cycle of the nematode *Capillaria* spp..

- *Faecal screening for GI helminths*

Routine screening of kakï began in 2001 to obtain baseline data to establish which diseases could pose serious risks to the source and wild population as well as people (Jakob-Hoff 2006). During the 5 year period from 2001 to 2006, 464 live kakï were screened for a suite of diseases and parasites and an additional 113 dead kakï were necropsied. Trematodes, cestodes and *Capillaria* spp. were all detected over the course of the study and captive kakï are now routinely screened for GI helminths prior to release using faecal analysis.

Faecal screening involves the identification of helminth eggs from a sample of faeces from several birds sharing the same aviary. The accuracy of this method is often variable for many reasons, including a need to validate laboratory diagnostic methods for individual host and helminth species (Geerts & Gryseels 2000) and spatial and temporal variation in both helminth prevalence and egg shedding rates (Misof 2004, Oju & Mpoame 2006, Villanua et al. 2006b). There is also concern that collecting samples from groups of birds can result in the loss of information at an individual level, and thus lead to unnecessary over-treatment (Vercruysse & Claerebout 2001, Ward et al. 1997). Faecal screening in kakï has been relied upon as the sole indicator of helminth presence and abundance and the results obtained determine the type of treatment that is applied. Hitherto, the reliability of current kakï faecal helminth screening methods has not been assessed.

- *Anthelmintic treatment in kakï*

Before release to the wild, all captive-reared kakï are dosed with praziquantel (PZQ; Droncit®, Bayer NZ Limited, Auckland, New Zealand) at a dose rate of 20 mg / kg body weight to treat cestode and trematode infections. PZQ is always administered, even if the faecal screening does not detect any trematode or cestode eggs. However, if faecal screening reveals the presence of *Capillaria* spp. in an aviary, the enclosed birds also receive a dose of levamisole hydrochloride (Aviverm, Westwood Enterprises, Auckland, New Zealand) at a dose rate of 16 mg / kg body weight. PZQ is regularly used to treat cestode and trematode infections in mammals and birds (Blankespoor et al. 2001, Dayan 2003, Jenkins 1998). Levamisole hydrochloride is used to treat GI nematodes in many animals, including chickens (El-Kholy & Kemppainen 2005) but it has caused fatal toxicosis in birds such as North Island brown kiwi (*Apteryx mantelli*) (Gartrell et al. 2005).

- *Efficacy of anthelmintic treatment*

Any good anthelmintic regime should include determination of its efficacy which is important for ensuring the costs of treatment are outweighed by measurable benefits. The aims of such evaluations should be to assess the ongoing need for treatment, drug administration success and the effectiveness of the anthelmintic, in particular monitoring for development of resistance to the anthelmintics used. This is especially relevant for captive management programmes where resources are often limited and individual animals are highly valued. The possible costs of anthelmintic treatment include the potential to inflict injuries by following and capturing the animals, administering the treatment (Ortiz et al. 2000), the inevitable development of anthelmintic resistance (Waller 1997, West et al. 2002), chemotherapeutic toxicity (Gartrell et al. 2005) and financial and time expenses (Ortiz et al. 2000). Although serious injuries incurred while treating black stilt have so far been relatively limited, broken bills and limbs occasionally occur. Fatal capture myopathy has also been reported in at least one instance (J. Hiscock 2007, personal communication). To date, the efficacy of the kakï anthelmintic regime has not been evaluated.

There are many ways to assess the efficacy of anthelmintic programmes. As most studies are conducted on farm animals, controlled experiments are those most frequently conducted. Such studies usually involve the culling of animals at the end of the trial for assessment of total worm burden (Yazwinski et al. 2003). This is not practical for endangered species management where individuals are extremely important and a variety of uncontrollable variables may exist. The faecal egg count reduction (FECR) test is a more appropriate research technique for determining anthelmintic efficacy in naturally-infected endangered species. The major advantage is that it permits survival of the experimental animals. The FECR test compares the number of eggs per gram (EPG) of faeces in control and treated groups before and after treatment (Cabaret & Berrag 2004). Other ways of assessing efficacy include following the post-treatment patterns of helminth egg excretion of individuals and or groups of animals over time (Villanua et al. 2006a); comparing the total worm burden of control and treated animals (for those that die incidentally during the course of the study) (Millan et al. 2002); and comparing the body condition, fecundity and survival of control and treated groups (Draycott et al. 2006, Millan et al. 2002, Woodburn et al. 2002).

3. Avian digestive organ morphology

i. Phenotypic plasticity

The avian body is a dynamic entity, and has the ability to change in response to different demands (Fox & Kahlert 2005, Starck 2003). Many avian species are subjected to seasonal fluctuations in environmental conditions (such as variation in food availability and nutrient composition) and internal physiological demands (due to increased energy requirements during reproduction, moulting, migration and during cold exposure) (Fox & Kahlert 2005, McWilliams et al. 1999, Moore & Battley 2006, Redig 1989, Starck & Rahmaan 2003). Avian digestive systems can respond rapidly and reversibly to these various ecological fluctuations in order to optimise digestive efficiency and exploit the full range of available resources in the face of change (Starck 1999, van Gils et al. 2003). Phenotypic plasticity of the digestive system is especially important for migratory birds and those that inhabit wide or highly variable habitats (Kehoe et al. 1988). The gizzard, small intestine and caeca as well as the liver (which is important in nutrient absorption) have all been reported to increase in size when food intake increases or food quality decreases (Kehoe et al. 1988, Starck 2003). Histological features of the gut as well as biochemistry and digestive physiology can also change in response to dietary changes (Karasov et al. 2004). Lengthening of the gut improves digestive efficiency in birds that eat poor quality diets by allowing longer transit times. Increased diameter increases food processing per unit body weight (Relyea & Auld 2004, van Gils et al. 2003). Whereas increasing the size of the digestive system leads to greater nutritional benefits, there are energetic costs involved with maintenance and carrying them around (van Gils et al. 2003). van Gils et al. (2003) suggest that the ecological setting determines the specific organ size at which the benefits of larger digestive organs must outweigh the costs. In their study, they found that red knots (*Calidris canutus*) optimised gizzard size in response to seasonal differences in prey items.

Hypertrophy of avian digestive systems occurs when birds are exposed to high intakes of low quality, high fibre foods (Piersma et al. 1993). Consequently, omnivorous, insectivorous and granivorous birds usually have longer guts than nectarivorous, frugivorous and carnivorous birds (Kehoe et al. 1988, Starck 1996). Similarly, captive-reared birds are often fed an easily digestible low-fibre diet and usually have shorter intestines than wild birds. An experiment by Starck &

Rahmaan (2003) with Japanese quail (*Coturnix japonica*) found that captive-reared birds conditioned to a low fibre diet increased gizzard and intestine size when fed a high-fibre diet. In addition to this, they observed an immediate decline in body mass after diet-switching. This phenomenon is described as "mismatching" between digestive load and digestive capacity. During the mismatching period, digestive organs are growing in size in response to reduced food quality and there is a lag period where digestive efficiency is reduced (Hilton et al. 2000, Moss 1989, Starck 1999). Starck (1999) found that it takes about 6 days for the gut of Japanese quail to become fully adapted to a new diet after which the rate of food intake returns to normal or elevated levels. Digestive optimisation has been reported to occur between three and four weeks in red grouse (Moss & Parkinson 1972) and mallards (Miller 1975). Birds that consume a wide range of food items may be adapted for rapid changes in digestive organs while specialist feeders may not have developed this ability to such a great extent (Hilton et al. 2000). Hilton et al. (2000) compared the digestive efficiency of the specialist common guillemots (*Uria aalgae*) and the opportunist lesser black-backed gulls (*Larus fuscus)* when fed on novel diets. The results indicated that there are often species differences in gastrointestinal plasticity. In this case, black-backed gulls had more flexible digestive systems than common guillemots.

ii. Studying phenotypic plasticity

Many studies of phenotypic plasticity in avian digestive organs use gut length as the main indicator of change. However gut length is not the only parameter in which changes may affect the resorption surface. Starck (1996) suggest that gut capacity (gut length × cross section of the gut interior) and the effective mucosal area (indicated by changes in villi length, width and number; changes in gut circumference and intestine length) to be the most appropriate measures of phenotypic plasticity. Moss (1989) observed the greatest increases in gut length in red grouse (*Lagopus lagopus*) occurring in the caeca. He subsequently concluded that the caeca was important in gut plasticity and therefore a useful organ to study changes. However, the use of this model is limited as not all bird species have well developed caeca and some do not possess these organs at all.

iii. Consequences of gut plasticity for captive management and translocations

The reduced digestive performance due to diet switching may have important implications for captive-reared birds released to the wild. As discussed, captive birds are often fed easily digestible, low fibre diets. In addition, they often have low food intakes because their daily energy expenditure is low (Moore & Battley 2006). After release, they may be unable to obtain adequate nutrients to survive whilst their atrophied digestive organs adapt to the new, often highly indigestible diet (Moss 1989). Reduced digestive performance could reduce body condition, resulting in increased vulnerability to stresses associated with the translocation (such as starvation, competition and exposure).

- *Relevance to kakï*

Captive kakï are fed a highly digestible diet of mainly minced ox heart (Cottam et al. 2001, Cottam et al. n.d.) (Appendix 1). However, the wild diet is comparatively high in fibre, consisting mainly of insects, small molluscs and small fish (Marchant & Higgins 1993, Pierce 1986). It is possible that kakï exhibit phenotypic plasticity in their digestive organs because they have a wide and variable diet (Pierce 1986) and it is probable they were migratory to a greater degree in the recent past (Pierce 1984). The time taken for digestive adaptation to occur is generally considered to be rapid (days to weeks), however, mortality rates of released animals are often highest soon after release. This is true for kakï as well as many other species, including ring-necked pheasants (*Phasianus colchicus*) (Wilson et al. 1992) and European rabbits (Calvete & Estrada 2004). The potential negative impacts of digestive inefficiency could therefore be amplified during the stressful establishment phase and could significantly influence post-release survival of kakï.

4. Aims of the study

The high mortality of captive-reared kakï released to the wild is the major factor limiting the success of the kakï recovery plan goals. It is therefore critical that the reasons for these high mortality rates are identified so that management can be driven to combat these factors in the future. Predation and starvation are suspected to be the most common causes of death in released birds, but the underlying contributing factors to these mortalities have not been fully evaluated. This research investigates the possible contribution of gastrointestinal (GI) helminth burdens and suboptimal digestive organs to the high mortality rates of released kakï. Emphasis is placed on evaluating the methods used to assess the importance of these factors and to make informed recommendations for future management of kakï. The results of this study will be applied specifically to the management of kakï but the methodology and principles used throughout have broad applications and will be valuable in many situations where endangered species are bred in captivity for ultimate release into the wild.

i. Efficacy of anthelmintic treatment

The primary objective of this part of the study was to assess the efficacy of anthelmintic treatment of naturally infected, captive-reared, subadult black stilt released to the wild. This was addressed within the following aims:

(1) evaluating the effectiveness of the current anthelmintic regime in controlling the common GI helminths of kakï;
(2) determining whether GI helminths increase in intensity after release to the wild;
(3) comparing the helminth burdens of control and treated kakï that die after release;
(4) assessing whether released kakï suffer an unusually high rate of helminth infection compared to captive and wild birds; and
(5) determining whether anthelmintic treatment results in increased survival of released kakï.

ii. Reliability of gastrointestinal helminth faecal screening methods

The primary objective of this part of the study was to evaluate the reliability of current faecal screening methods in detecting potentially harmful helminths in kakï. I also aimed to make informed recommendations for future health screening protocols. This was addressed within the following aims:

(1) determining whether faecal egg counts reflect the total worm burden of adult GI helminths;
(2) assessing the accuracy of the laboratory methods commonly used to detect trematode eggs;
(3) investigating whether the results of faecal egg counts could be affected by temporal and temperature-dependent variation in helminth egg shedding; and
(4) evaluating whether individual faecal analysis is less sensitive than pooled faecal testing for detecting GI helminths eggs.

iii. Gut morphology

The main objective of this part of the study was to determine whether reduced digestive efficiency is contributing to the high mortality rates of captive-reared kakï released to the wild. Specifically, my aims were to determine whether:

(1) captive and released birds have smaller and lighter guts than wild birds;
(2) the digestive organs of released birds increase to optimise digestive efficiency after release;
(3) emaciated birds are suffering from reduced digestive efficiency; and
(4) making a comparison of fresh and formalin preserved kakï organs was reliable. Chicken digestive organs were used as a model.

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Chapter 2

Efficacy of anthelmintic treatment in captive reared black stilt (*Himantopus novaezelandiae*) released to the wild.

"Giraffing" subadult kakï at Tasman delta

(Photo by J. Hiscock)

(Submitted to the Journal of Wildlife Management)

Abstract

Gastro-intestinal (GI) helminths have the potential to reduce the success of captive breeding for release programs both directly and indirectly. Of particular concern to wildlife managers is the possibility that GI helminths can increase in prevalence due to immunosuppression of the host during translocations. To help combat this, anthelmintics are commonly administered prior to release. There are risks associated with anthelmintic treatment, such as injuring the animals, anthelmintic resistance, toxicity, loss of parasite biodiversity and financial and time expenses. It is therefore advantageous to test the efficacy of anthelmintic programs to ensure their value. We investigated the efficacy of anthelmintic treatment and the significance of GI helminth infections in captive-reared black stilt (*Himantopus novaezelandiae*) released to the wild. Black stilt are critically endangered New Zealand endemic waders. High mortality of released birds is the major factor limiting significant increases in the wild population. In 2007 we dosed half of the 80 captive birds with praziquantel (PZQ) prior to release, leaving the other half untreated. We collected pooled and individual fecal samples from the birds before and after anthelmintic treatment; and before and after release to the wild, analyzing them using modified fecal flotation and sedimentation methods. We also conducted post-mortem worm counts on those birds that died following release whose digestive organs were intact. Overall, GI helminths were spread in the typical negative binomial distribution, where just a few individuals in the population harbored most of the parasites. PZQ had high efficacy against trematodes but not *Capillaria* spp.. We did not observe any increase in GI helminth prevalence following release to the wild as hypothesized. Similarities in the helminth burdens of captive and released birds suggest that the higher trematode burden of released birds is due to the captive environment rather than immunosuppression. The presence of immature trematodes in both control and treated birds indicates that anthelmintic treatment does not prevent re-infection. Moreover it confirms exposure of released birds to GI helminths at the release site. Treated birds did not have greater survival than control birds. Overall, our results suggest that anthelmintic treatment is an unnecessary cost and we recommend the cessation of this practice or at the very least, a reduction in the intensity of treatment. We also recommend continued health screening and place high emphasis on the need for annual efficacy testing to monitor the emergence of anthelmintic resistance if treatment is to be continued.

Key words: anthelmintic, black stilt, captive breeding, fecal analysis, gastrointestinal helminths, *Himantopus novaezelandiae*, New Zealand, survival, translocations.

Introduction

Anthelmintics are commonly used in endangered species captive management programs for the prevention and treatment of gastrointestinal (GI) helminth infections. Sublethal helminth infections can depress the immune defenses of an individual, leaving it susceptible to external pressures such as malnutrition, cold exposure, predation and translocation stresses (Hatcher et al. 2006, Kristan & Hammond 2000, McCallum & Dobson 2002, Villanua et al. 2006). More severe and acute infections can be direct causes of mortality (Bailey et al. 1996, Krone et al. 2003). Determination of the efficacy of anthelmintic strategies is an important element of any captive management program in order to ensure that the costs of treatment are outweighed by measurable benefits. The possible costs of anthelmintic treatment include the potential to inflict injuries during capture and administration of the treatment (Ortiz et al. 2000), anthelmintic resistance (Waller 1997, West et al. 2002), chemotherapeutic toxicity (Gartrell et al. 2005) and financial and time expenses (Ortiz et al. 2000). Controlled experiments are frequently conducted to determine the efficacy of anthelmintics in research operations and usually involve the culling of animals at the end of the trial (Yazwinski et al. 2003). This is not practical for endangered species management where individuals are highly valued and a variety of uncontrollable environmental variables may exist.

A rare captive-reared species that has an active anthelmintic regime is the black stilt or kakï (*Himantopus novaezelandiae*). Black stilt are a small (200 to 240 g) New Zealand endemic wader and are classified as critically endangered by the International Union for Conservation of Nature and Natural Resources (IUCN) (BirdLife International 2000). These birds were once widespread in New Zealand but are now confined to the Upper Waitaki Basin in the high country of the South Island. The latest wild population estimate (February 2008) was 78 adult birds (S. Cleland, Department of Conservation, unpublished report). Habitat loss and degradation, predation by both mammalian and avian predators together with hybridization with the pied stilt (*Himantopus h. leucocephalus*) are the primary causes of past declines and still remain major threats (Maloney & Murray 2002, Pierce 1984, Reed & Murray 1993). An intensive captive management program,

involving the artificial incubation and rearing of all wild and captive laid chicks is currently operating to supplement the wild population. Captive-reared black stilt are released to the wild as juveniles (ca. 2 months old) or subadults (ca. 9 months old) and are supplementary fed at release sites for 4 to 8 weeks. It is the poor survival of these released birds that is one of the main limitations of the black stilt recovery program in increasing the number of adults in the wild population (Maloney & Murray 2002).

The common GI helminths in black stilt are trematodes (flukes), cestodes (tapeworms), and *Capillaria* spp. (nematodes, roundworms). They are spread by ingestion of fecal contamination or infected intermediate hosts (invertebrates and freshwater snails). All three types of parasite are thought to affect the survival of black stilt when present in high numbers (R. Jakob-Hoff, Auckland Zoo, unpublished report). There is also concern that the stress of translocation and release may increase the intensity of subclinical helminth infections to pathogenic levels. This has been observed in some European game bird species such as ring-necked pheasants (*Phasianus colchicus*) (Villanua et al. 2006). For these reasons, since 2001, black stilt have been routinely screened for GI helminths prior to release using fecal analysis since 2001. Regardless of the results, all birds are dosed with praziquantel (PZQ; Droncit®, Bayer NZ Limited, Auckland, New Zealand) before the translocation at a dose rate of 20 mg / kg body weight to reduce the density of cestodes and trematodes. If the eggs of *Capillaria* spp. are detected in an aviary, the enclosed birds are also treated with levamisole hydrochloride (Aviverm, Westwood Enterprises, Auckland, New Zealand) at a dose rate of 16 mg / kg body weight. PZQ is routinely used to treat cestode and trematode infections in mammals and birds (Blankespoor et al. 2001, Dayan 2003, Jenkins 1998). Levamisole hydrochloride is used to treat GI nematodes in many animals, including chickens (El-Kholy & Kemppainen 2005) but it has caused fatal toxicosis in birds such as North Island brown kiwi (*Apteryx mantelli*) (Gartrell et al. 2005). Serious injuries incurred while catching and handling black stilt during handling have been infrequent, although broken bills and limbs do occur and there has been at least one reported case of fatal capture myopathy (J. Hiscock, Department of Conservation, personal communication). To date, the efficacy of the black stilt anthelmintic regime has not been evaluated.

Our study was carried out in conjunction with the annual release of black stilt by the New Zealand Department of Conservation (DOC). We aimed to assess the efficacy of anthelmintic treatment of naturally infected, captive-reared, subadult black stilt released to the wild by 1) evaluating the effectiveness of the current anthelmintic regime in controlling the common GI helminths of black stilt; 2) determining whether GI helminths increase in number after release to the wild; 3) comparing the helminth burdens of control and treated black stilt that died after release; 4) assessing whether released black stilt suffer an unusually high rate of helminth infection compared to captive and wild birds; and 5) determining whether anthelmintic treatment results in increased survival of released black stilt.

Study Area

We conducted our research in the Upper Waitaki Basin, South Canterbury, in the South Island of New Zealand, the only region that black stilt remain in the wild (Maloney & Murray 2002, Pierce 1996) (Fig. 1.).

i. Location and Number of Captive Birds

Our studies on captive birds took place at the Ruataniwha aviaries in Twizel, South Canterbury (44°16.5'S, 170°05.7'E), and at Peacock Springs in Christchurch, North Canterbury (43°27.8'S, 172°31.3'E). The Ruataniwha aviary complex consisted of a captive-rearing centre together with three large outdoor flight aviaries, whereas Peacock Springs had just one outdoor aviary. The aviaries at both locations were further divided into 4 to 12 compartments that housed different numbers of birds. Each aviary was fed by a separate water supply that was independent of the other aviaries. However, the source of water for all three Ruataniwha aviaries was a single small pond fed from a natural spring. In 2007, 64 subadult and 8 adult black stilt were housed at the Ruataniwha aviaries and a further 13 subadults and 4 adults were held at Peacock Springs.

ii. Releases and Release Sites

There were two black stilt release sites in 2007. The first (August) release site was at Mailbox Inlet on the shore of Lake Tekapo (43°53.4'S, 170°29.2'E). The second (September) release site was the Tasman River delta near Mount Cook (43°54.4'S, 170°07.7'E) (Fig. 1). Mailbox inlet was surrounded by a mammalian predator reducing fence and a widespread predator trapping program was in operation at the Tasman delta. Both release sites were high altitude braided river and glacial lake systems on the leeward flanks of the main axial ranges; the Southern Alps. Situated in the 'rain shadow' zone, both release sites experienced low annual rainfall (600 – 800 ml), regular summer water deficit and intermittent severe drought (McGlone & Moar 1998). Summers were hot and dry with an average maximum temperature of 21°C (1971 – 2000). Winters were cold and frosty, with some snow and a maximum average temperature of 7°C (1971 – 2000) (National Institute of Water and Atmospheric Research [NIWA] 2008).

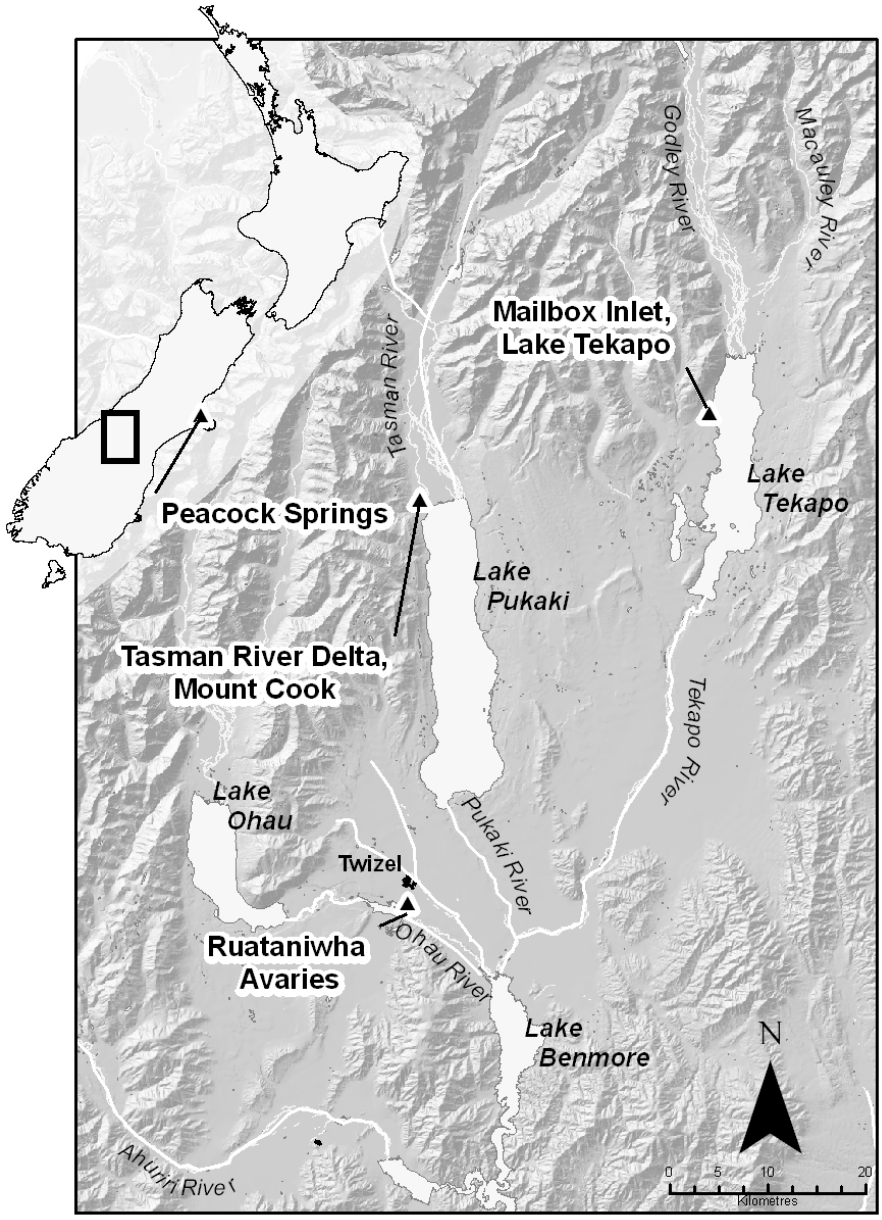

Figure 1. The Upper Waitaki Basin, South Island, New Zealand. We show the locations of the Twizel captive breeding complex (Ruataniwha Aviaries) and the two release sites (Tasman River Delta and Mailbox Inlet) where we studied the efficacy of anthelmintic treatment in subadult black stilt released to the wild in August and September 2007. The Peacock Springs captive complex is 215km north east of Twizel and is shown on the map on New Zealand to the left.

Methods

i. Source of Birds, Treatment and Releases

In August and September (late winter to early spring) of 2007, DOC released 77 subadult, captive-reared black stilt in two groups to two different release sites. The first release (n = 38), on 16 August, was to Mailbox Inlet, Lake Tekapo whereas the second release (n = 39), 5 September, was to the Tasman River delta, Mount Cook. Thirteen of the 38 birds released in August were raised at Peacock Springs. The remaining 25 birds in the August release and all 39 birds in the September release were raised at the Ruataniwha aviaries.

One week before release and again of the day of release, we dosed approximately half the birds in each release group with PZQ (August n = 18 of 38, September n = 19 of 39). We randomly choose half of the aviaries to dose, leaving the other half of the aviaries untreated. For the "treatment" birds, we ground PZQ tablets to a fine powder, mixed 4 mg with a small ball of minced ox heart and force fed one ball to each bird (a dose rate of 20mg/kg body weight). To reduce the level of stress applied to the "control" birds, we did not administer placebos.

ii. Fecal Sample Collection

- *Pooled samples*

We collected fecal samples from groups of black stilt in single aviaries by placing two clean plastic sheets approximately 1 m × 2 m in each aviary in areas favored by the birds (indicated by accumulation of feces on the gravel). We scraped the fecal samples from the plastic sheets using clean knives and stored them in sterile airtight containers at 4°C.

- *Individual samples*

We collected fecal samples from individual black stilt in captivity by handling the birds over plastic sheets while health checks were being conducted or transmitters attached. Alternatively, we collected samples from the birds while they were held in individual compartments of translocation boxes (approximately 40 × 20 cm) lined with plastic sheets. To collect samples from birds recently released to the wild, we placed plastic sheets (approximately 1 m × 2 m) beneath feeding plates. We then observed the birds individually and collected feces as soon as they were passed.

iii. Fecal Examination for Helminth Eggs

- *Pooled Sample Flotation*

We employed modified fecal flotation methods (Charleston & Pomroy 1984) for the identification of cestode and nematode eggs. We weighed approximately 0.5 g of each thoroughly homogenized sample into a bowl (unless < than 1 g was collected and then we used about half of the sample). We mixed the sample with $ZnSO_4$ solution of 1.2 specific gravity and strained it through a 1 mm sieve. We then ground each sample using a metal spatula and discarded any feces retained in the sieve. We poured the remaining mixture into a 10 ml glass centrifuge tube and filled it to the top with $ZnSO_4$ solution. We placed a cover slip on top of the tube and centrifuged the sample at 1200 RPM for 5 mins. After spinning, we placed the cover slip onto a glass slide and counted the number of eggs microscopically at 20 × magnification. The remaining supernatant and pellet were discarded.

- *Pooled Sample Sedimentation*

We used modified fecal sedimentation methods (Charleston & Pomroy 1984) to detect the heavier trematode eggs. We weighed approximately 0.5 g of each homogenized fecal sample into a bowl (unless < than 1 g was collected and then we used approximately half of the sample) and strained it through a sieve with tap water. We then ground each sample using a metal spatula and discarded any retained feces. We poured the remaining mixture into a 10 ml glass centrifuge tube and filled it to the top with water. We then inverted each tube 4 times to ensure proper mixing. We left the samples to sediment for 5 mins, before pipetting off the supernatant, leaving the pellet intact. After topping up the tube with water we left the sample to sediment for a further 5 mins and again, removed and discarded the supernatant. We poured the pellet into a metal bowl and added a drop of standard methylene blue solution to stain the eggs. Finally, we pipetted the sample into a marked Petri dish in manageable amounts and counted the eggs systematically under a dissecting microscope (3.5 × magnification).

- *Individual Sample Flotation followed by Sedimentation*

Individual samples were often very small and could not be practically split in half for analysis. We used the entire (thoroughly mixed) sample (or 0.5 g if it was large enough) for the flotation as

described above and examined the cover slip for cestode and nematode eggs. Instead of discarding the sample after centrifugation, we carefully poured off the supernatant. The pellet then became available for sedimentation. We mixed this with water and allowed it to sediment twice for 5 mins before using the standard examination methods previously described.

iv. Efficacy of Anthelmintic Treatment

We conducted this experiment at the Ruataniwha aviaries from 6 – 15 August 2007 (August release) and 24 August – 3 September 2007 (September release). We collected one pooled fecal sample from each aviary one day before the birds received the first dose of anthelmintic, one day after treatment and then every two days until the day of release (and second dose) so that samples were obtained for day 1 pre-treatment and days 1, 3, 5 and 7 post-treatment.

- *Fecal egg count reduction (FECR) test*

To calculate percent efficacy, we used the following two fecal egg count reduction (FECR) test formulae:

1. Controlled FECR = $100 \times \{1 - [T_2 / T_1] [C_1 / C_2]\}$ (Dash et al. 1988, Presidente 1985)
2. Critical FECR = $100 \times \{1 - [T_2 / T_1]\}$ (Kochapakdee et al. 1995)

The parameters T, C, $_1$ and $_2$ refer to treated, control, pre-treatment (day -1) and post-treatment (day 7) mean worm egg counts respectively.

The "controlled" test compared helminth numbers of control and treated birds before and after treatment and is considered preferable for use in poultry studies (Yazwinski et al. 2003). The "critical" test compared helminth numbers before and after treatment in the treated group only (Yazwinski et al. 2003). We used both arithmetic and geometric means to calculate each FECR.

- *Pattern of helminth egg excretion following treatment*

We analyzed pooled FEC after $\log_{10}$ (FEC+1) transformation to normalize the data. We used the MIXED procedure of SAS 9.1© (Cary, NC, USA), with a linear model that included the fixed effects of treatment status, collection day and treatment status × collection day interaction.

v. Pattern of Helminth Egg Shedding Following Release

We carried out this study from 7 August – 13 September 2007 (August release) and from 24 August – 8 September 2007 (September release). We collected individual fecal samples from all black stilt immediately prior to PZQ treatment (on the day of the first dose) and on the day of release (day of second dose). Following release to the wild, we aimed to obtain one fecal sample from each bird every week for two (September release) or four weeks (August release). We used the MIXED procedure of SAS 9.1, performing $\log_{10}$ (FEC+1) transformations due to skewness of the data. We used a linear model including the fixed effects of week of collection, treatment status and week × status interaction

vi. Total Worm Burdens of Captive, Released and Wild Birds

We conducted total worm counts on 4 captive, 11 released and 11 wild birds that were submitted for necropsy in 2007. These were made up of 15 black stilt, 8 black – pied stilt hybrids and 3 pied stilt (*H. himantopus leucocephalus*). Pied stilt and hybrid birds were used in this analysis because no wild black stilt were available. We stored all of the birds frozen straight after necropsies were conducted and thawed them immediately before our analysis. We cut the esophagus, proventriculus, gizzard, small intestine, cecae and rectum of each bird in half longitudinally, scraped the contents out and stored them separately in 10% buffered formalin solution. We counted the helminths of the upper and the lower halves of the small intestine separately. We examined and counted the parasites microscopically at 3.5 × magnification and identified them to class. We also used the historical total worm count data (1997 – 2006) for 21 captive, 10 released and 2 wild pure black stilt accessed from the Massey University pathology database. We compared the total worm counts (TWCs) of captive, released and wild *Himantopus* sp. using the MIXED procedure of SAS 9.1. The linear model took into account the fixed effects of treatment status, sex and age. We $\log_{10}$ (TWC+1) transformed all the data to achieve normality. We also compared the locations of GI helminths along the alimentary tracts of the birds (see Appendix 2 for methods and results).

vii. Total Worm Burdens of Control and Treated Birds

Eleven of the subadult black stilt that died after release to the wild in 2007 (control $n = 6$, treated $n = 5$) were in a suitable condition for total worm burden analysis. For these birds, we used the worm counting methods described in the previous section. We compared the total worm burdens of control and treated birds using the MIXED procedure (SAS 9.1). We $\log_{10}$ (TWC+1) transformed the data before analysis to normalize the distribution.

viii. Survival of Control and Treated Birds

We recorded the fate of black stilt after release for 31 (August release) and 84 days (September release). The birds in the September release were fitted with radio transmitters so we could track them for a longer period of time. Birds in the August release had no tracking devices, so we could only track them while they remained at the release site. After the 4 week supplementary feeding period, they dispersed elsewhere. We obtained data for 66 birds; the fate of the other 11 birds was unknown. We used Sigmaplot 11.0 © (Systat Software Inc., San Jose, California, USA) to compare survival (Kaplan Meier survival analysis, Gehan – Breslow test), hazard ratios (Cox regression – proportional hazards model) and mean survival times of black stilt in control and treated groups in each release month.

Results

From the fecal analysis, we detected the eggs of trematode and *Capillaria* spp. but we did not find any cestode eggs. From the total worm burden analysis, we recovered adult trematodes, cestodes, *Capillaria* spp. and other unidentified nematodes. We also identified immature trematodes in some birds.

i. Efficacy of Anthelmintic treatment

Pre-treatment fecal screening undertaken by a commercial laboratory in 2007 did not detect any *Capillaria* spp. eggs so none of the birds were treated with levamisole hydrochloride. Consequently, we could only assess the efficacy of PZQ.

- *Fecal egg count reduction (FECR) test*

When considering the arithmetic means, pertaining to the flock as a whole (McKenna 1997b), PZQ was found to have high efficacy against trematodes (Table 1.). The FECR proportions of 92% and 97% in August were inside the high efficacy threshold of >90% as outlined by Tucker et al. (2007) and Yazwinski et al. (2003). The values calculated from the geometric means and thus the average bird (McKenna 1997b) showed slightly lower FECR values of 80% and 87%. In contrast, PZQ had low efficacy against *Capillaria* spp.; the arithmetic means producing lower estimates of FECR (34% and 10%) than the geometric means (40% and 19%). The low prevalence of both helminth types in September meant we could not conduct FECR analysis reliably for this month.

Table 1. Mean pre-treatment helminth egg counts (no. eggs / g of feces [EPG]) and fecal egg count reductions (FECR) calculated from controlled and critical tests of the efficacy of praziquantel in the treatment of trematodes and *Capillaria* spp. in black stilt. Results are expressed using both arithmetic and geometric means. The study took place in August and September 2007 at the Ruataniwha aviaries, Twizel, New Zealand.

Helminth	Month	Test	Arithmetic mean		Geometric mean [a]	
			Mean EPG	FECR (%)	Mean EPG	FECR (%)
Trematodes	Aug	Controlled [b]	962	92	22	80
	Sep		0		0	
	Aug	Critical [c]	418	97	10	87
	Sep		0		0	
Capillaria spp.	Aug	Controlled	19	34	3	40
	Sep		0		0	
	Aug	Critical	12	10	1	19
	Sep		0		0	

[a] Fecal egg counts transformed $y = \log_{10}(FEC + 1)$, geometric mean = antilog (y)- 1

[b] Controlled FECR $= 100 \times \{1 - [T_2 / T_1] [C_1 / C_2]\}$ [d] (Dash et al. 1988, Presidente 1985)

[c] Critical FECR $= 100 \times \{1 - [T_2 / T_1]\}$ [d] (Kochapakdee et al. 1995)

[d] FECR parameters T, C, $_1$ and $_2$ refer to treated, control, pre-treatment and post-treatment mean worm egg counts respectively.

- *Pattern of helminth egg excretion following treatment*

There were substantial differences in the FECs obtained for the August and September releases for both trematodes and *Capillaria* spp. (trematodes: $F_{1, 104} = 67.73$, $P = <0.001$; *Capillaria* spp $F_{1, 104} = 77.15$, $P = <0.001$). Despite these differences, we chose to combine the FECs of both release months. This increased the power and reduced the error of our analysis in the presence of a high proportion of negative FEC results that were obtained for both release months.

The FECs of pooled samples were highly variable. Trematode FECs ranged from 0 – 1176 EPG in the August and 0 – 35 EPG in the September releases. *Capillaria* spp. FECs ranged from 0 – 104 EPG in the August and 0 – 5 EPG in the September releases.

There was no effect of treatment status on trematode FECs although the *P*-value was small ($F_{1, 17} = 4.08$, 0.059). There was also no effect of day ($F_{4, 68} = 1.40$, $P = 0.245$) or the interaction between status and day ($F_{4, 68} = 1.79$, $P = 0.142$). No trematode eggs were detected at days 1 and 3 following treatment, but FECs increased slightly by days 5 and 7 (Fig. 2). There were more trematode eggs in control than treated groups at days 1 ($t_{68} = 2.11$, $P = 0.039$), 3 ($t_{68} = 2.31$, $P = 0.024$) and 5 ($t_{68} = 2.19$, $P = 0.032$) following treatment, but not day 7 ($t_{68} = 1.81$, $P = 0.074$).

There was no effect of treatment status ($F_{1, 17} = 1.17$, $P = 0.294$) and day ($F_{4, 68} = 1.05$, $P = 0.388$) or their interaction ($F_{4, 68} = 1.44$, $P = 0.2431$) on *Capillaria* spp. FECs. No *Capillaria* spp. eggs were detected 1 day after treatment but FECs increased to approximate pre-treatment levels by day 3 (Fig. 2). No differences in the FECs of control and treated groups were observed at any of the collection days.

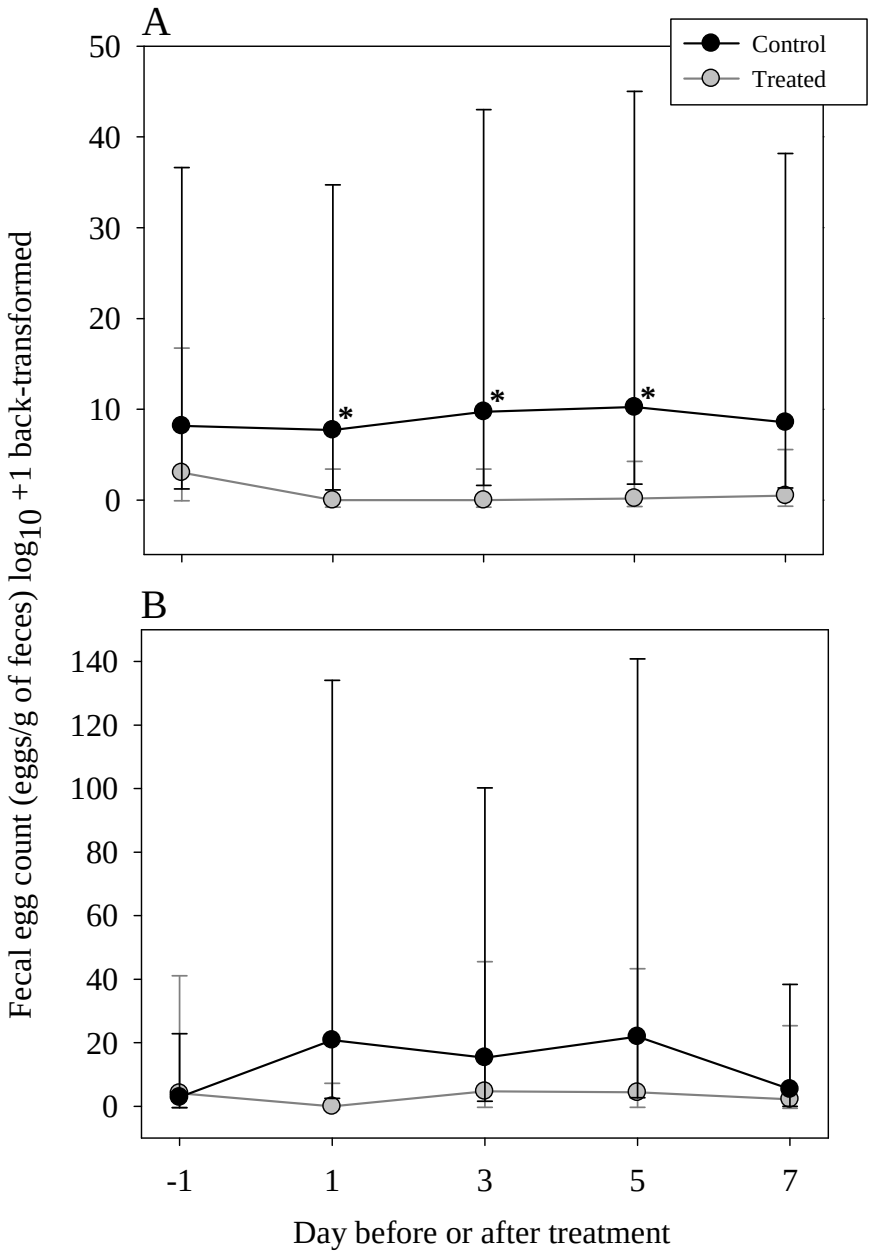

Figure 2. Least squares means of black stilt (*Himantopus novaezelandiae*) trematode (A) and *Capillaria* spp. (B) fecal egg counts (FECs) from pooled samples before and after treatment with praziquantel. Treatment was on day 0. Results are the combined FECs for the August and September releases, Upper Waitaki Basin, New Zealand, 2007. Asterisks denote significant differences in the FECs of control and treated groups ($P < 0.05$). Bars are back-transformed 95% confidence intervals from the $\log_{10}$ (FEC+1) transformed data.

ii. Pattern of Helminth Egg Excretion Following Release

Significantly fewer helminth eggs were found in September release birds than in August release birds (trematodes $F_{2, 233} = 15.81$, $P = <0.001$; *Capillaria* spp. $F_{2, 233} = 26.66$, $P = <0.001$). However, we chose to analyze the release months together to increase the power of our analysis.

Trematode FECs in the August released birds ranged from 0 – 1767 EPG. September FECs were low (0 – 37 EPG). There were no differences in mean FECs across the weeks of the study ($F_{4, 138} = 1.03$, $P = 0.393$), between control and treated birds ($F_{1, 67} = 1.55$, $P = 0.218$) nor was there an effect of the interaction between week and treatment status ($F_{4, 138} = 0.45$, $P = 0.769$). The FECs of treated birds did decrease noticeably one week after treatment (Fig. 3). This was not significant ($t_{138} = 1.51$, $P = 0.132$) but the analysis we performed on the August data alone did reveal significant differences at week 0 ($t_{60} = 2.02$, $P = 0.048$).

Overall *Capillaria* spp. FEC ranged from 0 – 2131 EPG in the August release. However, only 1 of 112 flotations conducted (< 1%) was positive for *Capillaria* spp. in the September release. Mean FECs did not differ significantly according to week of collection ($F_{5, 154} = 1.21$, $P = 0.307$), treatment status ($F_{1, 66} = 0.01$, $P = 0.918$) or their interaction ($F_{5, 154} = 1.56$, $P = 0.176$) (Fig. 3).

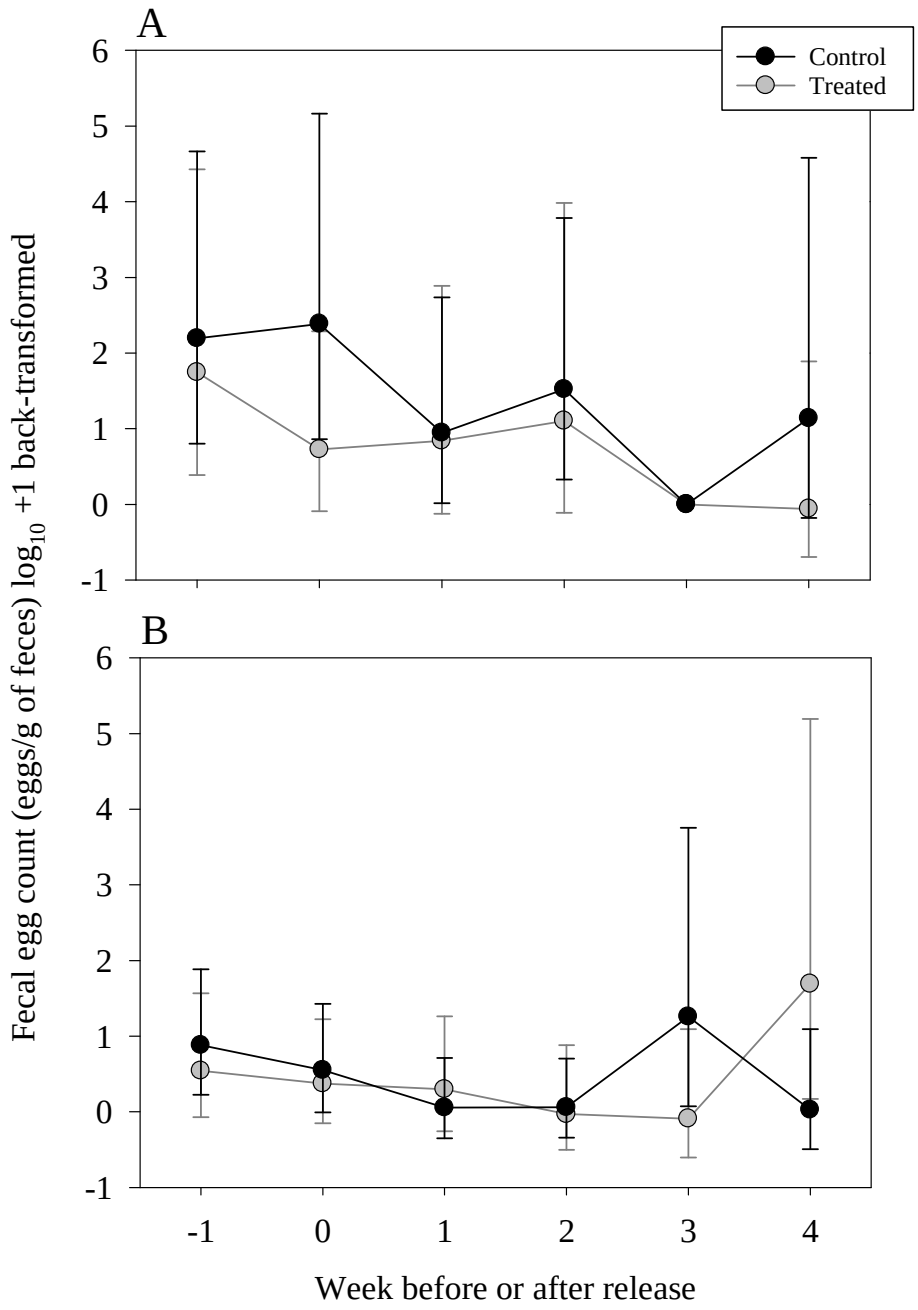

Figure 3. Back-transformed least squares means of trematode (A) and *Capillaria* spp. (B) fecal egg counts (FECs) from individual black stilt before and after release to the wild. Week 0 is the week of release. Results are combined for the August and September releases, Upper Waitaki Basin, New Zealand, 2007. Bars are back-transformed 95% confidence intervals from the $\log_{10}$ (FEC+1) transformed data.

iii. Total Worm Burdens of Captive, Released and Wild Birds

The total worm burdens of individual birds varied greatly. Trematode counts ranged from 0 – 6375; cestodes from 0 – 220; and nematodes from 0 – 30 worms. Released birds had higher trematode TWCs than wild birds. Wild birds had higher numbers of cestodes than captive birds. Wild birds also had higher nematode numbers than both captive and released birds (Table 2.).

The average number of trematodes found in female black stilt was 36.6 (back-transformed least squares mean) (back-transformed 95% CI = 11.9 – 108.9), about 11 times greater than in males with an average of 3.2 (0.4 – 11.5) worms per bird (t_{41}= 2.90, P = 0.006). Trematodes were about 7 times more abundant in subadult than adult birds (t_{56} = –2.49, P = 0.016). Average nematode burdens of adult birds were more than 25 times greater than those of juvenile birds (t_{55} = 3.46, P = 0.001).

Table 2. Differences in the total worm counts (TWCs) of captive (n = 25), released (n = 21) and wild (n = 13) black stilt, black–pied stilt hybrids and pied stilt from necropsy data (1997 – 2007). Least squares means and 95% confidence intervals (CI) are back-transformed from the $\log_{10}$ (TWC+1) data. A significant difference was accepted when $P < 0.1$.

				Captive		Released	
Helminth	Source	$\bar{x}$	95% CI	t_{df}	P	t_{df}	P
Trematodes	Captive	12.15	3.62 – 36.39				
	Released	20.70	5.54 – 71.07	t_{56} = –0.63	0.531		
	Wild	2.61	0.52 – 7.60	t_{56} = 1.91	0.062	t_{56} = 2.43	0.019
Cestodes	Captive	0.83	0.10 – 2.03				
	Released	1.64	0.43 – 3.89	t_{54} = –0.93	0.356		
	Wild	3.27	1.20 – 7.27	t_{54} = –2.04	0.046	t_{54} = –1.06	0.293
Nematodes	Captive	0.15	–0.06 – 0.40				
	Released	0.50	0.04 – 1.32	t_{55} = –1.11	0.273		
	Wild	2.12	0.83 – 4.33	t_{55} = –3.52	0.001	t_{55} = –2.13	0.038

iv. Total Worm Burdens of Control and Treated Birds

We had 11 birds suitable for this analysis, 6 of which died within the first 8 days of release. The remaining 4 birds died at days 30, 50 and 68 (n = 2) following release. There were no significant differences in the total helminth loads of control and treated birds for adult or juvenile trematodes ($F_{1, 7}$ = 0.27, P = 0.622 and $F_{1, 7}$ = 0.59, P = 0.466 respectively), cestodes ($F_{1, 7}$ = 1.33, P = 0.287) or *Capillaria* spp. ($F_{1, 7}$ = 0.78, P = 0.407) (Fig. 5.).

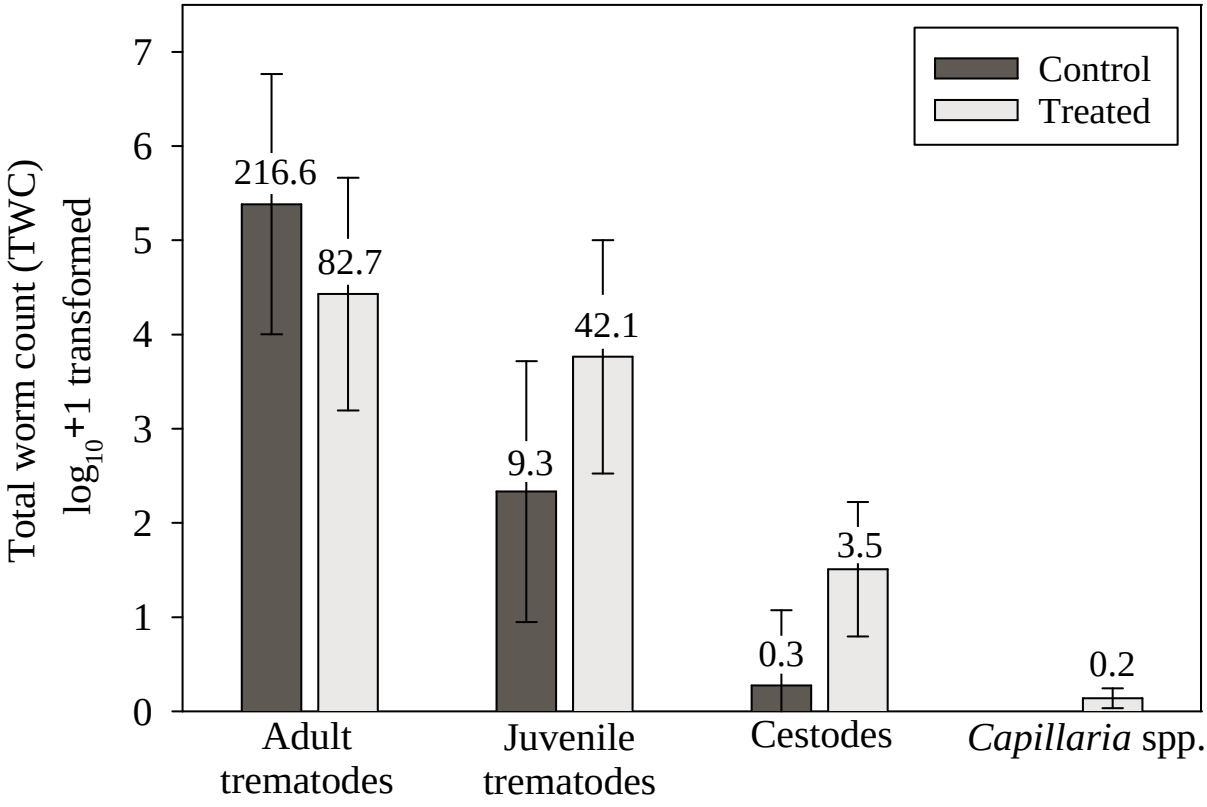

Figure 4. Least squares means of helminth total worm counts (TWC) for control (n = 5) and treated (n = 6) black stilt released to the wild in the Upper Waitaki Basin, New Zealand in 2007. Bars indicate the $\log_{10}$ (TWC+1) transformed values. The back-transformed least squares means are displayed above each bar. The individual standard errors from the $\log_{10}$ (TWC+1) transformed data are presented. Times to death after release ranged from 0 – 68 days

v. Survival of Control and Treated Birds

Of the black stilt released in August that we were able to locate, 8 (23.5%) died within the first week of release and 12 (38.7%) died during the first 4 weeks. However, 11 of the birds released in August dispersed immediately after release so the overall mortality rate is likely to be higher. Only 2 (5.7%) of the birds released in September died within the first 1 to 4 weeks after release; totaling 9 (25.7%) at the end of 12 weeks (Fig. 6). Causes of mortality of the 21 recovered black stilt that died in 2007 were predation (n = 13) (avian and mammalian), wire trauma (n = 4), starvation (n = 1) and unknown (n = 3). In many cases we could not make a completely confident diagnosis due to extensive predation and or scavenging.

Survival did not differ between treatment groups in the August (χ^2_1 = 0.42, P = 0.517) or September (χ^2_1 = 0.37, P = 0.545) releases. The total survival rates of control and treated birds to 31 days in the August release were 65%, SE = 12% and 57%, SE = 13% respectively. The total survival rate of birds in the September release to 84 days was 80%, SE = 9% for control and 67%, SE = 12% for treated groups. Mean survival time of birds in the August release over 31 days was 22.9, SE = 3.4 days for control and 26.9 days, SE = infinite no. for treated birds. Mean survival of birds in the September release over 84 days was 76.0, SE = 4.9 days in control and 74.4, SE = 5.7 days in treated birds. There were no significant differences in the hazard rates (how rapid the birds were dying) between control and treated birds for either of the releases (Aug: hazard ratio = 0.63, 95% CI = 0.19 – 208; Wald χ^2_1 = 0.50, P = 0.450. Sep: hazard ratio = 0.63, 95% CI = 0.17 – 2.35; Wald χ^2_1 = 0.48, P = 0.491).

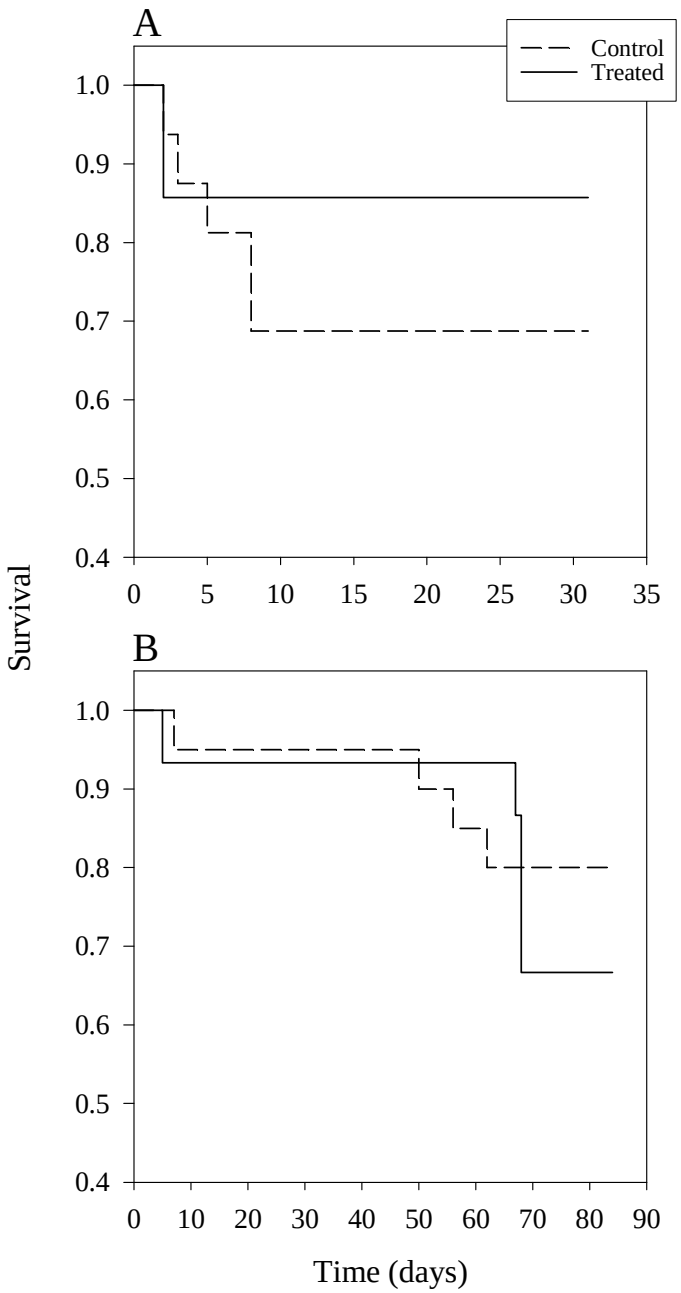

Figure 5. Kaplan-Meier **s**urvival curve for captive-reared subadult black stilt in control and treated groups for the August release (A) (0 – 31 days post-release, *n* = 31) and September release (B) (0 – 84 days post-release, *n* = 35). Both releases were to different regions in the Upper Waitaki Basin, New Zealand. There were no significant differences in the survival rates or times taken for birds to die after release in the control and treated groups for either release month. We give time in days, starting at the day of release to the wild.

Discussion

The main findings of our research were 1) the current anthelmintic program is effective at controlling trematodes but not *Capillaria* spp. in captive black stilt; 2) helminth egg excretion rates did not increase after the translocation as we hypothesized; 3) released birds had significantly higher trematode burdens than wild birds but did not suffer higher burdens of cestodes or *Capillaria* spp. than captive or wild birds; 4) there were no significant differences in the total worm burdens of control and treated birds; and finally 5) birds treated with anthelmintics before release did not have better survival than birds that were untreated.

i. Efficacy of Anthelmintic Treatment

We were not able to assess the efficacy of the full anthelmintic program. Typically, PZQ and levamisole hydrochloride are both administered to black stilt before release but levamisole hydrochloride was not administered in 2007 because no *Capillaria* spp. were detected by the commercial laboratory. The difference in the detection rate of *Capillaria* spp. between the commercial laboratory and our own work highlights the unreliability of currently used fecal screening methods. As we did not detect any cestode eggs, we could not assess the efficacy of the anthelmintic regime against these helminths. The presence of mature cestodes in many of the released birds that were later necropsied suggests they were probably present at the time of fecal sampling. Cestode eggs can be difficult to detect in flotations because they have relatively high specific gravities (David & Lindquistt 1982). In addition, many cestodes (including the Cyclophyllideans which have been identified in black stilt) do not shed individual eggs. Instead they release gravid body segments (proglottids) (Khalil et al. 1994) that are probably too heavy to float in the flotation medium.

We used two FECR calculation methods to calculate the percent reduction in fecal egg excretion following treatment. There is some debate about the use of arithmetic or geometric means to calculate FECR. The literature indicates that arithmetic means generally refer to the population as a whole whereas geometric means refer to the average individual in a population (Dash et al. 1988, McKenna 1997b). We used both arithmetic and geometric means to calculate FECR. In poultry, the threshold for high efficacy is considered to be a FECR of >90% (Tucker et al. 2007, Yazwinski et al. 2003). In our study, the arithmetic means for trematode FECR were 92% and 97% for the

controlled and critical tests respectively. The geometric means produced lower estimates of 80% and 87% but Dash et al. (1988) suggest that differences between the FECR estimates are of little consequence as long as both are high (>80%). Efficacy of PZQ against *Capillaria* spp. was low; 10 – 40%. This is not surprising as PZQ is not recognized as having good activity against nematodes (Martin et al. 1997).

Treatment with PZQ rapidly reduced trematode and *Capillaria* spp. numbers. In fact, prevalence of both helminth types in the treated group was 0% just one day after treatment. Trematode egg numbers in the treated group did not increase over the 7 day study period after this initial reduction. It is probable then that the reduction in trematode egg numbers is a true reflection of the vast majority being killed by the treatment. Commercial poultry FECR tests are also conducted over 7 days (Tucker et al. 2007, Yazwinski et al. 2003) suggesting we did allow enough time for trematodes to recover from the treatment if they were going to do so. The initial reduction in *Capillaria* spp. egg numbers was not maintained over the course of the study, suggesting that PZQ merely induced temporary suppression of egg excretion and did not actually kill the parasites (McKenna 1997a).

Although mean GI helminth FECs were relatively low, the large 95% confidence intervals indicate that some individuals within the aviaries had very high helminth burdens. This was especially true for *Capillaria* spp..

ii. Pattern of Helminth Egg Excretion Following Release

The lack of increase in post-release egg excretion rates was in contrast to the findings of other studies. For example, Villanua et al. (2006) observed post-release increases in egg excretion of *Eimeria* spp. (coccidia), *Heterakis* spp. and *Capillaria* spp. (nematodes) by farm-bred ring-necked pheasants. Our study suggests that black stilt do not suffer stress-related immunosuppression and increased parasitism following release but there are other possible explanations for the low excretion rates we observed. Firstly, the samples collected from individual black stilt were very small compared to other commonly studied avian species (e.g. chickens, turkeys, pheasants and grouse). Consequently, it is probable that they had lower sensitivity and we may have encountered a high number of false-negative results. This is supported by the differences between the maximum

mean FECs of pooled and individual samples that were collected from the same birds. It was impractical to collect pooled samples from released birds because they diverged somewhat from their aviary groups following release to the wild. Another factor which may have reduced the reliability of the post-release fecal samples is that we were not able to obtain samples from all birds following release, reducing the effective sample size. Some birds dispersed and we were not always able to observe each bird defecating individually. The prepatent period (time taken for a newly acquired parasite to reach maturity within the host and start producing eggs) could also have affected out results (Gemmill et al. 1999). Data are lacking on the prepatent period of avian trematodes but averages 50 – 60 days for *Fasciola hepatica* in rodents (Terasaki et al. 2003) and 2 – 4 weeks for *Opisthorchis felineus* in dogs (Schuster et al. 2007). The prepatent period for avian Capillarid species ranges from 3 – 8 weeks (Lloyd 2003). It is possible then, that our study was not long enough to properly investigate newly acquired infections.

We chose to analyze the FEC data from the August and September releases together to increase the power of our analysis. However, there were significant differences in helminth egg numbers between the months that may relate to captive management practices. The sources of birds for the August release were three long established aviaries (aviaries A, B and BS). In each of these aviaries, there was an adult breeding pair present in two of the four compartments. Birds for the September release were the first inhabitants of their newly built aviary (aviary C) and adults were not held in any of the compartments. The compartments in aviary C were smaller than in the other aviaries, and as crowding facilitates disease transmission (May 1995) we might expect these birds to have higher, not lower helminth burdens. However, the lower helminth prevalence could be explained by the new aviary habitat which may not have been suitable for the survival of the intermediate parasite hosts. It would be interesting to see if helminth prevalence increases in these aviaries over time as the habitat becomes more established. Young birds are more susceptible to helminth infections than are adults (Lloyd 2003, Tongson & McCraw 1967). Adult black stilt could be acting as helminth reservoir hosts, having superior immunity than their younger counterparts. This is commonly observed in agricultural systems. Ewes are considered to be the main sources of infection in young lambs (West et al. 2002).

iii. Total Worm Burdens of Captive, Released and Wild Birds

Abnormally high parasite loads may indicate naivety of released birds to the wild parasite fauna or stress-induced immunosuppression as we have discussed previously . Captive and released birds had significantly higher trematode burdens than wild birds. As released birds had the highest trematode loads this could be a sign of a stress-related helminth increase. However it is uncertain why this was only observed with trematodes. Due to the similarities in the trematode burdens of captive and released birds, it is probable that the higher burdens of these birds were due to concentration of the intermediate hosts and increased disease transmission, both assisted by the captive environment. It is interesting to note that for both cestodes and nematodes, captive birds had the lowest burdens; released birds had intermediate burdens; and wild birds had the highest burdens. This pattern suggests that to some degree, helminth intensity does increase with exposure to the wild environment. There are however, alternative explanations for the differences in helminth burdens between the source groups. All of the wild birds we analyzed were black – pied stilt hybrids or pied stilt whereas all the captive and released birds were black stilt. There could be differences in the helminth burdens of each genotype. Increased prevalence of parasitism in hybrids has been observed in ducks, crustaceans, rodents and invertebrates (Derothe et al. 2001, Mason 1990, Sage et al. 1986, Whitham 1989, Wolinska et al. 2004). The proposed reasons for this are reduced resistance of the hybrid through outbreeding depression and or differences in the ethology and ecological preferences of hybrids to one or both of their parents (Le Brun et al. 1992). Black–pied stilt hybrids tend to associate more with pied stilt, even following their northward migratory path to winter feeding grounds whereas black stilt remain *in situ*. Research by McDonald (1998) suggests that the parasite fauna is similar between the black and pied stilt. However, his sample size was small (black stilt $n = 11$, pied stilt $n = 3$) and he did not consider hybrids in the analysis.

Females had significantly greater trematode infections than males. This was an unexpected result as males and females were housed under the same conditions and all were sexually immature (thus eliminating female breeding stress as a contributing factor (Nagy et al. 2007)). The raw data revealed two individual females with atypically high adult trematode numbers of 3612 and 7329. Both were in good nutritional condition. The highest male total worm count was just 120 trematodes. It is therefore likely that the higher intensity of helminth infection in female black stilt

is due to over dispersion of helminths in the population rather than an effect of sex.

The results of our research call attention to the potential need to conserve the parasite fauna of hosts that are themselves endangered (Perez et al. 2006). This is becoming increasingly important as our definition of biodiversity also increases (Hugot et al. 2001). If black stilt do indeed share their helminth fauna with pied stilt, parasite conservation may not be required but the preliminary work of McDonald (1998) in identifying the helminths of both species should be furthered to confirm this. As a paradox to the classical view that parasites are always undesirable, Hudson et al. (2006) paper that a healthy ecosystem is one that is rich in parasites. Under this theory, it is possible that conservation of the native black stilt helminth fauna would enhance survival of the birds in the long term.

iv. Total Worm Burdens of Control and Treated Birds

Our sample size for this analysis was limited to fresh and intact birds that died following release in 2007 and was correspondingly small (n = 11). The times from release to death differed but because of the small sample size we could not factor this effect into our analysis. It is interesting to note that control birds had greater trematode burdens than treated birds. Conversely, treated birds had higher numbers of immature trematodes. Although these differences were not significant, there is some indication that the treatment was effective in reducing trematode numbers. Furthermore, there seems to be a higher rate of re-infection in treated birds than in control birds. In an effort to reduce the problem of re-infection in the wild, Newborn and Foster (2002), Woodburn et al. (2002) and Draycott et al. (2006) successfully dosed free-living game birds with anthelmintic laced grains. There is potential for medicated meat to be provided to black stilt after release. However this operation would probably not be worthwhile because the benefits of treatment for survival appear to be low and it is likely that problems would arise with administering the correct dosage and to the correct species over a large area.

v. Survival of Control and Treated Birds

For both releases, there were no differences in mean survival time or time from release to death (the hazard rate) between the two treatment groups. For the August release, mortalities were greatest very soon after release, a characteristic that is shared with many reintroductions (Sarrazin

& Legendre 1999). Why birds in the September release had far greater initial survival is uncertain but we cannot rule out the possibility that the lower prevalence of GI helminths enhanced their survival. An alternative explanation is that the release sites varied in their suitability. Factors such as the level of predator control and amount of natural food at the release site, degree of dispersal by birds from the release site and the timing of releases are all thought to affect the success of black stilt translocations (Adams, 1995, unpublished thesis; Cline, 2007, unpublished thesis; Keedwell et al., 2002; Sanders, 1996, unpublished thesis). Previous releases have also been met with mixed success. Overall, the 2005/06 release had high survival rates whereas the 2006/07 release had relatively poor survival rates. The 2007/08 release (the focus of our study) was a little different as one release site had low survival and the other had high survival.

Management Implications

The results of our research indicate no increase in helminth intensity after release to the wild; no benefits of treatment for post-release survival; and the inevitable exposure to GI helminths following release to the wild. On the other hand, we did find a small number of individuals to be highly infected. This result may be of little importance however, as we did not find a direct causal relationship between increased helminth burden and increased post-release mortality. Based on these results, and the risks associated with treatment, the current anthelmintic program appears to be an unnecessary cost. We therefore recommend the cessation of regular anthelmintic treatment of captive-reared black stilt released to the wild. We do however, advocate continuation of health screening in order to monitor changes in the composition and distribution of GI helminth infections and in particular, the emergence of potentially pathogenic species. An alternative to terminating anthelmintic treatment altogether would be to reduce the intensity of treatment applied to such a level that the benefits would effectively outweigh the costs. This could be done by administering anthelmintics in some years only; to a selected sample of birds annually; or by alternating the type of anthelmintic used. If this management approach were to be adopted, we feel it would be prudent to incorporate annual efficacy testing into the black stilt anthelmintic regime to monitor the development of anthelmintic resistance (a phenomenon that is considered to be an unavoidable outcome of the continued use of anthelmintics (West et al. 2002)). Our research also shows that there is a need to improve the sensitivity and reliability of helminth egg detection mechanisms to ensure that misdiagnoses do not occur in the future.

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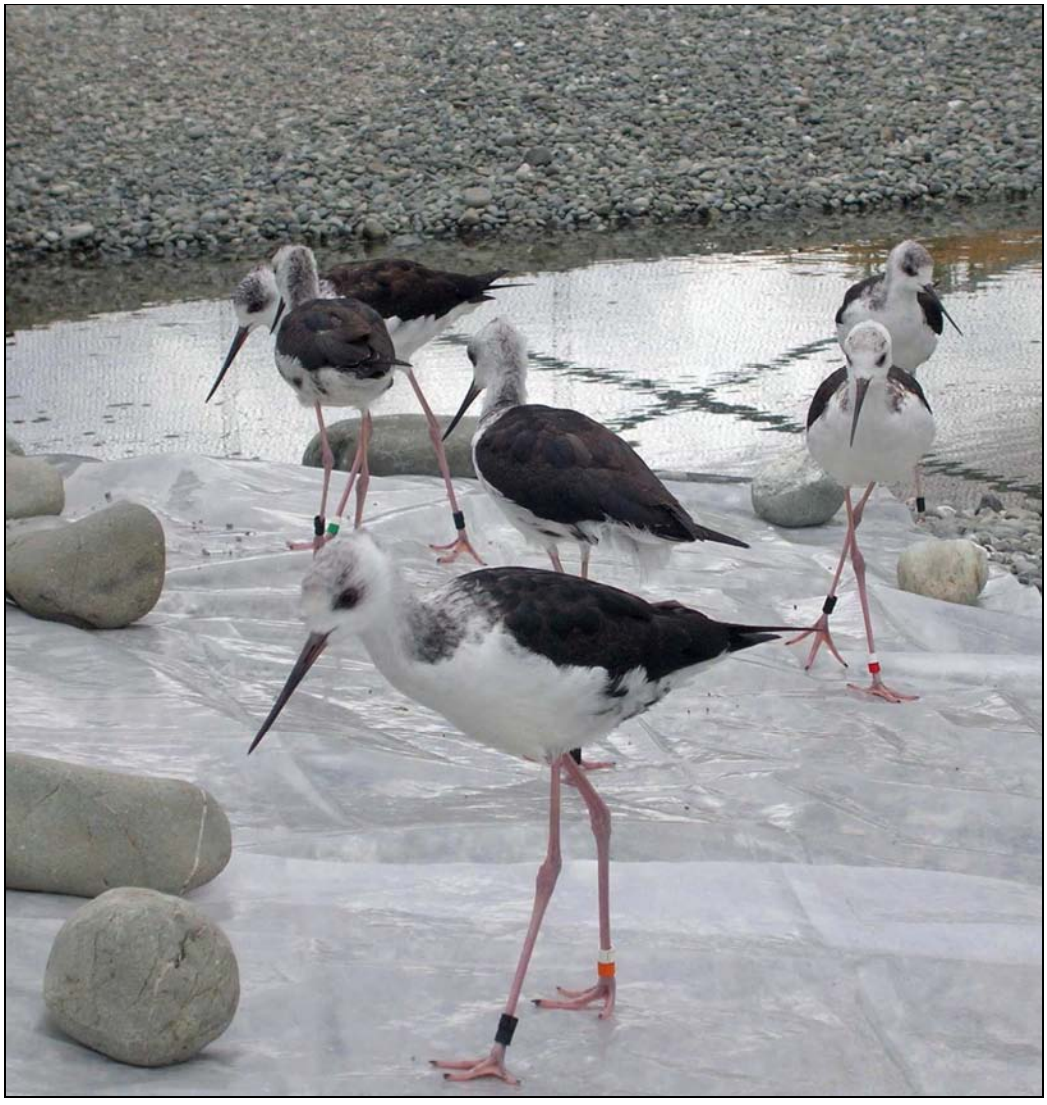
Chapter 3

Assessing the reliability of faecal helminth screening in captive-reared black stilt (*Himantopus novaezelandiae*)



Juvenile black stilt inspecting the faecal collection sheets

(Photo by L. Robertson)

(Submitted to the Journal of Helminthology)

Abstract

Gastrointestinal (GI) helminth infections may pose a risk to captive management programmes of endangered species by reducing productivity and the animal's ability to cope with physiological and environmental stresses. Black stilt or kakï (*Himantopus novaezelandiae*), a rare New Zealand endemic wader, are thought to be adversely affected by GI helminths and so are treated with anthelmintics before they are released into the wild as juveniles and sub adults. This study aimed to assess the reliability of helminth screening protocols in black stilt and make recommendations for future management. Faecal egg counts were found to be poor indicators of total worm presence and abundance. The two modified sedimentation methods used to detect trematodes provided relatively low egg recovery rates (31.72 ± 9.08% and 39.35 ± 14.53%). We also tested whether daily, aviary and temperature-dependent variation in helminth egg shedding could affect the reliability of faecal egg counts. Trematode egg shedding varied between days (P = 0.007) but not by hour of the day or temperature. The collection and analysis of pooled faecal samples proved to be more cost and time-effective than samples from individual birds and the larger masses collected resulted in greater sensitivity. In conclusion, faecal analysis of pooled samples remains a useful qualitative indicator of helminth presence and absence but is quantitatively unreliable.

Key words: Black stilt, captive breeding, faecal screening, gastrointestinal helminths, *Himantopus novaezelandiae,* kakï.

Introduction

Gastrointestinal (GI) helminths are endoparasitic worms belonging to three groups: Trematodes (flukes), cestodes (tapeworms) and nematodes (roundworms). At low intensities, these parasites can inflict a small but constant demand on their host, posing a cost through the maintenance of immune defences and continual repair of damaged tissues. The activity and behaviour of infected individuals can also be significantly altered. As a consequence, these sub-lethal infections can hinder an animal's ability to cope with physiological and environmental stresses such as nutritional demands, cold exposure, predation pressures, habitat loss and translocations (Hatcher et al., 2006; Kristan & Hammond, 2000; Mathews et al., 2006; McCallum & Dobson, 1995; McCallum & Dobson, 2002). High GI helminth loads are also an occasional cause of death (Bailey et al., 1996; Krone et al., 2003). Gastrointestinal helminth infections are especially important in endangered species captive management programmes, due not only to the rare status of the host but also because infectious disease transmission may be facilitated by crowded conditions (May, 1995). Where species are bred for release to the wild, there are additional risks; helminths could proliferate to pathogenic levels due to immunosuppression of the host during translocations; and parasites could also be spread from the captive to the wild population (and vice versa) (Mathews et al., 2006)

A common method used for detecting gastrointestinal helminth infections in captive-reared birds is faecal analysis, whereby helminth eggs are identified from a sample of faeces. The accuracy of this method is often variable for many reasons, including a need to validate laboratory diagnostic methods for individual host and helminth species (Geerts & Gryseels, 2000). Spatial variation in the helminth burdens of individuals and populations, as well as temporal variation in helminth intensities and egg excretion rates are other sources of variation (Misof, 2004; Oju & Mpoame, 2006; Villanua et al., 2006). There is also concern that collecting samples from groups of birds results in the loss of information at an individual scale, leading to unnecessary over treatment (Vercruysse & Claerebout, 2001; Ward et al., 1997).

One New Zealand native species thought to be adversely affected by GI helminths is the black stilt or kakï (*Himantopus novaezelandiae*) (Jakob-Hoff, unpubl. report, 2001), which has a wild population of around 80 adults (Cleland, unpubl. report, 2008) with a further 80-90 juveniles bred

in captivity for release to the wild each year. Black stilt were once widespread in New Zealand but have declined dramatically in number and range due to habitat destruction and degradation along with avian and mammalian predation and hybridisation with pied stilt (*Himantopus h. leucocephalus*) (Maloney & Murray, 2002; Pierce, 1986; Pierce, 1996). Nowadays, they are confined solely to the Upper Waitaki Basin in the South Island and are classified as 'critically endangered' by the International Union for the Conservation of Nature and Natural Resources (IUCN) (Birdlife International 2000). High mortality of captive-reared black stilt released to the wild has been identified as the main factor limiting the success of the recovery programme (Maloney & Murray, 2002), emphasising the high value placed on the continuation and improvement of this operation into the future.

There is concern that GI helminths may play a role in the low survivorship of released black stilt. Because of this, captive-reared juvenile and sub adult black stilt are routinely screened for parasites before release to the wild. Pooled faecal samples (from groups of birds) are collected from each aviary and sent to a laboratory for analysis. Regardless of the results, all birds are dosed with praziquantel (Droncit®, Bayer NZ Limited, Auckland, New Zealand) (20 mg / kg body weight) to treat cestodes and trematodes and if the nematode *Capillaria* spp. is detected in an aviary, the enclosed birds are dosed with levamisole hydrochloride (Aviverm, Westwood Enterprises, Auckland, New Zealand) (16 mg / kg body weight). Faecal screening is therefore relied upon as the sole indicator of helminth presence and abundance and the results determine the type of treatment that is applied.

The main aim of our study was to assess the reliability of current faecal screening methods in detecting potentially harmful helminths in black stilt and to make informed recommendations for future health screening protocols. This was addressed by asking 4 fundamental questions. (1) Do faecal egg counts reflect the total worm burden of adult helminths? (2) How accurate are the laboratory methods commonly used to detect trematode eggs? (3) Could the results of faecal egg counts be affected by temporal and temperature-dependent variation in helminth egg shedding? (4) Is individual faecal analysis less sensitive than pooled faecal testing for detecting GI helminths?

Methods

i. Faecal sample collection

Pooled samples (from groups of birds within aviaries) were collected by placing two clean plastic sheets approximately 1 m × 2 m within each aviary in areas favoured by the birds (indicated by accumulation of faeces on the gravel). Individual samples (collected from single birds) were collected in captivity from plastic sheets whilst birds were being handled by aviary staff and in the wild by placing plastic sheets beneath feeding plates. All samples were stored at 4°C until they were analysed.

ii. Examination methods

- *Pooled samples*

Modified faecal flotation methods (Charleston & Pomroy, 1984) were used to detect and count cestode and nematode eggs. Approx. 0.5 g of each sample (which was first thoroughly homogenised) was mixed with $ZnSO_4$ solution (1.2 specific gravity), strained through a 1 mm sieve and ground using a spatula. The resulting mixture was poured into a 10 ml glass centrifuge tube and topped up with $ZnSO_4$ solution. A cover slip was placed on top of the tube and the sample was centrifuged at 1200 RPM for 5 min. After spinning, the cover slip was placed onto a glass slide and the attached eggs were counted microscopically (20×). The remaining pellet was discarded.

Modified faecal sedimentation methods (Charleston & Pomroy, 1984) were used to detect and count the heavier trematode eggs. Approx. 0.5 g of each faecal sample was strained through a sieve with tap water and ground with a spatula. The resulting mixture was poured into a 10 ml glass centrifuge tube, filled to the top with water and inverted 4 times to ensure proper mixing. The samples were left to sediment for 5 min, before the supernatant was pipetted off and discarded, leaving the pellet intact. The tube was then topped up with water and left to sediment for a further 5 min after which time the supernatant was again removed. The pellet was transferred to a metal bowl, stained with a drop of standard methylene blue solution, then pipetted in small amounts into a marked Petri dish. Finally, the trematode eggs were counted using a dissecting microscope at 3.5×.

- *Individual samples*

Due to small quantities collected, the entire amounts were used in the flotation and the coverslip was examined for cestode and nematode eggs just as described above. Instead of discarding the remaining pellet after centrifugation, the supernatant was carefully poured off, leaving the pellet available for the sedimentation process. This was mixed with water and then allowed to sediment twice for 5 min before the standard sedimentation examination methods previously described were carried out.

iii. Relationship between total worm burdens and faecal egg counts

Of the birds that were submitted for necropsy in 2007, 9 black stilt, 6 black-pied stilt hybrids and 3 pied stilt were suitable for this analysis. Causes of death in these birds were predation (n = 7), trauma (n = 2) and euthanasia (n = 9). The mature helminths of each bird were identified microscopically (3.5×) to class (and genus for *Capillaria* spp.) and counted so that a total worm count (TWC) was obtained for each individual. A faecal sample was also collected *post mortem* from each bird. To obtain a faecal egg count (FEC), the helminth eggs were counted using the methods described above for individual faecal samples. The number of birds found positive for helminths using TWC and FEC was compared using Analysis of Variance (ANOVA).

iv. Accuracy of the sedimentation methods

The accuracy of the two faecal sedimentation methods used to detect trematodes in (a) pooled and (b) individual samples was assessed. Fifty trematode eggs were added to each of 24 negative 0.5 g faecal suspensions. The standard sedimentation methods described above for pooled and individual samples were carried out on 12 of the prepared samples each. The recovery rate of each test was calculated as the mean proportion of eggs recovered and they were compared by ANOVA. The recovery rate of the flotation method could not be assessed as the methods did not allow for easy recovery and subsequent storage of cestode and nematode eggs.

v. Variation in helminth egg shedding

Pooled faecal samples were collected from two aviaries housing subadult birds (aviary A2 n = 6, aviary A3 n = 5). Samples were collected at the time intervals of 17:00 – 05:00, 05:00 – 08:00, 08:00 – 11:00, 11:00 – 14:00 and 14:00 – 17:00 hrs for 5 consecutive days. ANOVA was used to

compare the mean number of trematode and *Capillaria* spp. eggs per gram of faeces (EPG) at different times of the day and between days. Correlations between temperature and time of day were performed. All data were $\log_{10}(x+1)$ transformed for analysis.

vi. Comparison of pooled and individual samples

The collected masses of pooled and individual faecal samples were compared by ANOVA. All data were $\log_{10}(x+1)$ transformed for analysis but we discuss the arithmetic means for pooled samples (due to a normal distribution) and the geometric means (antilog of the transformed mean) for individual samples (due to skewness of the data). The analysed masses and subsequent sensitivity of pooled and individual samples were also compared using ANOVA. Sensitivity was calculated as the number of helminth eggs that had to be present in each faecal sample for 1 egg to be detected. We discuss the geometric means for all of the sensitivity data.

vii. Statistical analysis

SAS Enterprise Guide 4.1 (©1999-2006 by SAS Institute Inc., Cary, NC, USA) was used for all statistical analysis.

Results

i. Eggs detected

This study only recovered the eggs of trematode (unknown genera and species) and *Capillaria* spp.. No cestode, or other kinds of nematode eggs were detected.

ii. Relationship between total worm counts and faecal egg counts

Significantly fewer birds were found to be positive for helminths from the faecal egg counts than from the total worm counts ($F_{1, 142}$ = 23.97, P = <0.0001) (Fig. 1). Overall, true presence or absence was found 56.2% of the time. False-negatives were encountered 42.5% of the time and false-positives 1.4% (one faecal sample). The faecal egg counts (FECs) and total worm counts (TWCs) of each helminth type were not monotonically related and because the sample size was small, the relationships from a regression coefficient were not inferred.

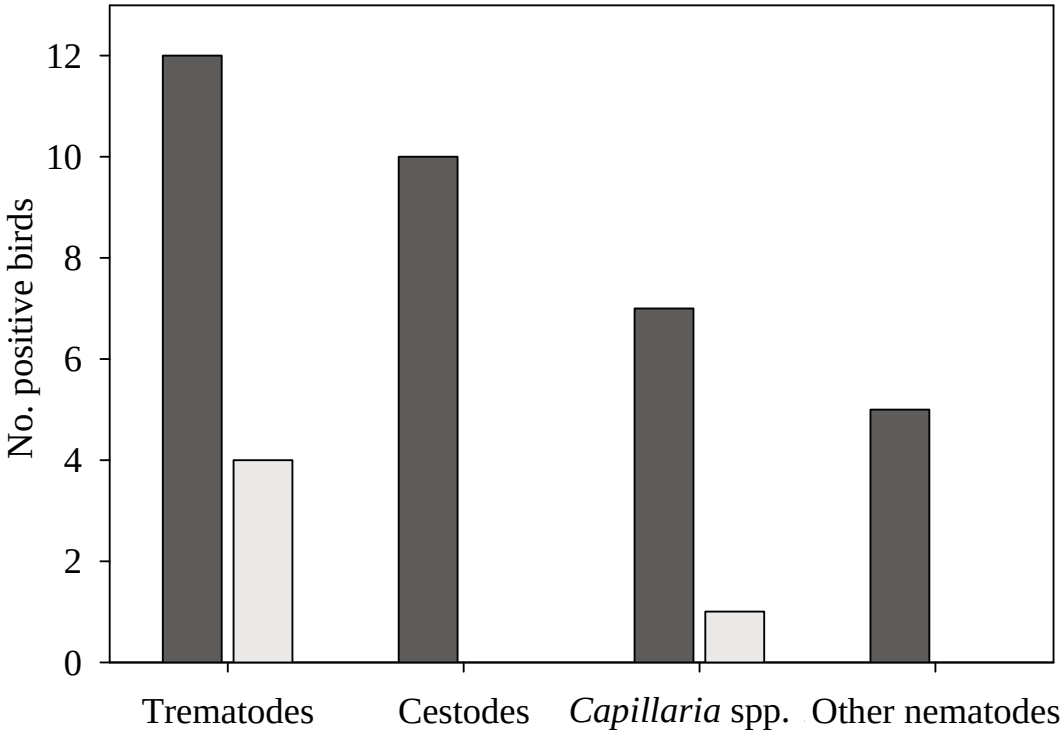


Figure 1. Number of black, hybrid and pied stilt (*n* = 18) positive for trematodes, cestodes, *Capillaria* spp. and other nematodes. The dark grey bars represent results from total worm counts (TWCs) and the light grey bars those from faecal egg counts (FECs) taken post mortem. The FECs produced significantly fewer positive results than the TWCs and failed to detect the eggs of cestodes and other nematodes altogether.

iii. Accuracy of the sedimentation methods

Both of the sedimentation methods used to detect trematode eggs yielded relatively low recovery rates but no false-negatives were observed. The mean recovery rates (± standard error [SE]) and 95% confidence intervals (CI) for individual and pooled samples respectively were 31.72 ± 9.08% [25.22– 38.21%] and 39.35 ± 14.53% [30.12 – 48.58%]. There was no significant difference in the mean recovery rates of the two tests ($F_{1, 22} = 2.08$, $P = 0.165$).

iv. Variation in helminth egg shedding

There were no significant differences in the mean number of trematode and *Capillaria* spp. eggs per gram (EPG) of faeces at different times of the day or between the two source aviaries but mean trematode EPG did differ as a function of day of collection (Table 1.). Temperature was not significantly correlated with the FECs of trematodes ($n = 47$, Pearson correlation coefficient (r) = -0.007, $P = 0.965$) or *Capillaria* spp. ($n = 17$, $r = -0.021$, $P = 0.420$).

Table 1. Differences in the mean number of (a) trematode and (b) *Capillaria* spp. eggs shed per gram of black stilt faeces (EPG) according to time of day and aviary. All data were $y = \log_{10}(x+1)$ transformed for analysis of variance but the back-transformed (antilog (y) – 1) descriptive statistics are

Helminth	Variable		EPG $\bar{x}$	EPG 95% C I	EPG n	ANOVA F-ratio	ANOVA P	ANOVA R^2
(a) Trematodes	Time of day (hrs)	17:00 to 05:00	6.81	2.97 – 14.37	10			
		05:00 to 08:00	14.74	11.99 – 18.07	8			
		08:00 to 11:00	7.16	4.24 – 11.71	10			
		11:00 to 14:00	7.50	4.74 – 11.61	9			
		14:00 to 17:00	10.16	5.71 – 17.56	8	$F_{4,42} = 0.610$	0.08	0.178
	Day	2-Aug-07	4.52	2.07 – 8.93	11			
		3-Aug-07	8.78	5.05 – 14.79	11			
		4-Aug-07	12.03	9.89 – 14.60	7			
		5-Aug-07	10.56	6.48 – 16.87	9			
		6-Aug-07	11.69	9.38 – 14.51	10	$F_{4,42} = 0.572$	0.01	0.278
	Aviary	A2	8.96	7.09 – 11.26	25			
		A3	8.83	5.94 – 12.93	22	$F_{1,45} = 0.650$	0.95	<0.001
(b) *Capillaria* spp.	Time of day (hrs)	17:00 to 05:00	0.76	0.00 – 2.10	10			
		05:00 to 08:00	1.28	0.58 – 2.29	10			
		08:00 to 11:00	0.59	0.00 – 1.88	10			
		11:00 to 14:00	1.22	0.13 – 3.35	10			
		14:00 to 17:00	0.62	0.00 – 1.90	10	$F_{4,42} = 0.761$	0.74	0.045
	Day	2-Aug-07	1.64	0.27 – 4.49	12			
		3-Aug-07	0.93	0.00 – 2.71	8			
		4-Aug-07	0.78	0.04 – 2.05	10			
		5-Aug-07	0.65	0.08 – 1.52	10			
		6-Aug-07	0.54	0.14 – 1.09	10	$F_{4,42} = 0.749$	0.51	0.074
	Aviary	A2	0.85	0.45 – 1.37	25			
		A3	0.92	0.29 – 1.86	25	$F_{1,45} = 0.752$	0.87	0.001

v. Comparison of pooled and individual samples

The mass of faeces collected was larger for pooled ($\bar{x}$ = 3.22 g, [95% CI = 2.89 – 3.85 g]) than individual samples (geometric $\bar{x}$ = 0.13 g, [0.11 – 0.14 g]) ($F_{1, 403}$ = 141.60, P = <0.0001). As a result, the proposed analysis of 0.5 g of faeces was attained in most instances for pooled samples ($\bar{x}$ = 0.48 g, [0.46 – 0.49 g]) but around 90% of individual samples were less than 0.5 g (geometric $\bar{x}$ = 0.09 g, [0.07 – 0.10 g]). Consequently, pooled samples had greater sensitivity i.e. they required a lower minimum number of eggs to be present in each sample for one helminth egg to be detected (geometric $\bar{x}$ = 0.66, [0.64 - 0.68]) compared to individual samples (geometric $\bar{x}$ = 1.79, [1.61 – 1.99]) ($F_{1, 397}$ = 247.38, P = <0.0001) (Fig. 2.). Individual samples had a noticeably higher urate component than pooled samples. They would therefore have been of poorer analytical quality due to lower faecal concentrations.

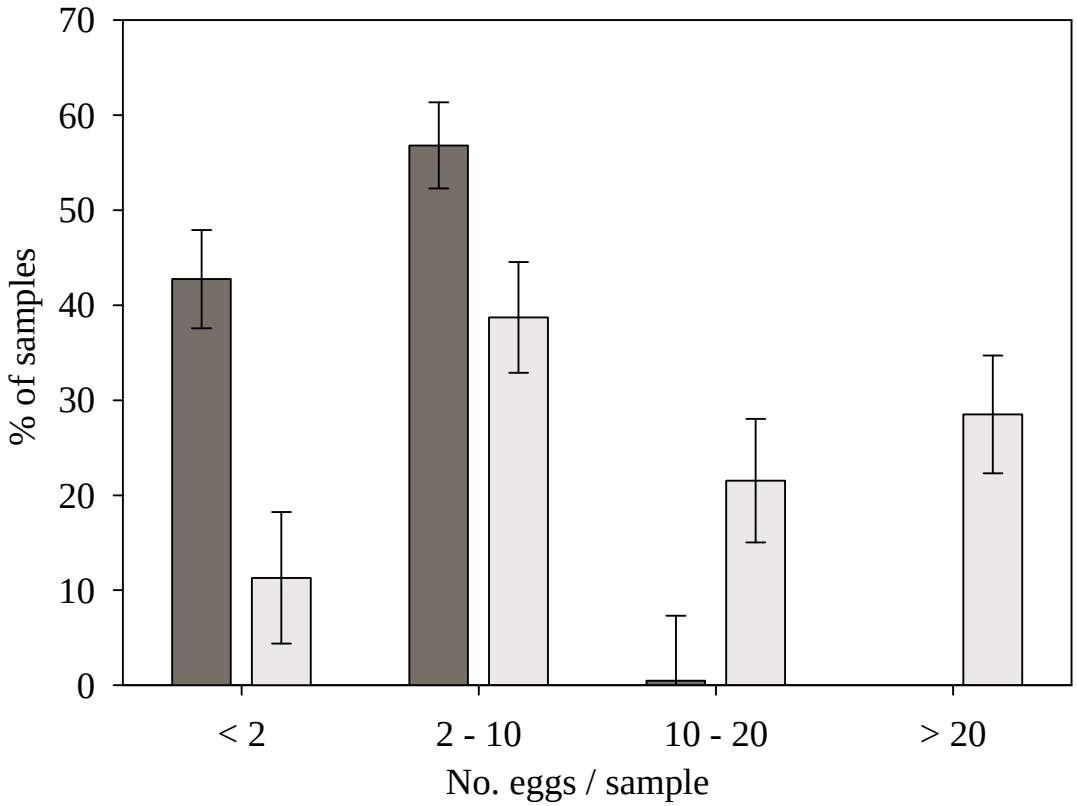


Figure 2. Number of helminth eggs that had to be present in each black stilt faecal sample before one egg could be detected. The dark grey bars represent the percentage of pooled samples in each of the 4 categories while the light grey bars represent individual samples. Bars correspond to standard errors. Individual samples had significantly lower sensitivity than pooled samples.

Discussion

Our study confirmed that in general, current faecal screening methods are not reliable quantitative and qualitative indicators of helminth presence and abundance. This is supported by the main findings of the study: faecal egg counts did not relate well with total worm burdens; there was a significant influence of day of sample collection on faecal egg counts; and the recovery rates of the sedimentation methods were relatively low. The detection of GI helminths, however, was better using pooled samples, which is current practice.

The underlying assumption of faecal examination as a means of detecting GI helminth abundance is that there is a definable relationship between eggs per gram of faeces and total worm burden (McKenna, 1981). Our study was not able to demonstrate such a relationship for black stilt. This indicates that caution should be placed on the reliance of faecal analysis as a quantitative measure of helminth abundance. In addition, we showed qualitative failure to detect the eggs of adult worms and observed false-positives indicated by the presence of eggs of adults not found in the GI tract. It is possible that total worm counts themselves are not reliable indicators of true helminth abundance, but they are generally considered to be the most accurate of the methods available.

The majority of studies on helminth screening have been conducted on poultry and domestic animals grazed under pastural conditions but it is likely that the general principals of helminth egg production and excretion in black stilt are similar. Previous studies have observed poor relationships between FEC and total worm abundance as we have. Examples of such studies are those of de Vlas et al., (1997) who studied *Schistosoma mansoni* in humans and Coop et al., (1985) who studied *Ostertagia circumcincta* in sheep. The postulated reasons for this include: (1) density-dependent effects (where an increasing helminth population leads to suppression of egg production either due to competition for resources or the hosts' immune response) (Bishop & Stear, 2000), (2) the aggregated distribution of parasites in a population (Monteiro et al., 2007), (3) disparity in the egg production of different helminth species (McKenna, 1981), (4) monosexual infection (de Vlas & Gryseels, 1992) , (5) phase of infection (Villanua et al., 2006), and (6) fluctuations in egg output (Seivwright et al., 2004; Villanua et al., 2006). Boes et al., (1997) suggest that false-positives can arise due to coprophagia and geophagia. This is a likely explanation for the false-positives observed in our study as supplementary feeding enforced aggregation of the birds and

subsequent fouling of the food provided. The studies that have shown good relationships between faecal egg output and total worm burden suggest that in some cases there is an absence, or at most a very weak effect of the above sources of variation (de Bont et al., 2002; Seivwright et al., 2004).

The estimates of trematode egg recovery rates observed in our study (31.72 ± 9.08% and 39.35 ± 14.53%) are similar to those of Rehbein et al. (1999) who achieved a recovery rate of 41.00 ± 1.50%) of the *Dicrocelium dendriticum* eggs experimentally added to sheep faeces. The considerably higher standard deviations in our study indicate that our methods have lower precision that those of Rehbein et al. (1999) i.e. repeated sampling may not yield similar recovery rates. Our estimates of egg recovery were lower than those of Antonia et al. (2002) who recovered a mean of 76.72 ± 15.42% *Fasciola hepatica* eggs from cow faeces. The discrepancy among studies probably relates to differences in the number of steps involved, sedimentation times and repetitions, quality of the preparations and amount of debris in the faeces (Kleiman et al., 2005; Rehbein et al., 1999). Leathwick et al. (2007) suggest the best way of increasing the accuracy of sedimentations is to increase the volume of faeces analysed . Agricultural studies such as those previously discussed can easily attain large amounts of faeces, (10 – 30 g compared to our maximum of 0.5 g) in contrast; black stilt are sparsely distributed and produce small stools, making it very hard to obtain large samples. The best way to increase the sensitivity of black stilt faecal analysis then is probably to increase the number of samples collected rather than the volume. While the trematode recovery rates were similar using the methods for pooled and individual samples, this was probably overestimated for individual samples. As we have demonstrated, individual samples have lower sensitivity due to the smaller masses collected (around 90 % were less than 0.5 g, in our study).

In our study, helminth egg output showed variation according to day of sample collection but was independent of time of the day and temperature. These findings contradict those of Villanua et al. (2006) and Oju & Mpoame (2006) who found significant variation in relation to sampling hour and temperature respectively. In contrast, Giver et al. (2000) observed daily variation of egg output of *Schistosoma japonicum* in pigs. The dissimilarity among studies probably reflects the differences in egg shedding dynamics of different parasite species whereby each strives to favour development

and transmission of their own infective stages (Misof, 2004; Oju & Mpoame, 2006). Alternatively, it could simply be due to variation in the detectability of eggs in the faeces (Misof, 2004).

In practice, only pooled faecal samples are collected from captive black stilt as a routine monitoring procedure. Our study supports the continuation of this and indicates that the costs of the poor reliability of individual samples outweigh the advantages of being able to obtain information on an individual basis. Collecting pooled samples from individual aviaries still allows greater precision than pooling aviaries together. In addition, the collection of composite samples reduces the need to exert stress on the birds by capturing or trailing them in aviaries and is more cost-effective. These findings are in agreement with a variety of captive ruminant studies by Eysker et al., (2008), Morgan et al., (2005), Ortiz et al., (2000) and Ward et al., (1997).

Our study was only conducted during the early spring season. As spring is the main release season, the dynamics of other seasons are probably of little relevance to the aims of this study. We only collected samples during one year and replication over other years would therefore be beneficial to confirm the current findings.

In conclusion, our study suggests that while faecal analysis may not be an accurate indicator of helminth prevalence in black stilt, it does remain a useful and cost-effective basic indicator of helminth presence and absence. We recommend that future pre-release health screening protocols continue the practice of analysing pooled faecal samples from each aviary before anthelmintic treatment is implemented. To help combat the possible effects of daily variation in egg shedding, special attention should be paid to collecting samples over a series of consecutive days. Future work could include the incorporation of a stochastic model, taking into account all possible sources of variation, as de Vlas et al. (1997) have done for human schistosomiasis infection however this would require the collection of baseline data over a period of time and much more information than is currently available.

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Chapter 4

Comparison of the gross morphology of the digestive organs of captive, released and wild black stilt (*Himantopus novaezelandiae*) and implications for management.

Adult black stilt at Mailbox Inlet

(Photo by J. Hiscock)

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Abstract

Black stilt (*Himantopus novaezelandiae*) are critically endangered New Zealand endemic waders. An intensive captive management program is in operation to supplement the very small wild population. A primary concern for managers is the high mortality rates of captive-reared birds released to the wild. The main purpose of our study was to determine whether reduced digestive efficiency resulting from mal-adaptation to the captive diet is contributing to these high mortality rates. We compared the digestive organs (both fresh and preserved) of healthy and emaciated captive, recently released (< 30 d), previously released (30 d – 5 months) and wild *Himantopus* sp. (including black-pied stilt hybrids and pied stilt). Captive, previously released and recently released black stilt had generally smaller digestive organs than wild birds. Moreover, released birds did not appear to increase digestive organ size to match the high fiber wild diet. However it is unclear whether the smaller organs of released birds' result in reduced digestive efficiency. Emaciated birds did not have atrophied organs, comparing favorably with captive, released and wild birds. The greatest mortality rates of released black stilt occur soon after release, while birds are being supplementary fed. Consequently, reduced digestive efficiency is probably not contributing significantly to mortality rates and the direct impacts of the translocation (e.g. predation, exposure, competition) are likely to be greater risk factors. We recommend the continued provision of supplementary food to released birds and an increased focus on habitat enhancement and predator control at release sites. Our secondary aim was to assess the reliability of comparing fresh and formalin fixed *Himantopus* sp. gut specimens and we used chicken digestive organs as a model. We found some significant differences in fresh and formalin fixed organ dry masses and variation in preserved organ lengths. Although chicken and stilt organs are very different, these sources of variation should be considered when interpreting the results of future studies.

Key words*:* digestive organs, phenotypic plasticity, captive breeding, translocations, avian, black stilt, *Himantopus novaezelandiae*, New Zealand.

Introduction

It has long been documented that the avian body is a dynamic entity, in a continual state of change.[1, 2] Avian digestive systems can respond rapidly and reversibly to seasonal fluctuations in environmental conditions and internal physiological demands.[1, 3-6] This enables optimized digestive efficiency and exploitation of the full range of available resources during times of change.[7, 8] Migratory birds and those that consume a wide range of food items may be better adapted to make rapid changes in digestive organs than more sedentary and specialist feeders.[9, 10]

Avian digestive systems undergo hypertrophy when exposed to high intakes of low quality, high fiber diets.[11] The gizzard, small intestine and ceca as well as the liver (which is important in nutrient assimilation) have all been reported to increase in size when food intake increases or food quality decreases.[2, 12] Lengthening of the gut improves digestive efficiency by allowing longer food transit times. Increased diameter increases food processing per unit body weight.[7, 13]

Starck & Rahmaan[3] observed that captive-reared Japanese quail (*Coturnix japonica*) conditioned to a low fiber diet increased their gizzard and intestine size when fed a high fiber diet. Additionally, they observed an immediate decline in body mass after diet switching. This phenomenon has been described as "mismatching" between digestive load and digestive capacity. During this period, digestive organs are in the process of enlargement in response to reduced food quality and increased food intake so there is a lag period where digestive efficiency is reduced.[8, 9, 14] Starck[8] suggest it takes about 6 days for the gut of Japanese quail to become fully adapted to the new diet. Digestive optimization has been reported to occur between three and four weeks in red grouse[15] and mallards.[16]

The reduced digestive performance due to diet switching has special implications for the release of captive-reared birds to the wild. Captive birds are often fed easily digestible, low fiber diets and because their daily energy expenditure is low, they have a low food intake.[6] Once released, they may be unable to obtain sufficient nutrients to survive whilst their atrophied digestive organs adapt to the new diet which is often highly indigestible.[14] Reduced digestive performance could reduce body condition, resulting in emaciation and increased vulnerability to pressures such as the translocation process, starvation, predation, competition and exposure.

Black stilt or kakï (*Himantopus novaezelandiae*) are New Zealand endemic waders. They are classified as critically endangered by the International Union for the Conservation of Nature and Natural Resources (IUCN)[17] and are severely restricted in both number and range. An intensive captive management program has been implemented in order to supplement the depauperate wild population (ca. 80 adults). Captive-reared black stilt are released to the wild as juveniles (ca. 2 months) or subadults (ca. 9 months). In an attempt to improve survival, released birds are supplementary fed the captive diet at the release site. The amount fed is gradually reduced over a four week period. Despite theses efforts, the mortality rates of released birds are unacceptably high. In captivity, black stilt are fed a highly digestible diet of a minced ox heart mixture [18, 19] (see Appendix 1. for full formulation). The wild diet in comparison is high in fiber, consisting mainly of insects, molluscs and small fish.[20] It is likely that black stilt exhibit phenotypic plasticity in their digestive organs because they have a wide and variable natural diet and they were migratory within New Zealand, in the recent past.[21] While the time taken for digestive adaptation to occur is generally considered to be rapid (days to weeks) this may be enough time to significantly influence black stilt survival after release.

Changes in digestive tract morphology are best characterized at necropsy. For studies on common species, controlled conditions can be easily attained and the experimental animals can be euthanized at the end of the trial.[22] In contrast, studies on endangered species are often conducted in variable conditions and dead animals can only be obtained if they die incidentally. To increase understanding of the causes of mortality in black stilt, the management team routinely sends deceased birds to Massey University, Palmerston North, New Zealand for necropsy. After these have been conducted, soft tissue samples, including the digestive tract, are preserved in 10% buffered formalin solution. Formalin preservation results in the shrinkage of biological tissues. The extent of shrinkage depends on formalin concentration, osmotic strength, time from death to fixation, size of the specimen and time in formalin.[23, 24] Changes in organ length and mass due to preservation can therefore affect the accuracy of comparisons with fresh specimens but correction factors can be devised for specific tissues or organs to combat this problem.[23]

The main purpose of our study was to determine whether reduced digestive efficiency is contributing to the high mortality rates of captive-reared black stilt released to the wild.

Specifically, we wished to determine whether captive and released birds had smaller and lighter digestive organs than wild birds; whether the digestive organs of released birds increased after release to optimize digestive efficiency; and whether emaciated birds were suffering from reduced digestive efficiency. Because some of the black stilt organs were preserved in formalin and others were fresh, our secondary aim was to evaluate the reliability of the comparing them to each other. We used chicken digestive organs as a model for those of black silt digestive organs.

Materials and Methods

i. Source of birds

Whereas captive and released black stilt were available for this analysis, no wild black stilt were obtainable. In their place, wild adult pied stilt (*H. himantopus leucocephalus*) and black-pied stilt hybrids were used. The limited size of the black stilt population and similarities in the morphology, habitat and behaviors of black and pied stilt means that these two species readily interbreed to produce viable hybrid offspring. [25]

- *Frozen specimens*

Specimens available for analysis were 4 captive juvenile and subadult black stilt; 11 captive-reared released subadult black stilt; 8 wild adult black-pied stilt hybrids; and 3 wild adult pied stilt (Table 1.). Causes of death among the captive and released birds included disease, trauma, predation and starvation. All the hybrid black stilt and pied stilt were euthanized by Department of Conservation (DOC) staff to break pair bonds with black stilt and avoid further hybrids. Three of the wild carcasses submitted were partial, due to gun shot damage and decomposition and a further two gut specimens became severely autolyzed after collection. For these birds only some of the larger, less decomposed, organs could be used.

- *Formalin fixed specimens*

These were stored in 10% buffered formalin solution for 1 to 15 years. The birds used were: 20 captive juvenile and subadult black stilt; 10 captive adult black stilt; and 12 captive-reared released subadult black stilt (Table 1.). Causes of death in these black stilt also included disease, trauma, predation and starvation.

- *Red shaver chickens*

We used red shaver chickens *(G. gallus domesticus)* as a model to measure the effects of formalin fixation on black stilt gut morphology. All 12 chickens analyzed were acquired fresh for necropsy at Massey University, Palmerston North, New Zealand.

Table 1. Summary of the sources of fresh and formalin fixed *Himantopus* sp. used in the gut morphology analysis. Parentheses indicate number of emaciated birds.

	Captive	Released ≤ 30 d	Released > 30d	Wild	Total
Juvenile & subadult black stilt	24	14 (4)	9 (1)	0	47
Adult black stilt	10 (3)	0	0	0	10
Adult hybrid stilt	0	0	0	8	8
Adult pied stilt	0	0	0	3	3
Total	34	14	9	11	68

ii. External morphometry

The lengths of the tarsus and bill of each *Himantopus* sp. specimen were measured with capilers (± 0.1 mm). Measurements of wing length were not able to be obtained for the wet formalin fixed birds so this measurement was excluded from the analysis.

iii. Digestive organ morphology

- *Frozen specimens*

As each bird was submitted for necropsy, the entire digestive tract (including the liver) was removed and weighed then stored frozen in an airtight bag. Immediately before analysis, the specimens were thawed, laid out straight (but not stretched) on a wet surface and the liver, fat and mesenteries were removed. Proventriculus length and gizzard length, width and depth were measured with calipers (± 0.1 mm). The lengths of the esophagus (from the posterior connection with the tongue to the proventriculus); small intestine (from the gizzard to the anterior junction with the ceca); each cecum (to the junction with the small intestine); and the rectum (from the anterior cecal junction to the cloaca) were measured using a steel rule (± 1 mm). The digestive tract was then divided into: Esophagus, proventriculus, gizzard, small intestine, cecum a., cecum b., and rectum (see Appendix 3). Each organ was cut in half longitudinally and the contents were scraped out and stored separately in 10% buffered formalin solution (for parasitological analysis). The digestive organs and liver were placed into individual pre-weighed aluminum foil containers,

weighed on electronic scales (± 0.01 g) and left to dry overnight in a 60°C oven. After this time, the specimens were cooled in a desiccator then reweighed. The drying process was then repeated until each organ achieved a constant mass.

- *Formalin fixed specimens*

The digestive organs were removed from the 10% buffered formalin solution and rinsed in tap water. The gut was divided into sections and measured in the same manner as the frozen specimens. Each organ was dried on tissue paper for approximately 1 min before being transferred to pre-weighed aluminum foil dishes. The organs were weighed before being dried to a constant mass at 60°C as for the frozen specimens.

- *Red shaver chickens*

Chicken digestive organs were divided into the same sections as those of the *Himantopus* sp.. Each organ was cut longitudinally to create two separate halves. The gut contents were then scraped out and discarded. One half of each digestive organ was weighed, placed in a pre-weighed aluminum foil dish and dried to a constant mass at 60°C. The length and mass of the other half was measured, before being preserved in 10% buffered formalin solution. After 30 days, the organs were removed from the formalin solution and length and mass measurements were taken again. Each organ was dried on tissue paper for approximately 1 min and then dried in a 60°C oven until a constant mass was achieved.

iv. Statistical Analysis

All statistical analyses were carried out using the Statistical Analysis System (SAS version 9.1© 2003, Cary, NC, USA).

Variation in the gut morphologies of individual black stilt due to different body size and mass was investigated to determine whether this needed to be accounted for in further analysis. Estimates of partial correlation coefficients were obtained for the correlations between organ lengths and dry masses with (1) each other, (2) fresh body mass, and (3) the two body size variables (tarsus and bill length). The GLM procedure with multivariate analysis of variance (MANOVA), correcting

for the effects of body mass, source (whether the bird was captive, released or wild) and sex was used for this analysis.

The digestive organ morphologies of captive, released and wild *Himantopus* sp. were compared with 8 birds that were emaciated using the GLM procedure. Released birds were divided into two groups; those that died during the first 4 weeks of release (released $\leq$ 30 d) and those that died between 4 weeks and 5 months of release (released > 30 d). The linear model included source as a class effect and body mass and sex as covariates. Sex was coded as 0 = female, 1 = unknown and 2 = male to form a numeric covariate. The initial model included the numeric covariate age but this was removed due to collinearity with body mass. Body size was not included as a covariate due to its limited correlations with digestive organ length and dry mass.

Differences in the percentage of mass lost during the drying process between fresh and formalin fixed chicken organs were analyzed using the MIXED procedure. Changes in the lengths of each organ were described using box plots.

Results

i. *Himantopus* sp. digestive organs

- *Descriptive statistics*

Captive birds (200.20 ± 9.61 g) tended to be heavier than released (188.79 ± 8.91 g) and wild (184.12 ± 9.30 g) birds (least squares means ± standard error [SE]). However, these differences were not significant (controlling for sex [$F_{3, 29} = 0.71$, $P = 0.555$] and age [$F_{1, 29} = 0.92$, $P = 0.347$]). Males were slightly heavier than females with average masses of 191.80 ± 8.64 g and 186.39 ± 4.79 g respectively (controlling for source: $F_{3, 30} = 0.56$, $P = 0.644$). Adult birds (194.63 ± 7.07 g) were heavier than juvenile and subadult birds (186.98 ± 3.93 g) (controlling for source: $F_{1, 46} = 0.79$, $P = 0.379$).

- *Digestive organ correlations*

Fresh body mass and size explained variation in the digestive organ dry mass and length measurements for some organs only (Table 2.). Body mass was significantly correlated with esophageal length and dry mass as well as liver dry mass. The only correlations with the body size variables were esophageal mass with bill length and rectal mass with tarsus length.

The lengths of the small intestine, ceca and gizzard were all correlated. The lengths of the small intestine and the ceca were also correlated with those of the rectum. Small intestine dry mass was correlated with gizzard and ceca dry mass. Cecal and rectal mass were also correlated and so were proventriculus and esophageal mass.

Most organ lengths (and volume for the gizzard) and dry masses were correlated with each other ([partial correlation coefficient, *P*-value]: gizzard [0.770, 0.001], small intestine [0.470, 0.071], ceca [0.546, 0.043] and rectum [0.582, 0.029]). However this was not the case for the esophagus (0.044, 0.880) and the proventriculus (0.289, 0.317).

Table 2. Correlations between digestive organs, body mass and body size in *Himantopus* sp. (black stilt [*n* = 48], black-pied stilt hybrids [*n* = 9], and pied stilt [*n* = 2]). Values are partial correlation coefficients obtained from MANOVA and are adjusted by body mass, source and sex. Body mass values are only adjusted by source and sex. Emaciated birds were excluded from this analysis.

	Eso–phagus	Proven–triculus	Gizzard	Small intestine	Ceca	Rectum	Liver
Organ size							
Esophagus							
Proventriculus	–0.438						
Gizzard[a]	–0.064	0.034					
Small intestine	0.213	–0.172	0.715**[b]				
Ceca[c]	0.027	–0.134	0.682**	0.754**			
Rectum	0.196	–0.143	0.435	0.694**	0.749**		
Body mass	0.490†	0.198	0.319	0.061	0.188	–0.017	
Bill length	0.321	–0.172	–0.060	0.157	0.223	0.035	
Tarsus length	–0.100	–0.2253	–0.152	–0.024	0.097	0.346	
Organ mass							
Esophagus							
Proventriculus	0.500†						
Gizzard	0.318	0.275					
Small intestine	0.315	0.445	0.714**				
Ceca[c]	–0.091	–0.082	0.381	0.516†			
Rectum	–0.196	–0.182	0.401	0.281	0.600*		
Liver	–0.308	0.367	0.211	0.426	0.449	0.261	
Body mass	0.515*	0.342	0.188	0.534*	0.019	0.283	0.748**
Bill length	0.515†	0.122	0.061	0.439	0.504†	0.114	0.275
Tarsus length	0.192	0.172	0.276	0.377	0.287	0.666**	0.623

[a] Gizzard size was measured in volume, all other organ size measurements were lengths.

[b] Significance levels denoted by: † $P < .1$; * $P < .05$; ** $P < .01$.

[c] Ceca values were calculated by obtaining the average length and mass of both ceca of each bird.

- *Comparison of captive, released, wild and emaciated birds*

Overall, there was a significant effect of source (whether a bird was captive, recently released, previously released, wild or emaciated) on proventriculus length ($F_{5, 12}$ = 4.79, P = 0.012), esophageal mass ($F_{5, 12}$ = 5.54, P = 0.007) and proventriculus mass ($F_{5, 12}$ = 6.93, P = 0.003). Liver mass differed as a function of source at the α <.1 level ($F_{5, 12}$ = 2.44, P = 0.096).

There were no differences in any of the organ length and volume measurements between the five source groups (Table 3.) When comparing the masses however, wild birds had heavier oesophaguses and proventriculuses than all four other source groups (Table 4.). Wild birds also had heavier gizzards than captive, recently released and emaciated birds and heavier small intestines than captive birds and both groups of released birds. Captive birds had heavier livers than recently released, previously released and wild birds. There were no differences in the masses of the ceca and rectum between any of the source groups.

There were no discernable differences in the gut morphologies of recently released and previously released birds (Tables 3. & 4.). Similarly, there were no differences between released and emaciated birds. However, emaciated birds also had similar gut morphologies to captive and wild birds.

Table 3. Least squares means (± standard error [SE]) for *Himantopus* sp. digestive organ size. We compared the means (adjusted by source, sex and body mass) for captive, released ≤ 30 days, released > 30 days, wild and emaciated birds. There were no significant differences in the organ sizes between any of the groups (P >.05).

Organ size	Source									
	Captive		Released ≤ 30 d		Released > 30 d		Wild		Emaciated	
	Mean ± SE	*n*	Mean ± SE	*n*	Mean ± SE	*n*	Mean ± SE	*n*	Mean ± SE	*n*
Esophagus	8.71 ± 1.35	9	11.47 ± 1.06	7	10.78 ± 1.04	5	10.09 ± 0.64	10	6.90 ± 1.96	1
Proventriculus	1.48 ± 0.11	24	1.27 ± 0.09	7	1.46 ± 0.08	6	1.60 ± 0.05	9	1.35 ± 0.16	4
Gizzard [a]	4.59 ± 1.93	22	5.97 ± 1.53	7	7.65 ± 1.50	6	7.32 ± 0.92	9	6.31 ± 2.82	3
Small intestine	31.04 ± 8.01	16	46.77 ± 6.33	10	46.80 ± 6.21	7	40.58 ± 3.81	10	42.27 ± 11.66	6
Ceca	2.77 ± 0.63	16	3.57 ± 0.50	9	4.68 ± 0.49	7	3.95 ± 0.30	10	4.53 ± 0.92	5
Rectum	3.08 ± 1.22	10	4.75 ± 0.97	8	5.71 ± 0.95	5	4.47 ± 0.58	10	4.06 ± 1.78	4

[a] Gizzard size was measured in volume (mm^3); all other measurements were lengths (mm).

Table 4. Least squares means (± standard error [SE]) for *Himantopus* sp. digestive organ dry masses (measured in g). We compared the means (adjusted by source, sex and body mass) for captive, released ≤ 30 days, released > 30 days, wild and emaciated birds. Means in the same row with different letters are significantly different (P <.05).

Organ mass	Source									
	Captive		Released ≤ 30		Released > 30 d		Wild		Emaciated	
	Mean ± SE	*n*	Mean ± SE	*n*	Mean ± SE	*n*	Mean ± SE	*n*	Mean ± SE	*n*
Esophagus	0.12 ± 0.02 a	8	0.12 ± 0.02 a	8	0.11 ± 0.02 a	5	0.18 ± 0.06 b	10	0.06 ± 0.03 a	1
Proventriculus	0.11 ± 0.02 a	19	0.11 ± 0.01 a	8	0.12 ± 0.01 a	6	0.18 ± 0.01 b	9	0.13 ± 0.02 a	3
Gizzard	0.69 ± 0.21 a	21	0.84 ± 0.17 a	9	0.99 ± 0.17 ab	8	1.37 ± 0.10 b	11	1.20 ± 0.31 ab	5
Small intestine	0.38 ± 0.10 a	16	0.35 ± 0.08 a	10	0.48 ± 0.07 a	7	0.67 ± 0.05 b	10	0.58 ± 0.14 ab	6
Ceca	2.77 ± 0.63	15	3.57 ± 0.50	9	4.68 ± 0.49	6	3.95 ± 0.30	10	4.53 ± 0.92	5
Rectum	0.02 ± 0.02	9	0.04 ± 0.02	8	0.06 ± 0.02	5	4.47 ± 0.58	10	0.06 ± 0.01	4
Liver	1.63 ± 0.16 b	22	1.12 ± 1.30 a	9	1.48 ± 0.13 a	7	1.20 ± 0.08 a	10	1.33 ± 0.24 ab	8

ii. Comparison of formalin fixed and fresh chicken digestive organs

- *Change in mass*

On average, organs that were preserved in formalin for 30 days gained 1.7% mass. The esophagus, proventriculus, small intestine, rectum and ceca all gained mass (range = 1.61 – 4.44%). However the gizzard and liver lost mass, averaging 0.96 and 0.71% respectively.

Average mass lost due to the drying process for all of the organs combined was 77.16% for fresh and 78.67% for formalin fixed specimens. The percentage of mass lost due to dehydration differed significantly between fresh and formalin fixed specimens for the proventriculus, small intestine and the rectum (Fig. 1.). In all three cases, formalin fixed organs lost a greater percentage of mass than fresh organs. The muscular gizzard lost the least proportion of mass.

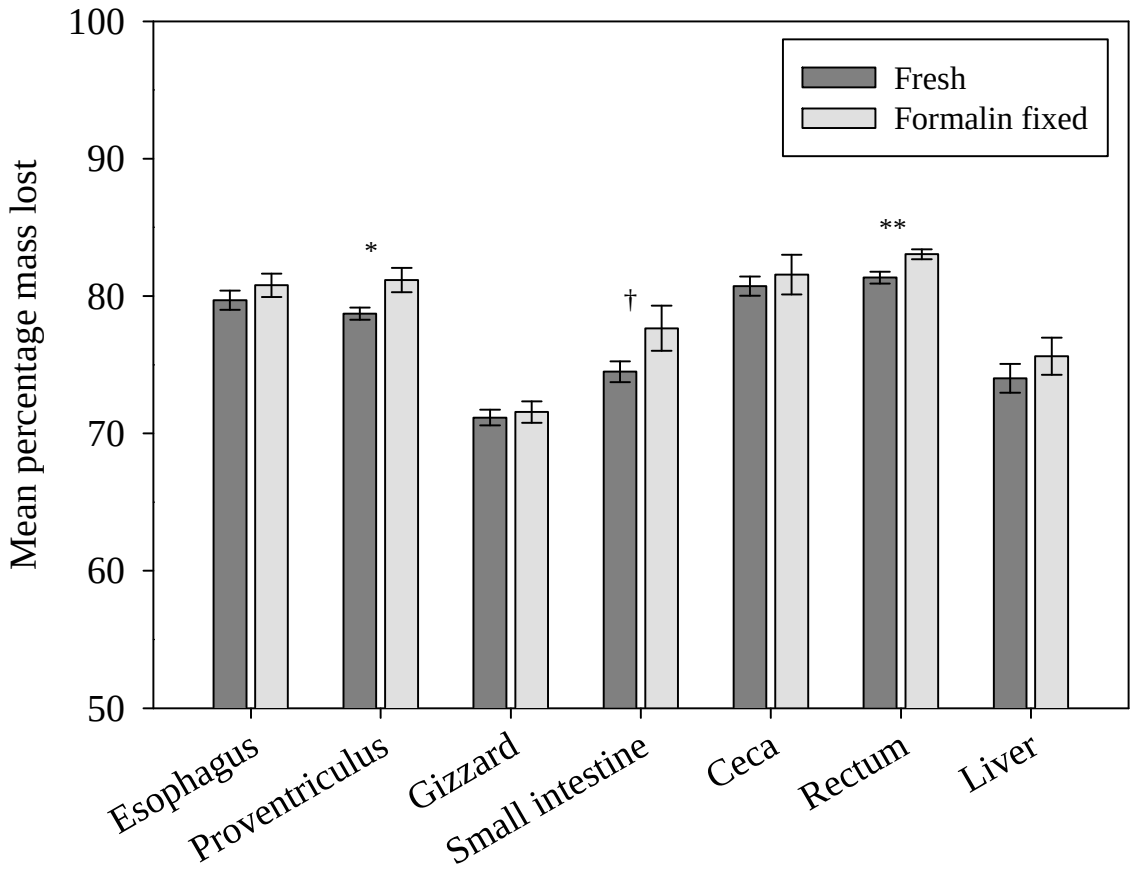

Figure 1. Mean percentage mass lost due to the drying process for fresh and formalin fixed (preserved in 10% buffered formalin solution for 30 days) chicken digestive organs. Bars indicate standard errors. Significant differences are indicated by † $P < .1$, * $P < .05$ and ** $P < .01$.

- *Change in length*

There were some variations in the percent length change of chicken digestive organs within and between organs due to preservation in formalin. The esophagus and small intestine were the most variable organs (Fig. 2.). The overall median length change was 3.96 ± 7.44% (standard deviation [SD]); ranging from 0.00% (gizzard length) to 10.94% (esophagus). Negative values were obtained for esophagus, gizzard (length and width) and rectum lengths.

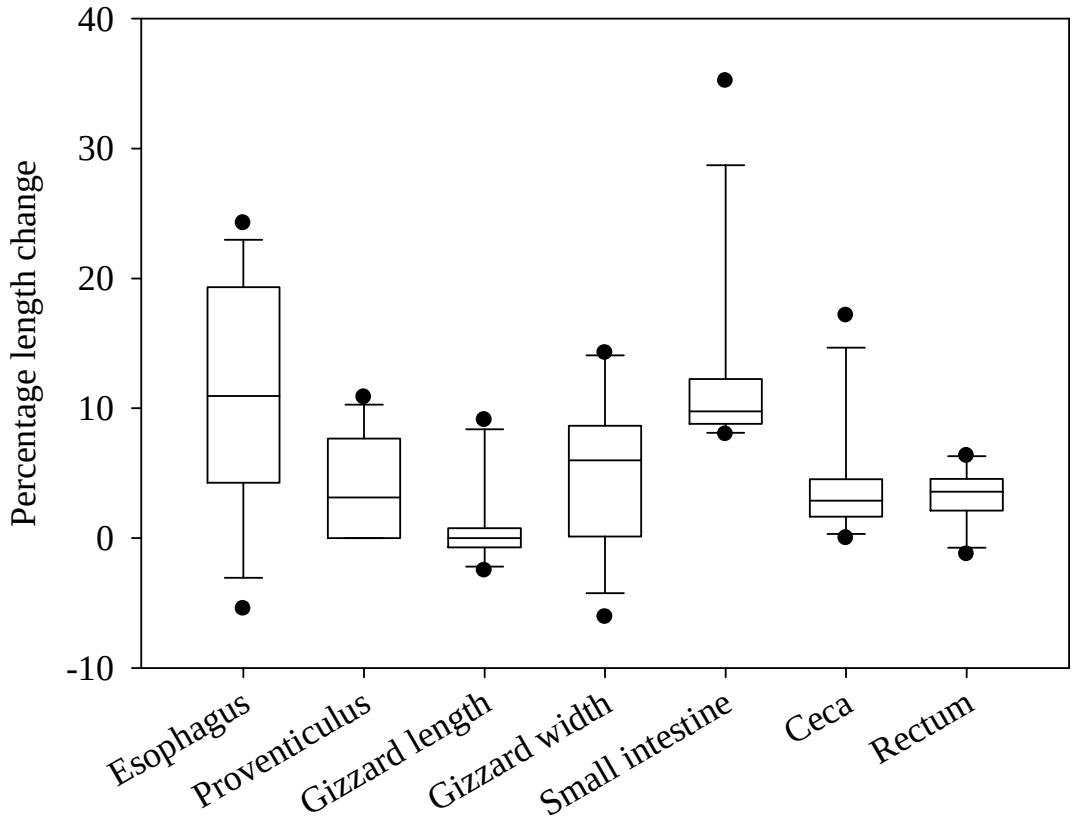

Figure 2. Percentage length change of chicken digestive organs (n = 12 for each organ) due to storage in 10% buffered formalin solution for 30 days. Boxes enclose the median, with 25th to 75th percentiles, and whiskers show the 10th and 90th percentiles. Outliers are indicated by black circles.

Discussion

i. Digestive organ morphology

This study has shown that, in general, captive black stilt had smaller digestive organs than wild hybrid and pied stilt. Released birds also had generally smaller digestive organs than wild birds but there were very few differences in the lengths and masses of captive, recently (≤ 30 days) and previously (30 days to 5 months) released birds. These findings suggest that the digestive organs of captive birds do not enlarge significantly following release to the wild. Emaciated birds had similar digestive organ morphologies to healthy captive, released and wild birds thus reduced digestive efficiency did not appear to be the primary cause of poor condition in these birds.

Wild *Himantopus* sp. probably had larger digestive organs than captive black stilt due to large intakes of the poor quality food available in their habitat.[7, 10-12] Possession of a larger esophagus and small intestine increases food transit times along the alimentary tract and increases the absorptive surface. A larger proventriculus increases glandular digestion and a larger gizzard increases the capacity of mechanical digestion.[7, 13, 26] Under the digestive optimization theory, the smaller digestive organs of captive black stilt could be explained as a response to the lower rate of food consumption and the low fiber captive diet.

The smaller organs of captive black stilt could also be explained by genetic factors. The black stilt population suffered a recent severe bottleneck; in 1983, only 23 adults remained in the wild.[27] It is possible that loss of genetic variation due to captive breeding has resulted in reduced capability for digestive organ phenotypic flexibility. Captive breeding may also be unintentionally selecting for genetically smaller digestive tracts.[6, 28] This theory is particularly relevant to black stilt as an estimated 93% of the current wild population have been raised in captivity.[27]

Released birds had smaller organs than wild birds suggesting that released black stilt may be suffering from reduced digestive efficiency. There are however alternative explanations for these differences. Firstly, some species are thought to show a greater degree of gut plasticity than others.[10, 12] It is possible that this ability is not well developed in black stilt. We would however expect some degree of phenotypic plasticity due to their generalist feeding behaviours, high fiber natural diet and previous migratory lifestyle.[21] Nowadays, black stilt are predominantly non-

migratory[20] and food sources may remain fairly constant throughout the year so there may be little need for them to optimize digestive organ size. Secondly, there are energetic costs involved with the maintenance of larger digestive organs and carrying them around.[7] Van Gils et al.[7] suggest that the ecological setting determines the specific organ size at which the benefits of larger organs must outweigh the costs. They reported red knots (*Calidris canutus*) to optimize gizzard size in response to different seasonal demands. Perhaps the physiological constraints associated with the release period (such as adaptation to the wild habitat, predation, competition, increased flight and exposure) prevent released black stilt from increasing digestive organ size significantly.[10] The smaller organs of these birds could therefore be representative of digestive organ optimization in the presence of a suite of ecological pressures. A third explanation is that the provision of supplementary food at the release site effectively acclimatizes released black stilt to the wild diet. McWilliams and Karasov[10] suggest that if birds are given sufficient time to adapt to the wild diet then considerable increases in food intake may not be expressed as increased digestive organ size.

Emaciated birds had similar gut morphologies to healthy wild birds and consequently did not appear to be significantly atrophied. Therefore, reduced digestive efficiency did not seem to be the primary cause of wasting in our sample of 8 emaciated birds, which probably makes them poor indicators of reduced organ size in captive and released birds. This is perhaps not surprising given that three of the emaciated birds were captive and 4 others were only recently released. Most of the birds therefore had access to the captive diet during the time they became emaciated. Emaciated birds did have similar gut morphologies to captive and released birds, but as discussed, it is unclear whether this was related to decreased digestive efficiency or other factors. A more appropriate study would have been to compare the digestive organs of captive and released birds with those of emaciated wild birds. We were not able to do this because all 11 wild *Himantopus* sp. available were in good nutritional condition. Moore and Battley[6] were able to make this comparison in brown teal (*Anas chlorotis*) and found similarities in the weights of healthy captive and emaciated wild digestive organs. This provided support for their hypothesis that captive birds had atrophied organs.

Captive black stilt had larger livers than released and wild birds, a finding that has also been observed in several waterfowl species.[6, 29, 30] This is probably explained by considering the liver's role in the storage of glycogen and lipids absorbed from the gut.[12] As captive birds generally have

low energy usage, they are able to accumulate surplus amounts of these nutrients. Recently-released birds had significantly lighter livers than captive birds indicating rapid mobilization of liver compounds during the translocation and release period.

Sources of error in our analysis could be responsible for the differences we found in the digestive organs of captive, released, wild and emaciated birds. The carcasses of wild black stilt are seldom found if they do not have radio transmitters attached to them. Consequently, the only wild birds available to us were those that had been euthanized by DOC staff and all 11 of these were black-pied stilt hybrids or pied stilt. Pied stilt differ morphologically from black stilt in that they have longer necks and tarsi and shorter bills, wings and tails[20]; it is possible that they differ in their internal morphologies as well. Because all the wild birds were adult, there could be a confounding effect of age in our analysis. We aimed to incorporate age as a covariate in ANCOVA but were not able to do so because of multicollinearity.

The time taken for avian digestive systems to adapt to a novel diet is generally considered to be days to weeks, but depends on the species and the extent of diet switching.[9, 10] Diet switching in black stilt is a gradual process. Released birds are provided with the captive diet for about 4 weeks after release. During this period, the amount of food supplied is progressively reduced until none is provided at all. The addition of supplementary feeding to release protocols in 1999, greatly increased the survival of released black stilt.[27] If reduced digestive efficiency does exist in black stilt, it was probably more important in the past, when the birds were subjected to more extreme dietary changes. Separate analysis of post-release survival rates showed the majority of deaths occur in the first few weeks after release (Robertson et al., 2008, unpublished data). Because birds are being supplementary fed at this time, reduced digestive efficiency is probably not reducing survival in the initial stages of release when the mortality rates are the highest.

Moss[14] observed that the greatest increases in gut length in red grouse (*Lagopus lagopus scotius*) occur in the ceca. He subsequently concluded the cecae were important in gut plasticity and a model organ in which to study changes. Moore & Battley[6] also observed phenotypic plasticity of the ceca in brown teal. Our study however, did not. Tetraonids and anatids are widely considered to have well developed ceca due to their mostly herbivorous diets. The ceca of these birds are

important for the microbial fermentation of cellulose.[14, 31] The ceca of black stilt are not required for cellulose digestion and so are probably less affected by changes in diet quality and or volume.

Many other studies have also used gut length and mass as the main indicators of phenotypic plasticity in avian digestive organs.[1, 6, 8, 12, 22] However, Starck[32] noted that gut length is not the only parameter in which changes may affect the resorption surface. He suggested that gut capacity (gut length × cross section of the gut interior) and the effective mucosal area (indicated by changes in villi length, width and number; and changes in gut circumference and intestine length) to be the most appropriate measures of phenotypic plasticity. We considered using these parameters in our analysis but after examination of archived histological specimens, we found great variation in cross sectional size and the number of villi at slightly different regions of the same digestive tracts.

ii. Formalin preservation

The most important findings of this experiment were (1) there are differences in fresh and formalin fixed organ dry masses that should be considered when interpreting the results and (2) variation in chicken organ lengths may explain why the lengths and masses of black stilt esophaguses and proventriculuses were not correlated. It could also account for the fact that there were differences in the organ dry masses between source groups but not in their lengths.

Desiccation resulted in formalin fixed chicken proventriculuses and rectums (and small intestines at the $\alpha < .1$ level) loosing significantly greater proportions of mass than fresh organs. We obtained relatively small standard errors for each of theses organs, indicating that the differences were small. It would have been more appropriate to quantify the actual differences in the mass lost between fresh and formalin fixed black stilt organs and to devise an appropriate correction factor. But, as is common when dealing with endangered species, we did not have surplus specimens to experiment with and used chickens because they were readily available. The degree of formalin shrinkage depends on the size of the organ,[23, 24] so it is likely that black stilt digestive organs would be more affected by preservation than the thicker chicken organs. Preservation time also affects the degree of shrinkage.[23, 33] Some of the black stilt specimens we used had been stored in formalin for 15 years and because of time constraints, the chicken gut experiment was only over 30 days.

There was considerable variation in the proportional length change within and between chicken digestive organs due to storage in formalin. We obtained some negative values, indicating some organs increased in length. Tucker and Chester[23] reported flounder larvae to increase in length after preservation in 4 and 10% buffered formalin. The large variations indicate that organ length can fluctuate over time and that it is a relatively subjective measure, questioning its reliability as a sole measure of phenotypic plasticity. The storage time of chicken organs (30 days) may not have been long enough to compensate for length fluctuations. Additionally, restrictive mesenteries and the malleable nature of the chicken digestive organs meant that it was difficult to take precise measurements.

Due to the sources of variation discussed above, we feel it is somewhat unreasonable to extrapolate correction factors for black stilt digestive organs from those of chickens.

iii. Conclusion

There is evidence to suggest that captive and released black stilt have smaller digestive organs than their wild counterparts. Moreover, released birds do not appear to increase digestive organ size to match the high fiber wild diet. Whether the small digestive organs of released birds manifest as reduced digestive efficiency is uncertain. However, is seems unlikely that digestive inefficiency is contributing appreciably to the high mortality rates of released birds. Released black stilt are probably more vulnerable to the direct pressures associated with the translocation such as predation, competition and exposure. This is supported by the high mortality rates of released birds in the initial stages of release, while the captive diet is still being supplied. Consequently, we feel that the continued provision of supplementary food to released birds and an increased focus on habitat enhancement and predator control at release sites should be the main priorities of the black stilt management plan. Increased attention to locating the carcasses of wild birds would help increase our understanding of phenotypic plasticity in black stilt, as would maintaining the practice of detailed necropsies of all deceased birds.

Our comparison of fresh and preserved chicken digestive organs suggests the need for quantification of mass and length changes due to formalin fixation if similar comparisons are to be made for black stilt digestive organs in the future.

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Chapter 5

General Discussion

The “watch and wait” faecal collection position at the Mailbox Inlet release site

(Photo by L. Robertson)

As outlined in the General Introduction, the main aim of this study was to assess the possible impacts of gastrointestinal (GI) helminths and digestive organ morphology on the low survivorship of captive-reared and released subadult kakï. An emphasis was placed on evaluating current management practices and the methods used to assess the importance of GI helminths and digestive organ plasticity. The main findings of this research are (1) GI helminths and reduced digestive organ size and mass are not contributing significantly to the low survivorship of released kakï, (2) the current anthelmintic regime is effective at controlling GI helminths but is probably unnecessary, and (3) faecal helminth screening is unreliable as a quantitative indicator of GI helminth abundance.

This research was limited by the number of experimental birds and digestive organs that were available for the study. As kakï are a critically endangered species, it was not feasible to obtain larger sample sizes. This meant that substitute species (chickens, pied and hybrid stilt) had to be used in some experiments. This situation was not ideal but did increase the validity of the results and allow a better understanding of the general principles under investigation.

Even though the results of this study do not implicate GI helminths and reduced digestive efficiency in the high mortality rates of released kakï, it is probable that intensive management is acting to buffer these effects. Supplementary feeding and predator control at kakï release sites reduce the stresses associated with the release period. Thus released kakï do not have to expend valuable energy evading predators and obtaining sufficient food in order to remain healthy. This in turn reduces the likelihood of immunosuppression of kakï and the subsequent proliferation of GI helminths (and other disease agents). In addition, management at the release site may increase the number of helminths that a bird can sustain whilst still remaining healthy. The progressive reduction in the amount of supplementary food provided at the release site minimises the likelihood of reduced digestive efficiency occurring. Pre-release anthelmintic treatment was found to reduce GI helminth burdens in the short-term. Hence, the physiological demands produced by intestinal parasites are probably cushioned by this temporary reduction. These possibilities are supported by the differences in survivability that were associated with different levels of management applied at the two release sites in 2007. The release site with greater predator control (Tasman delta) also had much higher survival rates.

Conservation biology is considered to be a multidisciplinary field that incorporates both pure and applied science in order to "solve" specific conservation problems (Latta 2000, Meine et al. 2006). Historically, most reintroduction programmes were evaluated subjectively, meaning little scientific information was gained from these exercises (Seddon et al. 2007). Seddon et al. (2007) suggest that reintroductions are most likely to be successful when multidisciplinary groups of scientists and field managers form close collaborations to devise the best course of management action. The current investigation provides an example of the application of the multidisciplinary principles of Conservation Biology to experimentally evaluate wildlife management practices and to help guide future management decisions. Specifically, the fields of biology, ecology, veterinary science and field management were incorporated to assess the value of several aspects of the kakï release programme. The conclusion that is of most importance to the kakï recovery programme is that current management practices are successful in ensuring that GI helminths and reduced digestive efficiency do not significantly lower the survivorship of captive-reared and released kakï.

Based on the results of this work, the following priorities for future research and management of captive-reared kakï released to the wild are recommended.

i. Terminate or reduce anthelmintic treatment.

There was little evidence found to suggest that the costs of treatment are being outweighed by the potential benefits. If the management team does not wish to totally abandon the treatment regime, an alternative would be to alter the level of treatment so that it is administered in some years only or to a sample of birds that are most at risk. The type of anthelmintic used could also be alternated between years. Nevertheless, health screening should remain an important tool for monitoring the emergence and distribution of GI helminths and to continually evaluate the importance and need for anthelmintic treatment.

ii. Incorporate regular efficacy testing to monitor the development of anthelmintic resistance.

Resistance is considered to be an inevitable outcome of any long term anthelmintic programme in domestic animals (West et al. 2002). Anthelmintic resistance poses a risk to both captive and wild kakï as resistant helminths will emerge and these could be introduced to the wild population

through the release of captive-reared birds. Efficacy testing can easily be carried out in conjunction with the current faecal helminth screening analysis.

iii. Identify the GI helminths of kakï to species.

It was beyond the scope of this study to identify the helminths of kakï to species level. However, doing this would greatly increase our understanding of the importance and pathogenicity of helminths to kakï. The parasites that were recovered in this study have been archived in 70% ethanol solution so that this research can be conducted in the future.

iv. Improve the accuracy of faecal helminth screening methods.

This research showed that there are high levels of variation in the standard faecal screening methods used in diagnostic laboratories. As faecal screening directly determines the type of anthelmintic regime applied, it is important that the tests are accurate. A stochastic model could be developed to incorporate these sources of variation but this would involve the collection of extensive baseline data.

v. Continue to collect pooled faecal samples from each aviary.

Faecal samples collected from individual kakï had lower sensitivity compared with those collected from groups of birds within aviaries. The current practice of collecting pooled samples has been shown to be the most reliable, safe and cost-effective way to collect faecal samples.

vi. Continue detailed necropsies of all kakï that die (both captive and wild) and increase the effort in locating wild carcasses.

This will allow more baseline data on helminths and their infections to be obtained. This study has highlighted the need to increase the sample size of carcasses for further studies. Good necropsy and field data will allow managers to continue to accurately monitor causes of death in kakï. Increased effort allocated to locating these carcasses in the wild will ultimately increase our understanding of how disease, gut physiology and other factors are impacting on released kakï in the long term.

vii. Continue supplementary feeding at the release site.

The advent of supplementary feeding has greatly increased the survival rates of released kakï (Maloney 2003) and the results of this study suggest that the provision of supplementary food reduces translocation stress and reduced digestive performance. Supplementary feeding also encourages birds to stay at the release site where they can be closely monitored.

viii. Correlate GI helminth burdens and digestive organ size with a quantifiable measure of body condition

This research relied largely on performance and survival based factors to assess the impacts of GI helminths and gut plasticity in released kakï. A quantifiable measure of body condition would enable a more direct physiological assessment of the impacts of these factors. Body condition of live birds can be measured using bioelectrical impedance analysis (BIA) and total body electrical conductivity (TOBEC) analysis. Both of these methods offer a quantifiable alternative to traditional morphometric analysis (Pitt et al. 2006, Ten Hwang et al. 2005). To determine body condition in predated or scavenged carcasses, a bone fat assay can be used. Because bone fat is one of the last nutrients to be depleted before death, the absence of medullary fat can be used as an indicator of poor nutritional condition (Guglielmo & Burns 2001, Hutchinson & Owen 1984). This method would be especially useful for wild kakï as complete carcasses are seldom recovered.

ix. Consider the role that starvation might play in high mortality of released kakï

Starvation of released kakï was not explored to a great degree in this thesis. As starvation has been implicated in the deaths of released kakï in the past (Johnston et al. 2007), its potential role in the high mortality rates of these birds warrants investigation. The bone fat assay could also be used for this research.

x. Increase the focus of management towards improved predator control and habitat enhancement

The captive management programme is currently very efficient at rearing and releasing large numbers of kakï to the wild each year. However, management at release sites to ensure the survival of the released birds appears disproportionately low. It should be noted though that research into increasing the survival of released kakï is currently in progress (with studies such as this

investigation). This research indicates that physiological constraints on released kakï are currently not contributing significantly to the high mortality rates encountered. It seems far more likely that direct risks at the release site, such as predation and habitat loss are having the largest impact. Therefore, reducing these risks should become the main focus of the kakï recovery programme.

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Appendices

Sunset in Aviary A2, Twizel

(Photo by L. Robertson)

Appendix 1

Composition of the captive kakï diet

Ingredient	Per kg (g)	Using 1 kg oxheart (g)
Oxheart	782.4	1000.0
Dry cat biscuits [a]	56.5	72.0
Water	43.5	56.0
Kiwi premix	4.3	5.5
Iodized salt	2.5	5.2
Mealworms	100.0	128.0
Ca supplement	10.9	13.9

[a] Go-Cat (dry cat food) chicken, beef, calcium and vegetable flavor, from Friskies Pet Care, 1 Broadway, Newmarket, Auckland, New Zealand.

Source:

Cottam Y, Hendricks W, Sancha E. *Captive diet of New Zealand black stilt held at Twizel*. Department of Conservation: Wellington; 2001.

Appendix 2

Supplement to Chapter 2:
Spatial variation in the distribution of gastrointestinal helminths along the alimentary tract of *Himantopus* sp. in New Zealand

The following outlines some additional research that I conducted during the course of my MSc. Because this facet of the research is not directly related to the aims of Chapter 2 (Efficacy of Anthelmintic Treatment), the methods and results are provided as a supplement to that chapter. It is my intention to publish these findings as a Letter to the Editor or a Short Communication in an appropriate journal.

Methods

Total worm counts were conducted on 4 captive, 11 released and 11 wild *Himantopus* sp. that were submitted for necropsy in 2007. These were made up of 15 black stilt (*H. novaezelandiae*), 8 black-pied stilt hybrids and 3 pied stilt (*H. h. leucocephalus*). All of the birds were frozen immediately after necropsies were conducted and then thawed immediately before worm counts were undertaken. The oesophagus, proventriculus, gizzard, small intestine, caeca and rectum of each bird were cut in half longitudinally and the contents were scraped out so that the helminths of the upper and the lower halves could be counted separately. The parasites were examined and counted microscopically at 3.5 × magnification and identified to class. The historical total worm count data accessed from the Massey University *post mortem* database (1997 – 2006) were also used for 21 captive, 10 released and 2 wild black stilt.

All statistical analyses were carried out using the Statistical Analysis System (SAS version 9.1© 2003, Cary, NC, USA). The MIXED procedure was used to compare the locations of GI helminths along the alimentary tract. All the data was $\log_{10}(x+1)$ transformed to achieve normality.

Results

There were significant differences in the anatomical locations of each helminth type along the alimentary tracts of the birds (trematodes: $P = < 0.001$; cestodes: $P = < 0.001$ and *Capillaria* spp. $P = 0.025$) (Table 1.). Fifty four percent of all trematodes were found in the upper and lower small intestines and 20% in the caeca. Cestodes were most abundant in the small intestine with 86% being found there. No trematodes or cestodes were recorded in the oesophagus or proventriculus. Forty one percent of *Capillaria* spp. were found in the oesophagus. Relatively high proportions were also found in the caeca (24%) but this was not significant. *Capillaria* spp. were absent from the lower small intestine and rectum altogether (Fig. 1.).

Table 1. Differences in the least squares mean number of trematodes, *Capillaria* spp. and cestodes found at different locations along *Himantopus* sp. digestive tracts (black stilt n = 15; black - pied stilt hybrid n = 8; pied stilt n = 3). Only significant differences are displayed. Means and 95% confidence intervals (CI) are geometric ($\log_{10}[x+1]$ back-transformed).

	Descriptive statistics	Differences between organs (P =)						
	$\bar{x}$ (95% CI)	OES[a]	PRO[b]	GIZ[c]	UPP SI[d]	LOW SI[e]	CAE[f]	REC[g]
Trematodes								
OES[a]	0.00				<0.001	<0.001	<0.001	0.019
PRO[b]	0.00				<0.001	<0.001	<0.001	0.008
GIZ[c]	0.12 (0.00 - 1.14)				<0.001	<0.001	0.001	0.007
UPP SI[d]	4.25 (1.70 - 9.23)	<0.001	<0.001	<0.001			0.029	
LOW SI[e]	3.68 (1.38 - 8.19)	<0.001	<0.001	<0.001				
CAE[f]	9.59 (4.39 - 19.79)	<0.001	<0.001	<0.001		0.029		0.001
REC[g]	2.04 (0.53 - 5.02)	0.019	0.008	0.007			0.001	
Cestodes								
OES	0.00				<0.001	<0.001		
PRO	0.00				<0.001	<0.001		
GIZ	0.10 (0.00 - 0.43)				<0.001	0.003		
UPP SI	1.26 (0.73 - 1.97)	<0.001	<0.001	<0.001			<0.001	<0.001
LOW SI	1.20 (0.67 - 1.90)	<0.001	<0.001	0.003			0.002	<0.001
CAE	0.06 (0.00 - 0.40)				<0.001	0.002		
REC	0.04 (0.00 - 0.38)				<0.001	<0.001		
***Capillaria* spp.**								
OES	0.44 (0.22 - 0.70)		0.047	0.007	0.015	0.002		0.002
PRO	0.15 (0.00 - 0.36)	0.047						
GIZ	0.07 (0.00 - 0.258)	0.007						
UPP SI	0.09 (0.00 - 0.29)	0.015						
LOW SI	0.00	0.002						
CAE	0.23 (0.04 - 0.45)							
REC	0.00	0.002						

[a] Oesophagus, [b] Proventriculus. [c] Gizzard, [c] Upper half of small intestine, [e] Lower half of small intestine, [f] Caeca, [g] Rectum

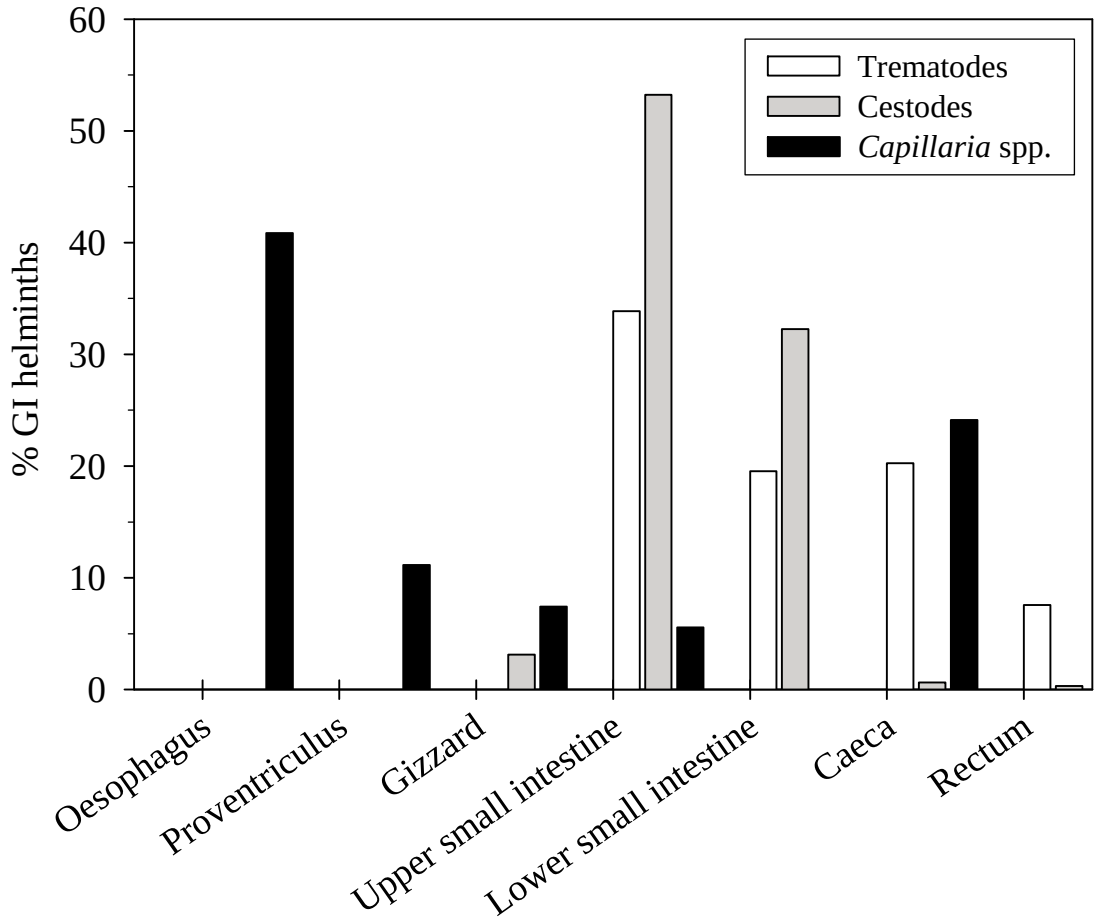

Figure 1. Percentage of gastrointestinal (GI) helminths found in different regions of the *Himantopus* sp. (black stilt n = 15; black – pied stilt hybrid n = 8; pied stilt n = 3) alimentary tract in New Zealand.

Appendix 3

Morphological measurements of the kakï digestive tract

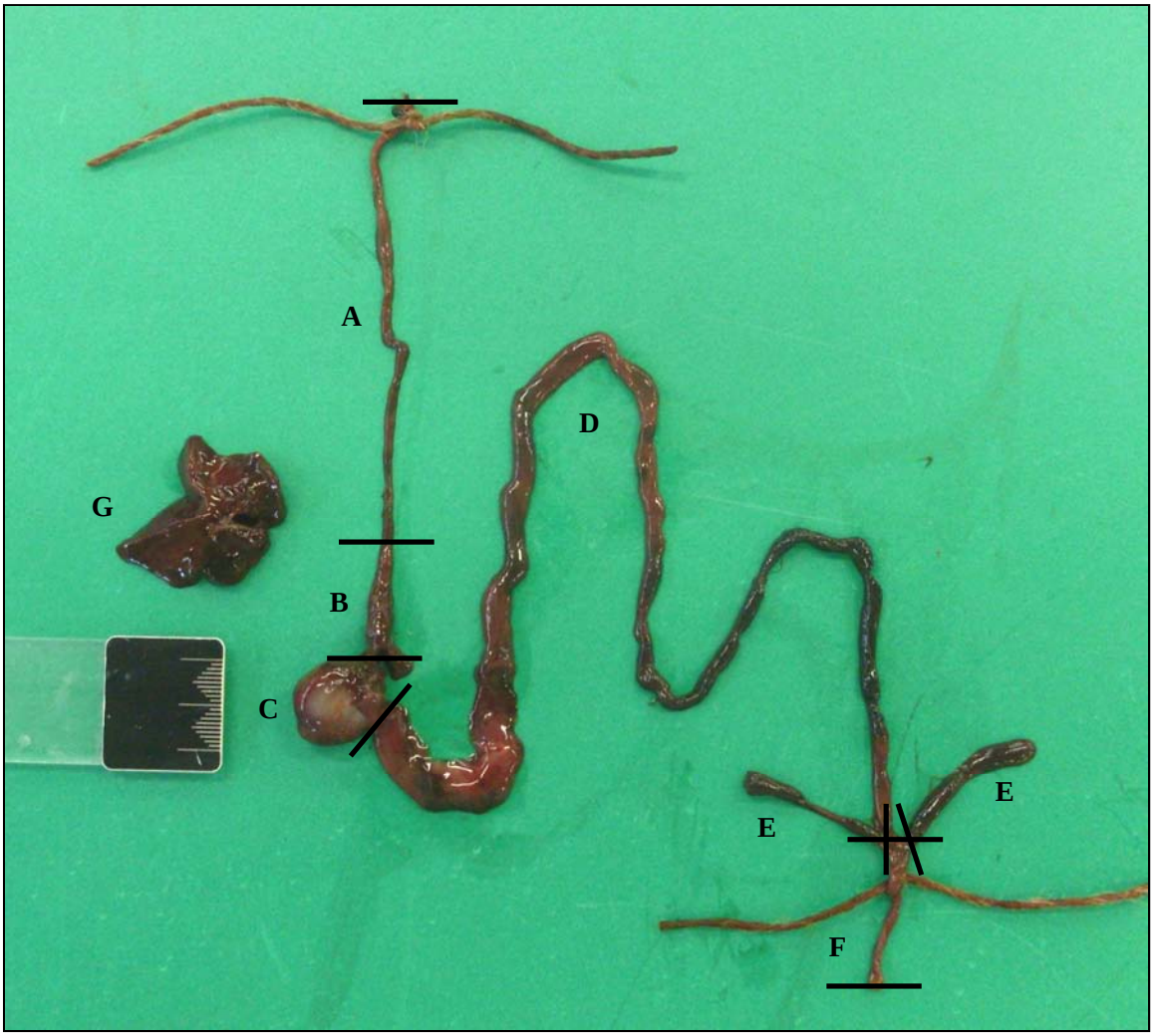

Figure 1. Intact digestive tract of a captive-reared and released subadult kakï. The gut is split into the sections measured as described in Chapter 4: A = oesophagus; B = proventriculus; C = gizzard; D = small intestine; E = caeca; F = rectum; and G = liver. The oesophagus and rectum are both tied with a piece of string to prevent loss of gut contents. Scale bar = 2cm.