Putting Conservation Medicine into Practice: Examples from Three Endemic New Zealand Bird Species

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From the moment I picked your book up until I laid it down I was convulsed with laughter. Someday I intend reading it.

Groucho Marx

Abstract

Conservation medicine is increasingly being viewed as an important component of conservation biology. While programmes focussing on wildlife health are generally limited to controlling the spread of infectious diseases, there is a need to evaluate the impacts of non-infectious diseases: in particular, a critical examination of invasive management practices is overdue. Marking or tagging animals for identification is one of the most common management tools employed by conservation managers, and yet their impacts have rarely been quantified. In the kakapo, *Strigops habroptilus*, metallic bands applied to the tarsus were implicated in joint problems in the banded leg; in contrast to this, subcutaneously implanted passive integrated transponders appear to be safe and effective in both adults and chicks. In the North Island robin, *Petroica longipes*, leg bands were directly implicated in leg injuries at a rate of 2% of adults per year. The most common injury was a result of the birds trapping their hallux (back toe) between a band and their leg; this forced the leg into a flexed position and resulted in tissue damage.

To accurately interpret clinical pathology data collected in wildlife health assessments, reference ranges for haematological and biochemical data should be generated for each species. In the kakapo, blood samples from 1996 and 2002 allowed these references to be produced; however, this exercise highlighted limitations that are often underappreciated in conservation medicine. Many factors can influence the results: two of these being sample storage and laboratory processing methods. Many conservation programmes cannot collect, store and process samples in an ideal environment and, thus, comparisons between ideally generated reference ranges and data from individuals collected in the field may be spurious. Similarly, opportunistic carcass collection and post-mortem examination provides valuable identification of disease agents, but the findings are difficult to interpret in terms of their importance or prevalence within populations. The description of aspergillosis in a North Island robin is a case in point.

The movement of animals for conservation purposes – translocations – is becoming widespread, and has the potential to introduce diseases into disease-free areas; the stitchbird, *Notiomystis cincta*, is currently the focus of conservation efforts that rely on translocations. Two poorly-understood diseases were examined: facial dermatitis and sub-lingual oral fistulas. The prevalence of facial dermatitis was influenced by season and sex, with males showing a higher prevalence of the condition than females during the breeding season. Histopathology, mite isolation and a therapeutic drug trial all suggest that a burrowing mite, *Knemidocoptes* spp is responsible for the condition. Sub-lingual oral fistulas are more widespread than previously thought, as they are not limited to birds with obvious tongue protrusions through the tissue deficit in the lower mandible. Evidence supports the hypothesis that these fistulas are acquired after fledging, and have a limited impact on bird productivity and survival.

Acknowledgements

The ideas and data collected in this thesis are the result of five years of collaborating with people from a range of disciplines and backgrounds. It has been simply brilliant to work with them – they are the proof that there is a large group of talented and dedicated people out there who are doing whatever it takes to help preserve biodiversity in New Zealand.

For introducing me to the world of robins, herring and central heating, thank you Åsa Berggren (well, not really thanks for the herring). Our partnership in both work and life has sustained me during the past four years.

Thanks to everyone in the Department of Conservation's National Kakapo Team. I enjoyed my time working with you and the birds, and had a fabulous few months wandering around the muddy tracks of Codfish Island. Thanks especially to those people with whom I worked on the projects mentioned in this thesis; Kate McInnes, Daryl Eason and Graeme Elliott (the only other person who appreciates a potato-topped pie as much as I do). Thanks also to Jo Wright for her patience, good humour and excellent company on the long cold nights we spent collecting blood from the chicks, and Joanne Paul-Murphy for her enthusiasm and help in getting the kakapo-blood data through to publication.

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At Massey University I received great help, advice and constructive criticism from my two supervisors: Ed Minot and Maurice Alley. Thanks to Maurice, Kerri Morgan, Ian Scott, Pat Davey and Evelyn Lupton for their help in all matters pathology and parasitology. It has been fun getting back to my veterinary roots and your knowledge and experience has helped bring comprehension to things I had forgotten years ago. Thanks Ed for your enthusiasm and belief in my abilities; it has always been good to know that help is always just down the corridor. Thanks also to Erica Reid and Barbara Just for keeping the finances ticking along and helping me whenever I would materialise in your offices. Thanks to the Palmerston North crowd for keeping me sane (or allowing me to express my insanity) as well as feeding and giving me places to kip when needed – Mike, Al, Viv, Tony, Kath, Marc, Alastair, Masha, Russell, Fiona, Dorothea, Yvan, Becky, Paul, Carol, Kirsty, Robin, Fiona, Jay and Richard. Thanks Ian and Heather for not turning away a waif in need of a bed and some food, and for making the best soups anyone could ask for. And thanks, of course, to my family – Mum, Dad, Stu, Date, Lou, Sheryl, Stephanie, Madeline and Nathan – for not rolling your eyes too much when I said I was embarking on yet another degree.

Three groups contributed financially to this project, and I am grateful for their assistance: the New Zealand Lotteries (Environment and Heritage fund), the Supporters of Tiritiri Matangi Inc. and the JS Watson Conservation Trust. I hope you'll agree the money was well spent. A final thanks to the Department of Conservation Biology at the Swedish University of Agricultural Sciences for giving me access to a desk and computer when I was writing up part of this thesis.

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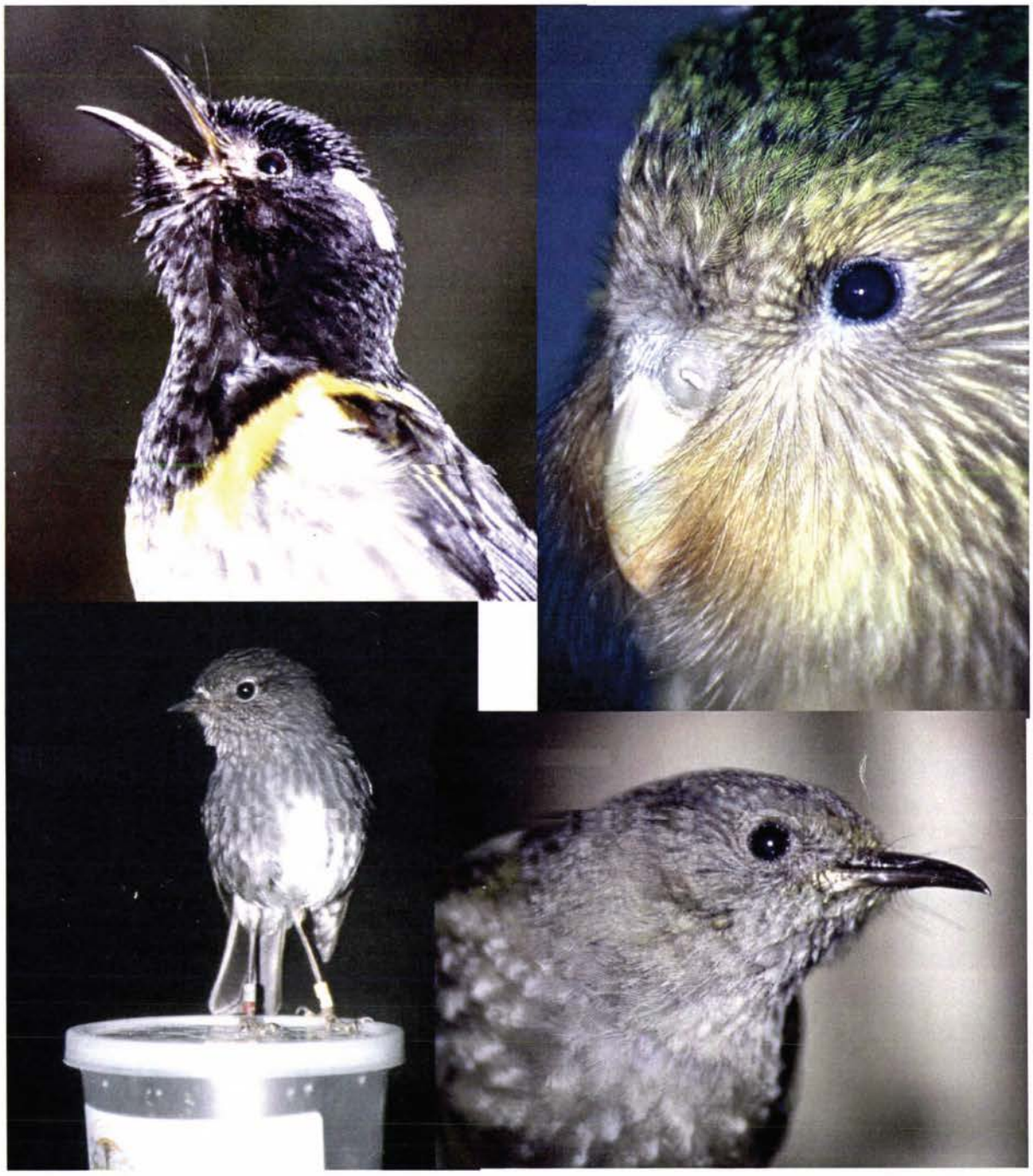
Note on text

Each chapter is set out in the style of the journal in which it has been published or submitted. Consequently, there are some minor stylistic differences between the chapters, differences in the language used (American versus Australian English) as well as some repetition. For all chapters, with the exception of chapter 2, my input into the study design, data collection, analysis and write-up of the manuscript was greater than that of my co-authors. For chapter 2, Åsa Berggren and I worked equally on all aspects of the study and manuscript. All photos reproduced in this thesis are mine.

Thesis Introduction



Clockwise from top left: Male stitchbird, juvenile female kakapo, female stitchbird, male North Island robin standing on container of mealworms

Increasingly, it is being recognised that morbidity and mortality associated with disease presents a significant threat to the conservation of many wildlife species (Daszak et al. 2000; Deem et al. 2001). One of the chief concerns of conservation biologists is that new diseases may be introduced into areas previously free from these maladies as a direct result of the movement of animals for conservation purposes (Viggers et al. 1993; Deem et al. 2001). Thus, veterinary perspectives in conservation biology – commonly called 'conservation medicine' or 'wildlife health' – have been proposed as being a necessary component of effective conservation management (Boyce et al. 1992; Meffe 1999).

It has been suggested that veterinarians wishing to enter this field need to posses an ecological perspective, coupled with a strong research background in addition to their working knowledge of veterinary medicine (Boyce et al. 1992). Previously, few people have possessed such an interdisciplinary background; because of this, conservation medicine has often been limited to collaborations between veterinarians and conservation managers. This collaboration, while often extremely effective, is prone to misunderstanding because veterinarians and conservation biologists have differing priorities (McInnes & Low 2001). Because of my background in veterinary medicine and conservation biology, **the aim of this thesis** is to use this dual perspective to resolve some of these conflicts and interpret health data from free-living populations of three endemic bird species – the kakapo *Strigops habroptilus*; the stitchbird or hihi *Notiomystis cincta*; and the North Island robin *Petroica longipes*. In order to address this aim I sought to answer the following questions:

1. What health effects are associated with methods of individually marking birds?
2. Can haematological and biochemical reference ranges be produced for the kakapo, a species where individual veterinary management is feasible?
3. What diseases are present in the robin and stitchbird populations on Tiritiri Matangi Island, and what impacts might these have on the conservation of those populations?

The Study Populations

For all three species, the populations studied had been intensively monitored since they were translocated to the islands they currently inhabit; stitchbirds were introduced to Tiritiri Matangi Island in 1995; North Island robins were introduced to Tiritiri Matangi

Island in 1992; and kakapo had been introduced to Codfish Island during the 1980s and 1990s. Thus, the populations had a number of ideal characteristics for answering questions relating to health issues. These included:

1. Population size. In all cases the populations were small enough (all < 100 breeding adults) to allow comprehensive monitoring, but large enough to enable meaningful results to be collected
2. Marked population. Since translocation, all birds have been individually banded; this allowed the progress of individual animals to be monitored over time.
3. Genealogical data. Population monitoring since translocation has recorded the sex and relationship of all birds in the populations; this was useful in comparing disease statistics relative to sex and age in the populations studied.
4. Well-established monitoring techniques. Kakapo are regularly monitored via telemetry, stitchbirds are monitored via supplementary feeding stations and nestboxes, and robins are monitored using calling tapes and mealworms. Thus, all individuals could be censused or captured if necessary.

Research Approach

Presently, conservation medicine is primarily concerned with limiting the spread of infectious diseases (Daszak et al. 2000; Deem et al. 2001). While limiting disease spread is important, there are other factors that potentially impact on the health of wildlife. Marks (e.g. leg bands) are commonly used to identify individual animals within populations for research and management (Calvo & Furness 1992). Despite their widespread application, little is known about their health consequences. In chapters one and two, I examine two types of common marking techniques – leg bands and passive integrated transponders – and their health consequences over a long-term period.

In order to interpret clinical pathology data for disease screening, it is generally recommended that each species have a set of reference values established for various haematological and biochemical parameters. These are derived from healthy animals, often from within captive institutions (Scope et al. 2000; Zais et al. 2000; Dutton et al. 2002), with little consideration given to their applicability to samples collected from animals in isolated field conditions where sample storage and processing may be less than ideal. In chapter 3, I create a set of reference values for kakapo, and question the applicability of these values outside of a narrow set of criteria. It is also common to

recommend the creation of national databases to record disease diagnoses in wildlife (Leighton et al. 1997; Sainsbury et al. 2001; Anon 2002). In chapter 4, I present a case of aspergillosis in the North Island robin – a first record in this species – and attempt to place this finding into a population context.

In New Zealand, translocations of endangered species, in order to establish new populations or boost vulnerable ones, present a mechanism by which diseases may be spread to locations where they do not naturally occur (Viggers et al. 1993; Reed & Stockdale 1994). Because of this, it is generally recommended that disease patterns at the source and release locations be clearly understood in order to make informed decisions about the risks involved when moving animals (Reed & Stockdale 1994; Jakob-Hoff et al. 2004). I examine one previously undescribed disease and one poorly understood disease in the stitchbird and discuss the implications of these conditions for current and future conservation efforts (chapters 5 & 6).

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CHAPTER 1

Evaluation of passive integrated transponders for identification of kakapo, *Strigops habroptilus*

Handling a 35 day old kakapo chick on Codfish Island, just prior to subcutaneously implanting a transponder

Chapter reference:
Low, M., Eason, D. & McInnes, K. 2005. Evaluation of passive integrated transponders for identification of kakapo, *Strigops habroptilus*. *Emu – Austral Ornithology* 105: 33-38.

Abstract

We assessed the reliability and safety of subcutaneously inserted passive integrated transponders (PITs or microchips) for individually identifying kakapo, *Strigops habroptilus*. Fifty-seven adult birds had the viability and location of their transponders assessed and their health evaluated after an average 16 months post-implantation. Also, 21 chicks implanted at five weeks of age had their growth rates monitored before and after implantation and their PITs assessed four months later. All transponders in both adults and juveniles were found to be working and located in the general area of implantation. No complications, health effects or growth rate changes were detected in either adult or juvenile birds. Kakapo are excellent candidates for this tagging system as their large body size allows the PIT to be deposited far from the insertion hole, and this combined with their flightlessness, possibly contributes to minimising transponder migration and loss. While PITs have now been evaluated in several bird species, we suggest caution in generalising the results from this study to smaller, flighted species. We encourage further studies into the use of PITs in birds in order to better elucidate the factors influencing PIT retention.

Introduction

Identifying animals for population studies, management purposes, or live trade by using unique individual markings has commonly relied upon numbered tags or bands externally attached to the animal (Calvo and Furness 1992; Freeland and Fry 1995). More recently, tags or transponders have been developed that can be internally attached through subcutaneous, intramuscular or intraperitoneal insertion via a sterile needle. These passive integrated transponders or "PITs" are increasingly being used in conjuntion with, or replacing, more traditional methods of individually marking animals. They are most commonly used in fish (for example Armstrong *et al.* 2001) but are increasingly being used for marking crustaceans (Bubb *et al.* 2002), amphibians (Brown 1997), reptiles (Boarman *et al.* 1998), birds (Jamison *et al.* 2000) and mammals (Braude and Ciszek 1998). Transponders are small (approx. 11 x 2mm) and consist of an electromagnetic coil and microchip sealed in a biologically inert glass capsule. When an external PIT-reader emits a low-frequency signal, this activates the transponder and it transmits a unique code back to the reader for display (for more details see the review by Gibbons and Andrews 2004).

While PITs generally offer a reliable and safe permanent mark, they can fail, most commonly due to them falling out of the insertion hole soon after implantation (Gibbons and Andrews 2004). Transponder loss rates from animals vary greatly between studies with it ranging in fish from 0.004% (Dare 2003) to 12.1% (Feldheim *et al.* 2002), and in birds from 5% (Jamison *et al.* 2000) to 41% (Becker and Wendeln 1997). While PIT implantation generally appears to have no significant effect on growth rates or survival (Carver *et al.* 1999; Jamison *et al.* 2000; Bubb *et al.* 2002), death associated with insertion of the PIT needle (Jackson and Bünger 1993) and transmission and exacerbation of bacterial infections associated with implantation (Elliott and Pascho 2001) have been reported.

Insertion technique and inappropriate post-insertion management are commonly blamed for PIT loss, however, the relative importance of factors influencing PIT retention and potential complications remain uncertain in many species (Gibbons and Andrews 2004). The majority of research addressing these questions has focussed on commercial aquatic species (for example see Elliott and Pascho 2001; Feldheim *et al.* 2002; Dare 2003), with 82/95 research papers retrieved by the authors via the computer database

search engine 'Web of Science' (The Thompson Corporation, USA) using the search term "passive integrated transponder" focussing on fish and crustaceans. While some research to address these questions has been undertaken on birds (for example Becker and Wendeln 1997; Clarke and Kerry 1998), the current research bias does highlight the relative lack of information available regarding PIT use in birds. Thus, as has been recommended for studying potential negative impacts of leg-banding or 'ringing' in birds (Calvo and Furness 1992), PIT-tagging should be critically reviewed in conjunction with the current research programmes utilising it. In this paper we describe our experiences in using PITs in the critically endangered endemic New Zealand ground parrot, the kakapo *Strigops habroptilus*.

The kakapo is intensively managed by the New Zealand Department of Conservation and, until recently, all birds were individually identified with a numbered stainless-steel leg band. A decision was made to remove all leg bands after concern was raised that their presence may predispose some birds to injury and the development of tarsometatarsal ankylosis (fused hock joint) in the banded leg ($n = 5$). Even though all birds are fitted with a backpack-mounted transmitter for individual monitoring, a safe permanent method of identification was sought in the event of transmitter failure. It was decided that subcutaneous implantation of a PIT was the best option available for individual identification. To evaluate the impact and efficacy of this change in management we sought to answer two questions. First, are PITs effective as a long-term marking tool for individual identification of kakapo? Second, is there any evidence of health consequences arising from their use in this species?

Methods

Study species

The kakapo is a large flightless nocturnal parrot endemic to New Zealand. Adult kakapo weigh between 1.2 and 3.5 kg with females weighing generally less than males. While they spend most of their time on the ground, they are excellent climbers and feed and roost in trees where their wings are used for balance. The introduction of exotic mammalian predators contributed to the extinction of the kakapo on mainland New Zealand by the 1970's. Its population is now restricted to 86 individuals on three offshore islands and is managed by the National Kakapo Team, a division of the New Zealand

Department of Conservation. The location of all birds is regularly monitored and each kakapo is caught at least once yearly for a health examination and to replace its backpack transmitter. While each transmitter broadcasts a signal unique to the individual bird, a permanent tag is also used as an identification backup in case of transmitter failure; in previous years individually-numbered leg bands have been used. If breeding occurs, all nests are located and an intensive monitoring effort is undertaken to ensure chicks and adult females are healthy by primarily monitoring their weight.

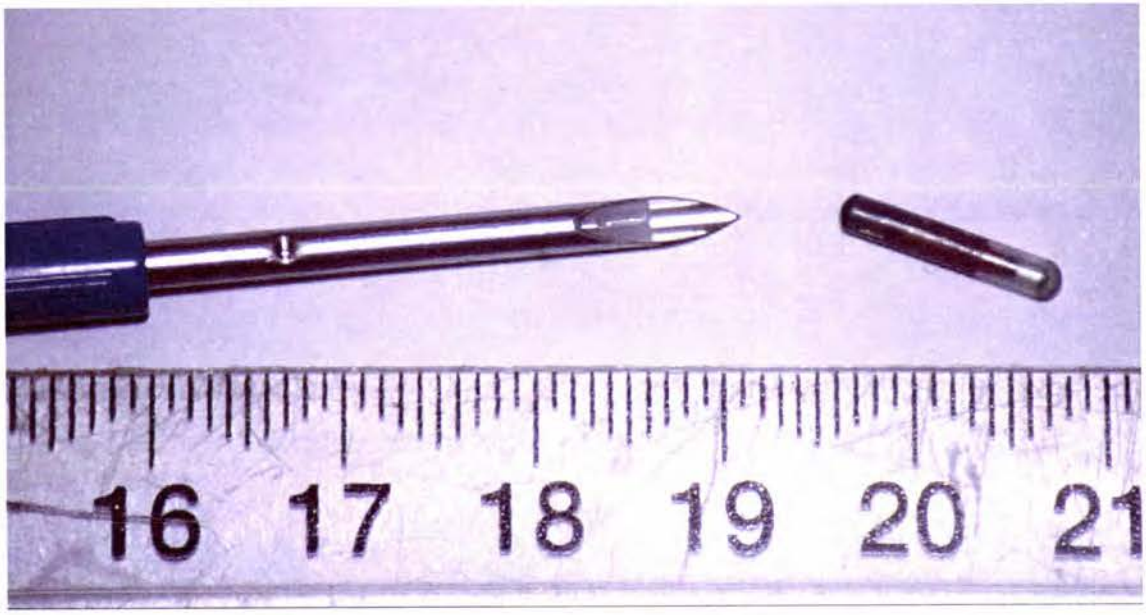

Fig. 1. Allflex™ transponder and insertion needle as used in this study for subcutaneously tagging kakapo.

PIT implantation

We used Allflex™ glass-encased implantable transponders (11.5 x 2.1mm; Fig. 1), and the associated battery-powered compact hand-held reader for detecting transponders in the field (Fig. 2). These hand-held readers are not powerful and require close approximation to the PIT (usually < 50mm) and a fully-charged battery to register the code. With one exception, all adult kakapo ($n = 61$) were caught between February and August 2001 and at this time had PITs implanted. Implantation was undertaken during routine health examinations when each bird was caught while roosting during the day. During the procedure, birds were held in an upright position with their legs and head restrained by a second person.

Fig. 2. Hand-held compact Allflex™ PIT-reader as used in this study. All PITs were scanned immediately post-implantation to ensure they were working and had not accidentally been withdrawn with the needle. At the left of the featherless area, the insertion hole can be seen (arrow).

Transponders were inserted subcutaneously with the associated sterile 12-gauge needle after the implantation site was located and cleaned with an alcohol swab. The site chosen for implantation was a loose-skinned featherless area located dorso-laterally at the right-hand base of the bird's neck (Fig. 3). The needle was inserted in a caudal to cranial direction under the skin for a distance of approximately 20mm before the transponder was injected and needle withdrawn (Fig. 4). On completion of the procedure, the transponder was visualised under the skin to ensure that it had not been accidentally withdrawn with the needle. All transponders were checked to be working before use and the implantation site scanned and checked after insertion to ensure the PIT was still functioning (Fig. 2).

Fig. 3. Dorsal view of an adult kakapo showing the featherless implantation site at the right hand side of the neck (arrow). This is the standard restraint position for an adult kakapo during routine health checks and transmitter changes, and it allows easy access to the implantation site.

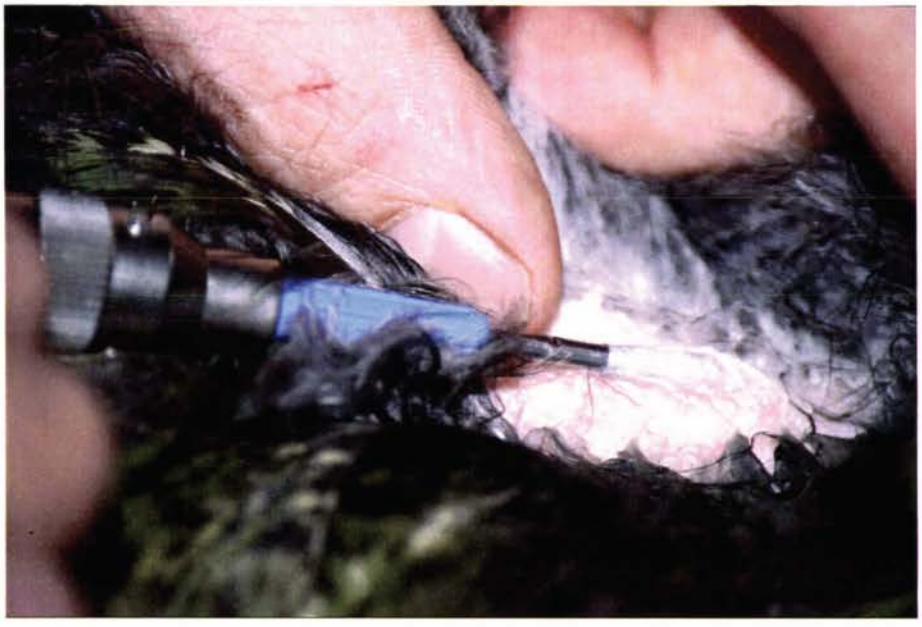

Fig. 4. Subcutaneous insertion of the needle containing the PIT immediately prior to implantation.

Between April and June 2002 all parent-reared chicks from the current breeding season (n = 21) had transponders inserted before fledging, at an approximate age of 35 days after hatching and a weight range of 0.9 - 1.8 kg (mean = 1.2kg). The same method used for implanting PITs in adult birds was used when implanting chicks, although chicks received their implant at night when the adult female was absent from the nest.

PIT assessment

All chicks were examined and weighed daily until two weeks of age and then every second day until fledging at approximately 70 days old. The rate of growth for each chick before and after implantation was compared to determine if PIT implantation affected growth rate. In all chicks, the PIT implantation site was visually inspected 48 hours post-implantation (Fig. 5). Adults were not rechecked immediately after implantation, as capture of the adult birds is stressful and logistically costly, with handling of these birds generally kept to a minimum.

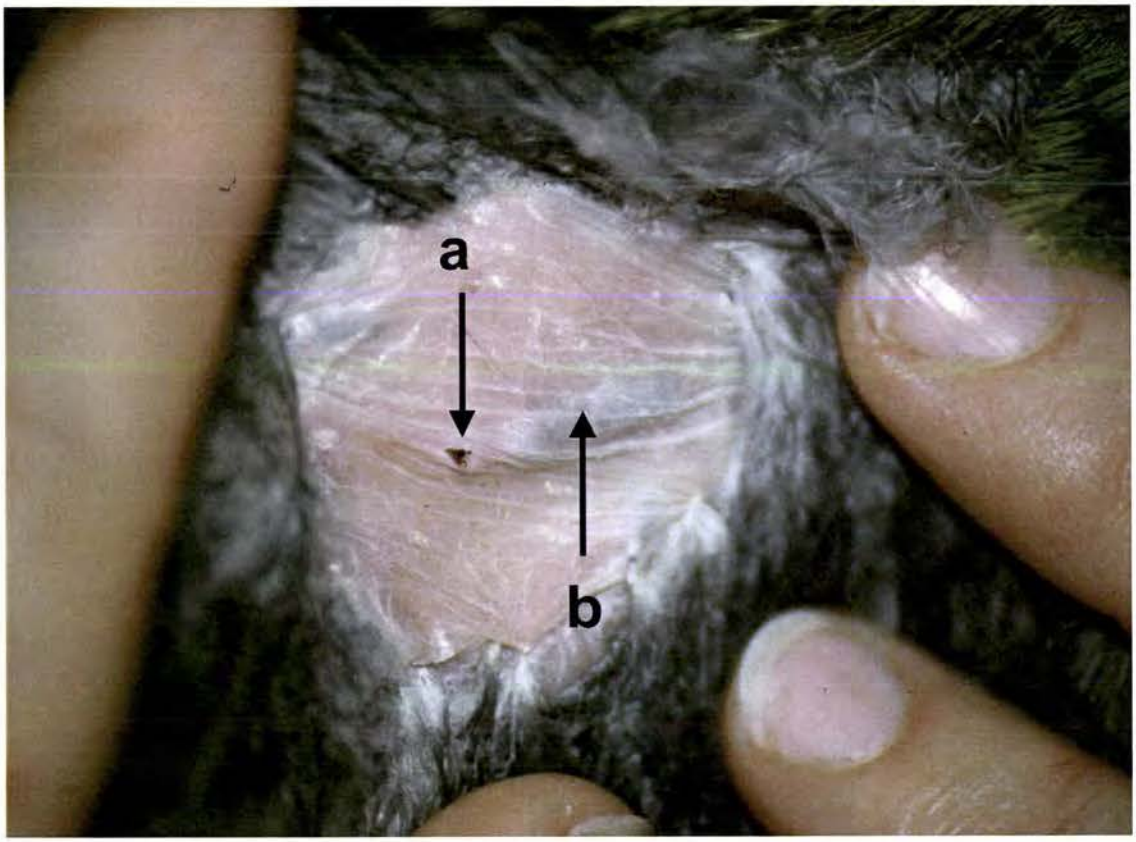

Fig. 5. View of the implant site 48 hours post-implantation. The local area shows no signs of inflammation or irritation, with the only evidence of the procedure being **(a)** a small scab at the site of insertion, and **(b)** the PIT visible under the skin.

PITs were assessed in 57 adults (males = 32, females = 25) during routine health checks at a mean time after implantation of 16 ± 3.3 months (mean ± SD), and in 21 parent-reared chicks at 4 ± 0.5 months (mean ± SD) post-implantation. During this assessment, four pieces of information were collected about each PIT. First, whether the PIT was present or absent. This was determined by using a PIT-reader to scan the implantation site. If the PIT was not detected within 30 seconds, a scan of a wider area over the bird was undertaken followed by a visual and tactile assessment to locate the PIT. Second, the time taken for the reader to display the PIT code. Third, the position of the PIT relative to the implantation site. This was to determine the degree of internal PIT-migration and was measured in two ways. The position of the hand-held reader was recorded when the PIT-code was registered, as well as a visual assessment of the implantation site and the surrounding area. Finally, the site where the PIT was implanted was examined for any pathology as well as recording the general condition of the bird.

Analysis

While all PIT-tagged kakapo were recaptured during the study, some data were not collected during the routine health examinations by field staff, resulting in lower values than the total number of PITs in some analyses. For the comparison of chick growth before and after implantation, a paired parametric test was used, as both data sets were normally distributed (Shapiro-Wilks $P > 0.05$). Means are expressed with standard errors unless otherwise stated.

Results

Adults

PIT implantation added less than a minute to the routine health check being concurrently undertaken, with the time from insertion of the needle to its withdrawal being less than 5 seconds. Bleeding was not a significant problem during implantation; blood vessels in the skin were easily seen and avoided. Surface capillary bleeding associated with the penetration of the needle was occasional and was quickly stopped with the application of direct pressure to the site. Pain associated with subcutaneous implantation in this region appeared to be minor, as most birds did not react to the insertion of the needle.

When assessed 16 months post-implantation, all transponders were detected by the reader at the implantation area (right shoulder) indicating that no PIT loss had occurred and any internal migration was minimal. In over half of the birds ($n = 35$), visual confirmation of the transponder was sought and of these 71% ($n = 25$) had the transponder visually confirmed under the skin in the featherless implantation area. No skin discolouration or pathology was detected in the vicinity of the implant and the general condition of all birds was considered to be normal. With the exception of one transponder, all ($n = 56$) were detected in less than 5 seconds of activating the reader (93% of PITs were detected immediately). The one transponder that failed to read in that time was visible at the implantation site and required approximately 60 seconds to register. It is likely that this was due to a low battery in the reader, as the PIT responded immediately when rechecked 4 months later using a fully charged PIT-reader. No mortality of kakapo occurred during the period of this study.

Chicks

The weight gain for all chicks in the 48 hour period prior to implantation (75 ± 12g) was not significantly different to their weight gain in the 48 hours after implantation (72 ± 15g; paired *t*-test, $t = 0.15$, d.f.= 19, $P = 0.87$). All chicks had their implantation sites visually checked 48 hours post-implantation and no infection or complications were noted during any of these inspections (see Fig. 5). In August 2002, an average of 4 months post-implantation, all transponders ($n = 21$) were detected within 5 seconds of activating the reader and were located at the site of implantation. No chicks died post-implantation and no pathology was noted at the implant site.

Discussion

Evaluating PIT tagging in kakapo

Gibbons and Andrews (2004) identified seven factors to be considered when selecting a method to individually mark animals. They recommend that the mark should be: 1) unique to each individual, 2) neutral in its behavioural, physiological and health consequences, 3) easily readable, 4) easily attached with minimal handling of the animal, 5) permanent for the period of study, 6) reliable, and 7) of relatively low cost. The individually coded passive integrated transponders used in this study satisfied all seven of

these recommendations and thus appear to be an excellent way of permanently marking individual kakapo. While it has been pointed out that the cost of PITs in large-scale projects may be prohibitively high (Gibbons and Andrews 2004), for a well-funded project targeting a small number of individuals, such as the kakapo recovery programme, the costs of PIT tagging are negligible.

We found no significant effects of PIT tagging on the health of kakapo as measured by changes in growth rate, mortality or evidence of pathology at the implant site. This was likely to be, in part, because of our decision to implant the PIT subcutaneously rather than intramuscularly; a site often recommended for birds (Elbin and Burger 1994; Ritchie *et al.* 1994; Rupley 1997). While intramuscular implantation may provide better PIT security, we deliberately avoided this insertion route as the kakapo has a significantly reduced pectoral muscle mass and we wanted to minimise any implantation associated trauma. Kakapo PIT implantation occurred in a standard position on the bird for ease of finding the transponder with the reader in future. This site was selected for its ease of access during standard restraint; loose skin for simple and quick subcutaneous implantation; lack of feathers to minimise pain, bleeding and the introduction of foreign material; and distance from vital structures and the backpack transmitter (Figs. 3, 4). The lack of significant migration of any PITs during this study suggests that this site provides an effective long-term standard marking and reading site on the birds.

The only problems encountered with reading PITs in the field appeared to be when the PIT-reader was powered by an old battery. The reader still functioned but it took longer to register the presence of a PIT than when the battery was new. This is likely to account for the one PIT that took over 60 seconds to register during the study but registered immediately when a PIT-reader fitted with a new battery was used to reread it 4 months later. Because of this, we recommend that a new replacement battery is carried into the field with all PIT-readers: to be fitted as an additional check in the event of a PIT failing to register.

Factors influencing PIT retention

No loss or significant migration of transponders has been detected in kakapo since their introduction approximately 23 months ago. This is unusual, in that most published studies document PIT failure at a rate higher than 0%. In birds, PIT loss rates are highly variable between studies (14%, Jackson and Bünger 1993; 21%, Elbin and Burger 1994; 4 – 47%, Becker and Wendeln 1997; 5%, Carver *et al.* 1999; 5% Jamison *et al.* 2000), with this variability also reflected in studies in non-avian species (0 – 50%, Freeland and Fry 1995; 5%, Harper and Batzli 1996; 0%, Bubb *et al.* 2002; 12.1%, Feldheim *et al.* 2002; 0.2%, Gries and Letcher 2002). Differing rates of PIT loss between studies and species suggests multiple factors are involved in influencing rates of PIT retention.

The majority of PIT loss occurs during or soon after implantation (Becker and Wendeln 1997; Renner and Davis 2000; Dare 2003), with the PIT being expelled out through the needle insertion hole before wound healing can adequately seal it off (Freeland and Fry 1995; Gibbons and Andrews 2004). The likelihood of a PIT being lost soon after implantation appears to be dependent on at least three factors. First, is the depth of PIT insertion. It is important that the entire PIT is contained within the animal and that it is as far as is practical from the insertion hole. Transponder loss is reported to occur because: the PIT fell out of the needle prior to insertion (Galimberti and Sanvito 2000), a portion of the PIT remained outside of the animal due to incomplete insertion (Hagen 1996), and the PIT migrated towards the insertion hole as the needle was withdrawn (Freeland and Fry 1995). The depth of PIT insertion is likely to be affected by operator experience as well as the size and temperament of the animal (Jamison *et al.* 2000). This makes it important for operators of this technology to be well practised at using the 12-guage needle implanters and to check the location of the PIT immediately post-implantation. If necessary, finger pressure can be used to move the tag away from the insertion hole (Freeland and Fry 1995). Obviously in a large bird such as the kakapo the needle can be advanced a relatively long way before the transponder is ejected, minimising the chance of it falling out of the insertion hole before healing has sealed it off. This is also the case in chickens and turkeys and may explain at least some of the differences in success between these species and smaller birds (for example see Becker and Wendeln 1997 versus Jamison *et al.* 2000).

The second factor influencing PIT retention is the location of implantation. Becker and Wendeln (1997) reduced PIT loss from 17% to just 4% by changing the site of

subcutaneous implantation in the common tern, *Sterna hirundo*, from the bird's back to its breast. While the ideal implantation site varies between species (Gibbons and Andrews 2004) the reason for why one site is superior to another, and why some species have higher rates of PIT loss, is not always clear. One possibility that we propose is that differences in PIT retention are correlated with the mobility of the skin and muscles around the implantation site. Boisvert and Sherry (2000) suggest that subcutaneously implanted PITs in black-capped chickadees, *Poecile atricapillus*, were lost because flight movements caused the PIT to move under the skin. One common feature of bird studies where PIT loss has been minimal is that those birds are ground dwelling (for example: quails, Carver *et al.* 1999; chickens, Jamison *et al.* 2000; kakapo, this study). It is possible that significant movement of musculature around the area of the transponder, in particular structures associated with flying, increase the chances of the transponder being ejected out of the insertion hole. This idea is supported by Freeland and Fry (1995) who found in a study of 14 mammal species transponders were only lost from species that fly or glide, and that these loss rates were not related to the animals' size.

The final factor affecting PIT retention is the post-implantation management of the insertion hole. To prevent external migration of the PIT soon after implantation the wound can be sealed immediately after the needle is withdrawn. Becker and Wendeln (1997) improved PIT retention in the common tern from 59% to 83% by using surgical glue to seal the insertion hole. Because of the low rate of PIT loss in kakapo, this additional measure of PIT security is unnecessary in this species.

The kakapo appears to be an excellent species on which to use PIT technology, as it is a large ground dwelling species and the implantation site is an easily accessible, loose skinned, featherless area. For kakapo management purposes, the use of subcutaneous passive transponders provides a safe, reliable and cost effective means of identifying individual birds; no complications have been noted and no loss or significant transponder migration has occurred. The relative importance of the factors influencing PIT retention is still poorly understood and it is unknown how they might vary between species. Thus we recommend an experimental approach be adopted in the future to ascertain methods of PIT insertion that maximise PIT retention in the long-term.

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CHAPTER II

Leg problems and banding-associated leg injuries in a closely monitored population of North Island robin (*Petroica longipes*)

Characteristic one-legged perching posture of a North Island robin suffering a band-entrapment injury. Here the bird has its left hallux caught between its leg and a colour band, and cannot place the foot to the ground.

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Abstract

Although plastic and metallic leg bands are widely used for identifying individual birds to assist population monitoring, the health risks associated with banding are quantified relatively rarely. We recorded the general occurrence of foot and leg injuries during a four-year study of the North Island robin (*Petroica longipes*) and assessed the probability of banding-injury relationships. While most leg problems were not obviously related to banding (transient lameness, congenital deformity, infection, fracture), on 10 occasions individuals experienced lameness or injury directly because of the presence of bands (~2.5% of individuals per year). In eight of these instances, individual robins caught their back toe (hallux) in between a band and their tarsus. This resulted in an inability to place the affected foot on the ground, and in some cases a pedal injury. We believe that this previously undescribed toe entrapment is made possible because of the robin's sideways perching behaviour on upright vegetation. This highlights that relationships between leg banding and injury may be species-specific and that the impacts of banding should be identified and quantified in all species in which it is used. This will allow more accurate assessments of the risks and benefits associated with this common marking technique.

Introduction

Identifying individual birds by marking them with coloured or metallic leg bands has been widely used by researchers for decades (reviewed in Calvo & Furness 1992). These bands are typically placed on the tarsometatarsus because the bands are easily applied here, it allows identification of birds from a distance and it is generally considered to be safe for the bird (Lowe 1989). While leg bands are an important research and conservation tool, their presence may not always be benign. Colour banding has been noted to affect mate attractiveness and mate choice in a number of species (Burley *et al.* 1982; Johnsen *et al.* 1997; Hansen *et al.* 1999). While the potential for these behavioural biases are often actively investigated, the possibility of band-induced injuries is acknowledged relatively rarely despite evidence suggesting that complications associated with marking animals may be widespread.

In wading species, banding injuries are reported to occur at a rate of 2-3 % of resighted birds in the years following banding and to be more likely associated with the presence of a metal band (Reed and Oring 1993; Amat 1999). In passerines, band injuries are poorly documented but have been reported to occur in as much as 9.6 % of the banded population, with most injuries associated with colour bands (Sedgwick & Klus 1997). Complications associated with leg-bands in birds include irritation and discomfort (Rose 1997; Sedgwick & Klus 1997), entanglement in vegetation (Bart *et al.* 2001), abrasions and open sores (Dilks & O'Donnell 1993) and complete amputation of the foot (Reed & Oring 1993; Sedgwick & Klus 1997; Amat 1999; Taylor & Castro 2001; see also the review by Calvo & Furness 1992). Alternative marking techniques in birds such as neckbands and collars (Castelli & Trost 1996; Schmutz & Morse 2000), and wing or flipper tags (Barlett & Rusch 1980; Froget *et al.* 1998) as well as the use of bands in other flying species such as bats (Baker *et al.* 2001) have also been associated with adverse effects. Because of the relative paucity of studies into the potential effects of marking, Calvo and Furness (1992) recommend that such work should be carried out in association with current research programmes.

The North Island robin (*Petroica longipes*) is an endemic New Zealand passerine that is currently the focus of numerous population-management projects in an attempt to mitigate some of the effects of introduced exotic predators. During a four-year behavioural study of an isolated island population of this species, data were also collected

on the incidence of leg and foot problems. In this study we paid particular attention to the role bands played in initiating or exacerbating the observed conditions. Here we describe our findings including a previously undescribed presentation of band-induced leg injury.

Methods

Species and study area

The North Island robin displays weak sexual dimorphism in both size and colour, is long-lived (12+ years) and sedentary, with monogamous long-term bonds and year round territories (Higgins and Peter 2002; Armstrong *et al.* 2000). Robins are insectivorous and spend a large portion of their time perching sideways on upright vegetation or hopping along the forest floor searching the leaf litter for prey. This feeding strategy is reflected in their upright posture and their disproportionately long legs (tarsometatarsus length = 36.2 ± 0.15 mm) when compared to body length (159 ± 1 mm) (Å Berggren, unpublished data). Robins are naturally curious and will approach closely, making them easy to observe and catch if necessary.

The study population is located on Tiritiri Matangi Island (36°36'S, 174°53'E), a bird sanctuary situated off the northeast coast of New Zealand's North Island. The population was translocated and established in 1992 and has been monitored by both the New Zealand Department of Conservation and university researchers since that time. An average population size of 97 birds was present on the island for each of the four breeding seasons when observations for this study were collected (September 2000 to February 2004).

Bands and banding

With only one year's exception since translocation, an attempt was made to band all robin chicks and any unbanded adults. This has resulted in a population with a high proportion of banded individuals (97% of the population in 2003). Because of various research projects and management priorities since the birds' introduction, numerous people (> 12) have been involved in banding the birds. Despite this, banding techniques and band types have remained consistent. Birds are generally banded as chicks in the nest with one metal band and three plastic colour bands being placed on the tarsometatarsus (one plastic on the same leg as the metal band and two on the other leg). Aluminium metal bands were

used, except in two years when stainless steel bands were used; both were applied using a standard set of banding pliers. Plastic (celluloid) colour bands are of a 'butt-end' or 'split-type', where the edges of the band opened using a fluted applicator (Lowe 1989) and brought together around the leg rather than being overlapped. According to the standard banding protocol for this species in New Zealand, the colour bands were not acetone sealed but squeezed together to ensure correction of any stretching that occurred during application. In 1992, the original translocated birds had 'C'-sized bands applied (inner diameter 3.5 mm), but after that all birds were banded with 'B'-sized bands (inner diameter 3 mm). Only two individuals during the study's first year were carrying C-bands and one individual was carrying a stainless steel metal leg band. All bands were sourced from the New Zealand bird-banding office.

Observations

All territories on the island were visited, on average, every fourth day during the breeding season (September – March), and on two other occasions (May and July) outside of this time. Playback calls were used to aid in the censuses, to maximise the encounter rate of individuals. When observing individuals any postural abnormalities were noted – as the most common response to a leg injury is to consistently favour one leg. In these cases the birds would be encouraged to move in order to observe the extent and cause of any injury sustained in the favoured leg. This was achieved by supplying mealworms (*Tenebrio molitor*) and observing the birds by using a pair of close focussing 8x30 binoculars as they moved while feeding. In all bird observations the individuals were identified and thereby data on known age and sex were collected. During the first three years of the study, data were collected on all observed instances of lameness and leg injuries. During the fourth breeding season, only data relating to banding injuries were collected.

Injuries

In the few cases where an injury was observed, actions were taken in addition to the regular observations. If a band was caught over a toe, or if there was any sort of wound where it was possible that the band might compromise healing, an attempt was made to catch the bird for examination and band removal on the day of discovery. While most of these birds were caught and treated within 24 h, one bird evaded capture for 17 days. For other less serious injuries or wounds, and any not associated with the band, the bird was

not caught and the progression of the lesion monitored with binoculars until it either resolved or progressed and a decision to catch the bird was made. The incidence of injury types was determined as a percentage of the average population found with that injury per year.

Catching and measurements

Catching involved using either a hand net or a spring-loaded trap. In both cases, mealworms were placed on the ground as a lure, and a net brought down over the bird when it came to feed on them. If a band was determined to be a direct cause of any injury or threatened to compromise wound healing, it was removed using a pair of fine-point circlip pliers. Open sores were cleaned and examined and then treated with a 10 % povidone-iodine solution before the bird was released. If possible, caught birds were weighed (± 0.5 g), and tarsometatarsal length (± 0.05 mm) and wing length (± 0.5 mm) were also measured. All injured birds were closely observed in the weeks after release and in some cases were recaptured to examine wound healing.

Additional captures of non-injured adults and juveniles provided measurements of the hallux, claw, and tarsometatarsus length, width and depth. These were measured using vernier callipers in order to evaluate the size of gap between bands and the leg in this species to help elucidate the aetiology to toe entrapment between bands and the leg.

Ethical note

The New Zealand Department of Conservation was informed of banding injuries during the period of this study and supported the ongoing banding and monitoring of this population. All work undertaken in this study was carried out under a research permit from the New Zealand Department of Conservation and had Massey University animal ethics approval.

Analyses

Where data were normally distributed (Shapiro-Wilks $p > 0.05$), *t*-tests were used for group comparisons. For non-parametric comparisons the Mann-Whitney U test was used. A Fisher's exact test was used to compare expected and observed frequencies of sex and occurrence of leg injury, the presence of the metal band and leg injury, the type of band (metal and plastic) involved in toe entrapment injuries, and whether survival differed

between injured birds and the population as a whole. A Chi-square test was used to assess differences between sexes in the type of injury the bird suffered. Sample sizes vary between some tests, as the sex of four juveniles is unknown and the survival of individuals from the 2003/04 breeding season were not known at the end of the study. Where multiple statistical tests were carried out on the same dataset, a sequential Bonferroni correction was used in evaluating *P*-value significance (Rice 1989).

Results

Types and rates of injuries

In all, 54 individuals exhibited some form of leg injury or lameness during the study period; the injuries of 44 of these could not be directly attributed to the presence of bands (Table 1). The majority of these birds ($n = 32$) exhibited a temporary lameness of unknown cause that manifested as a consistent favouring of one leg during an observation session, which resolved before the bird was next observed. Other problems included swellings or sores on toes ($n = 5$), current or healed leg fractures ($n = 3$), paralysis ($n = 2$) and poor gripping ability ($n = 2$).

Leg injury symptom	*n*	% per year	Likely cause
No evidence of band-induced pathology[A]			
Transient lameness (no lesion)	32	11.5	Unknown
Swelling / sores on foot	5	1.8	Infection
Current / healed leg fracture	3	1.1	Trauma
Poor gripping ability	2	0.7	Trauma / Infection
Semi-paralysed	2	0.7	Congenital
Band-induced[B]			
Back toe caught in band	8	2.0	Band
Band abrasion / laceration	2	0.5	Band

[A]Data collected during 2000-03.
[B]Data collected during 2000-04.

Table 1. Categories of observed leg injuries in the Tiritiri Matangi robin population, 2000-2004

A further 10 birds were lame as a direct result of wearing bands (Table 1). Of these, eight were because of the hallux (back toe) was caught in between a band and the bird's leg (Fig. 1a). This resulted in the tarsometatarsal joint being permanently forced into a flexed position and prevented the bird from placing that leg on the ground. Another two birds became lame due to long-term wear on a plastic colour band (both of which had been applied six years previously) resulting in it either partially slipping inside a second band and causing pain due to pressure and abrasion, or it cracking and opening, with the lower edge of the band being forced into the dorsal surface of the foot.

Causal factors in leg injuries

Observed leg injuries occurred evenly throughout the breeding season. The age of injured or lame birds (3.40 ± 0.38 years) did not differ significantly from the age of birds in the general population (3.28 ± 0.19 years) (Mann-Whitney U test, $z = -0.10$, $n = 211$, $P = 0.92$). We could not detect any significant difference between the ages of birds with the various injury classes and the general population (Mann-Whitney U, all $P > 0.05$). However, any real effect of age on injury type could be masked due to the large variance and small sample sizes for the different injuries being tested. Females ($n = 23$) were just as likely as males ($n = 24$) to experience some type of leg problem (Fisher's exact test, $p = 0.99$). The sexes did not differ in the type of injuries exhibited ($\chi^2 = 4.85$, $n = 47$, $P = 0.43$). However, females were younger (2.7 ± 0.56 years) than males (4.2 ± 0.54 years) when suffering from a sore leg (Mann-Whitney U, $z = -2.18$, $n = 47$, $P = 0.029$).

There was no correlation between the presence of the metal band on the injured leg with any type of problem (Fisher's exact, $P > 0.99$). This was also the case when the subset of temporary lameness was tested ($P = 0.36$). The significance of the result was not altered if the position of the metal band on the leg (above or below the plastic band) was considered ($P > 0.99$).

Seven out of the eight bands that caught birds' back toes were plastic, though this did not represent a significant bias towards plastic bands being involved in this type of injury – three of every four bands on every bird are plastic (Fisher's Exact test, $P > 0.99$). In all but one case it was the distal (lower) band that caught the toe. In one bird, the distal band was bypassed and the toe was caught underneath the proximal band. In both plastic and metal bands that caught toes, the band was properly positioned and had not opened in

any way to explain why these individuals would be more susceptible. Six of these birds were female and the birds were of varying ages (see Table 2).

For leg and toe morphometrics, adult toe, nail and leg measurements did not differ from juveniles (Mann-Whitney U, n= 24, all $P > 0.05$). The nail length was 8.57 ± 0.07 mm, toe length 9.34 ± 0.13 mm, leg width 1.54 ± 0.02 mm and leg depth 2.14 ± 0.03 mm. This gave a space of 0.86–1.46 mm between the band and the bird's leg to catch the nail and toe, with pressure from the band under these conditions potentially affecting the lower half of the tarsometatarsus. In captured birds, the rear toe was quite easily extended along the leg when the leg was positioned at a similar angle as is found when perching in the sideways-on-trunk position. From here it was quite easy to move a band over the nail and onto the toe.

Sex	Age	Band type	Band location	Toe pathology
F	1	Plastic	Distal	***Minor irritation to ventral surface***
F	3	Plastic	Distal	Minor irritation to ventral surface [A]
F	3	Plastic	Distal	None [B]
F	1	Plastic	Distal	Severe swelling and ulceration
M	1	Plastic	Proximal	None [B]
M	7	Metal	Distal	Severe swelling and ulceration
F	1	Plastic	Distal	Severe swelling and ulceration
F	2	Plastic	Distal	None

[A] Found dead
[B] Self-corrected

Table 2. Details of the eight birds that trapped their hallux (back toe) between a band and their tarsometartarsus

Effects of injury

Body measurements (weight, tarsus and wing length) in injured and lame males, females and juveniles were compared with the same sex and age group of non-injured birds for which we had data. We found no significant differences between the two groups (injured and healthy) (*t*-test, all $P > 0.05$). Yearly survival of injured or lame juveniles (0.21) and adults (males 0.96 and females 0.75) did not differ significantly from the known percentage of yearly survival of the general population (juveniles 0.35, males 0.81 and females 0.76, Fisher's exact test, all $p > 0.05$) (Armstrong and Ewen 2002; Å Berggren unpublished data). However, this lack of effect on survival was probably influenced by our quickly remedying the cause in band-induced injury cases.

Birds with their back toes caught between the band and the leg displayed a range of injury severity (see Table 2). The length of time that a bird had had the toe caught did not completely explain the seriousness of the injury, as birds with no lesions had their toes caught for at least 2–17 days, and birds with minor to severe lesions (Fig. 1*b*) a maximum of 21 days (i.e. the bird had not been seen for the 20 days prior to the discovery of its band-injury). With the exception of two birds, the affected toe and leg recovered complete function within two weeks of removal of the band. One bird recovered function in the foot but lost the distal half of the toe and associated claw (Fig. 1*c*). A second bird was lame for several weeks after the band was removed and, while the toe survived, there was evidence of reduced function due to scarring or nerve damage.

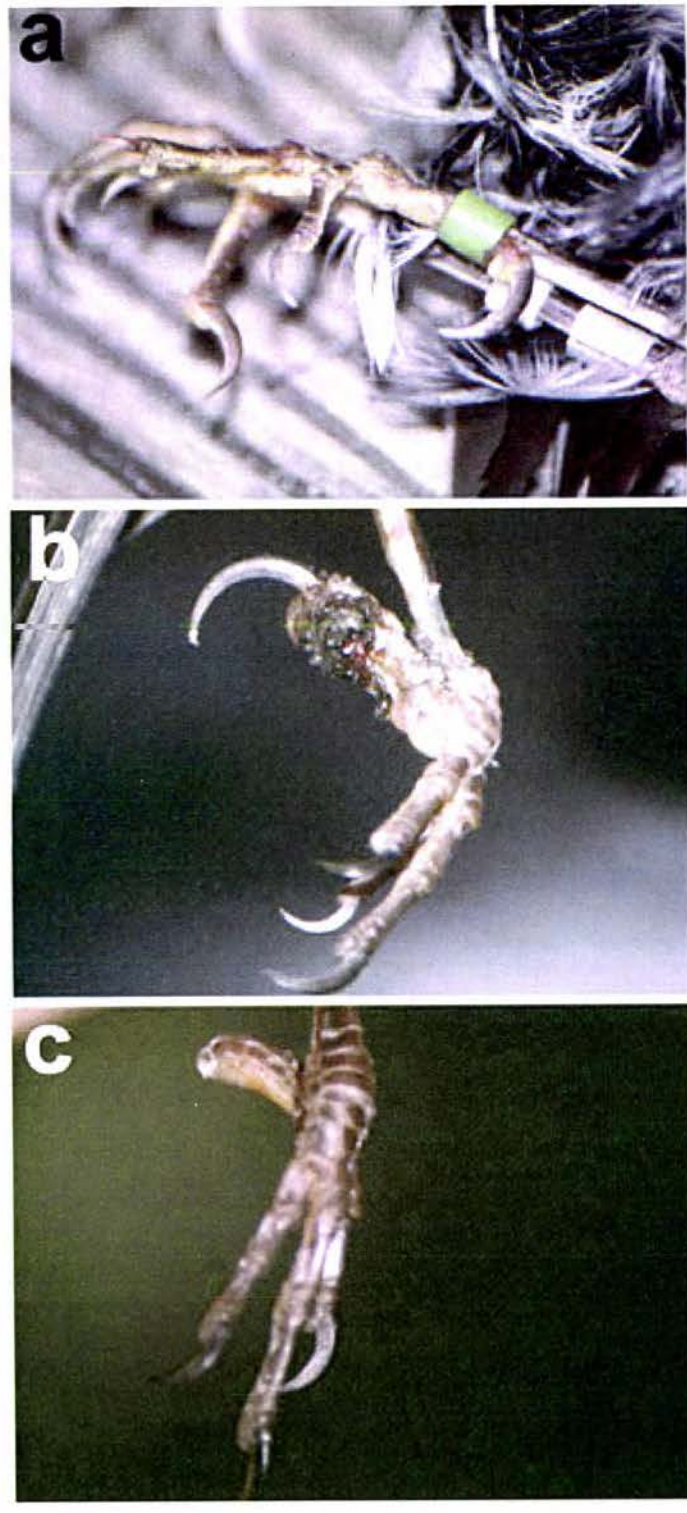

Fig 1. Presentation and sequelae of a band-related hallux-entrapment in a North Island robin. **(a)** Rear-pointing toe caught between a band and the tarsometatarsus (arrow). **(b)** Ulceration and swelling of the rear toe as a result of being trapped between the band and leg. **(c)** Distal toe amputation as a complication of pressure necrosis on the rear toe.

Discussion

Investigations into the negative health impacts directly attributed to leg banding are relatively few considering the number of research and management projects that rely on banded individuals (Calvo & Furness 1992; but see Reed & Oring 1993; Gratto-Trevor 1994; Brooker & De Rebeira 1996; Rose 1997; Sedgwick & Klus 1997; Amat 1999; Bart et al. 2001). In New Zealand, reports of adverse effects of bird banding appears limited to unpublished reports and documents that are difficult to source and thus lack a wide readership (Dilks & O'Donnell 1993; Taylor & Castro 2001). If this phenomenon occurs more widely through either a reluctance of the researcher or of international journals to publish banding-injury data, this might foster a general perception that adverse affects from banding are much lower than the reality.

Most reported studies of banding injury have relied on numbers of resighted birds in migratory populations (Reed & Oring 1993; Gratto-Trevor 1994; Brooker & De Rebeira 1996; Sedgwick & Klus 1997; Amat 1999). The figures quoted in these studies might significantly underestimate band-induced problems as many more birds potentially could have suffered band problems during migration and did not survive to reach the breeding grounds. One of the strengths of our study is that we were able to continuously monitor all individuals in the population over a long period, allowing us to accurately evaluate the relative incidence of various foot and leg problems.

Most leg and foot conditions encountered during this study appeared unrelated to the presence of bands. Foot sores, transient lameness and evidence of leg fractures have also been observed in unbanded individuals in this and other North Island robin populations (Å Berggren, unpublished data; R Lewis, R Boulton and Y Richard personal communication). However, further work is required to determine if these conditions occur more frequently in banded individuals. This study also shows that when examining the effect of banding on birds, time is an important factor. The likelihood of finding injured birds, even if they are present, is very small in short-term studies or occasional surveys (Weiss & Cristol 1999) and under these conditions the negative effects of banding may be underestimated.

Banding injuries in the North Island robin

The most surprising result from this study was the relatively high number of birds ($n = 8$, or 2% per year) that managed to entrap their hallux within a leg band, a seemingly impossible achievement. Two of these birds self-corrected before intervention, suggesting that a number of additional instances of temporary toe-entrapment might have gone unobserved. While the possibility exists that some birds suffered chronic toe-entrapment during the winter months when the population was not regularly monitored, the very chronicity of the problem suggests that most or all cases during this time would have been detected during the May and July surveys. Thus 1.5% appears to be a reasonably accurate estimate of the numbers of birds that suffer chronic toe-entrapment per year within this population, with this figure rising to 2% if other banding injuries are included.

The effect of a toe becoming trapped in a leg band impacts on the bird in two ways. The first is the acute problem of the leg becoming effectively useless as it cannot be placed on the ground, and thus limiting the bird's ability to forage and perch. The second is more chronic, and relates to progressive negative effects on the bird's health. It appears that the pressure of the band can cause swelling of the distal toe and this, in turn, exacerbates the problem and results in further swelling and ultimately pressure necrosis and ulceration of the ventral hallux (Fig. 1*b*). In a situation where the band is not removed, such as in an unmonitored population, we expect a number of sequelae. Infection of the foot may result in ongoing foot problems and increased morbidity and mortality. Ongoing pressure to the ventral toe is likely to result in partial or complete toe amputation (Fig. 1*c*) and possible foot amputation if the swelling under the band compromises blood supply to the foot. This is similar to the cause of foot loss in wading species where an accumulation of mud and debris under the band compromises circulation to the foot (Amat 1999). Under this scenario, the individual would ultimately correct the condition, but at the expense of a loss of function to the foot or the foot itself.

Cause, impact and management of banding injuries in robins

We hypothesise that the robin's foraging and perching behaviour makes it unusually susceptible to toe-entrapment as described in this study. When foraging and engaging in territorial behaviour, robins often perch sideways on upright vegetation (Fig. 2). If the trunk of the tree is large, the toes are completely extended in the uppermost foot and the

tarsometatarsus lies alongside and parallel to the back toe. It appears that only under these circumstances could the band could catch on the nail and slip down, entrapping the toe. Compared with many other birds, robins are unusual in that they would find themselves adopting this posture tens to hundreds of times per day. We believe that it is because of the robin's sideways perching behaviour that this type of band injury becomes possible. During the four-year study, one stitchbird was also found with the same problem, a back toe caught in a plastic colour wrap-around band. Stitchbirds will also adopt a similar posture when foraging for insects, although not nearly as commonly as robins. We recommend that birds with similar perching habits be closely looked at to see if this type of band problem occurs in other species and whether it is correlated with perching behaviour.

Figure 2. A North Island robin in a common perching position. We believe that it is during this time it becomes possible for the rear nail and toe on the upper leg to become trapped between the band and the tarsometatarsus.

The ten birds found with band-induced lameness were banded over a space of eight years (from 1995 to 2002), involving six different experienced bird-banders. Toe-entrapment occurred with both colour and metal bands, and in all cases the bands responsible were found to be normal in all respects and were completely closed at the time of investigation and removal. The two remaining cases of the band injury were due to the colour bands becoming worn and brittle, allowing them to slip and induce problems through pressure and abrasion. Thus it appears that banding technique is not at fault in these cases and thus a technique modification such as acetone-sealing the bands (Lowe 1989), would not solve the problem. It is probable that acetone-sealing would exacerbate complications from toe-entrapment in ~25% of cases, as those birds that otherwise would have self-corrected by forcing their hallux through the split in the band would now be prevented from doing so.

Currently there is no agreed upon acceptable level of band-induced injury in birds. For bats, a group of animals with very high levels of band injuries, Baker *et al.* (2001) suggest that recaptures with a higher than 2% band injury rate are unacceptable. While the cumulative figures for banding injury per year in the North Island robin are 2%, and thus could be considered 'acceptable', we believe that consideration of its impact on both population viability and individual welfare needs to be carefully considered. It is unlikely that the viability of the Tiritiri Matangi population is significantly affected by a morbidity factor affecting such a small proportion of the birds, especially since the majority of juveniles die because of density dependence effects due to a lack of available habitat for juveniles to disperse into and colonise (Dimond 2001). However, in marginal mainland populations or establishing translocated populations the impact of banding injuries may not be so easily absorbed by the population. It needs to be realised that while many band injuries do not directly kill individuals, they may reduce the ability of a parent to invest in offspring and reduce the reproductive success of that pair.

Alternative marking methods have either been shown to have significant problems in other species (e.g. neck and wing bands), are inappropriate for observational work (e.g. passive integrated transponders) or lack permanence (e.g. colour dying) (Calvo & Furness 1992). Because of this we do not advocate changing the current method of marking this species just for change's sake. Primarily because of welfare concerns for individual birds, we recommend that researchers and conservation managers working with this species consider a number of general banding guidelines. Firstly, robins should only be banded

when necessary to answer scientific or conservation questions that cannot be answered adequately with an unmarked population. Second, acetone-sealing of split bands should not be adopted as it is possible that this will prevent some self-correction of toe-entrapment. Third, in long-term studies, replacing worn or old (> 4 years) bands at times of recapture should be done routinely to minimise the likelihood of worn bands causing pressure or abrasion problems. Fourth, all researchers and managers need to be made aware of the propensity for individuals of this species to trap their back toe within a band, and regularly monitor all banded individuals, paying particular attention to any bird favouring one leg. Finally, a cost-benefit analysis should be undertaken at the end of any investigation using banded individuals to assess whether the birds should be caught and the bands removed.

Identifying and quantifying risks with research techniques needs to be undertaken proactively by researchers and should be encouraged by the research community and management organisations (Calvo & Furness 1992). This will allow the improvement of techniques, and if risks are still present, mechanisms can be put in place to improve animal welfare. From this position, better-informed decisions can be made regarding the value, risks and future use of banding as a research and management technique.

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CHAPTER III

Hematology and biochemistry reference intervals for the kakapo *Strigops habroptilus*: generation and interpretation in a field-based wildlife recovery program

Marama at approximately 80 days of age – a kakapo chick requiring hand-rearing at Burwood Bush near Te Anau during the 2002 breeding season.

Chapter reference:
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Abstract

The purpose of this study was to generate a set of hematological and biochemical reference values for the critically endangered kakapo (*Strigops habroptilus*) and assess the effect of blood storage, processing and bird parameters on interpretation of these values. The majority of the world's kakapo population was sampled in both 1996 and 2002. The medians and variances of twelve hematological and eight biochemical parameters collected in both sampling periods were compared. Handling and processing of samples differed between the two sampling periods contributing to significant differences in the medians for two hematology parameters - packed cell volume (PCV) and hemoglobin (Hb) - and five biochemical parameters: total protein (TP), aspartate aminotransferase (AST), alkaline phosphatase (AP), phosphorous and creatinine.. In 2002, a paired sample experiment examined the effect of separating plasma from the cellular component of the blood at either 3.5 or 36 hours. Significant differences between early and late separated paired samples were found for glucose, calcium, creatine kinase (CK), AP, AST and lactate dehydrogenase (LDH). A dramatic change in potassium was also noted but this could not be compared statistically due to a small sample size. Sex had no significant effect on parameter medians or variances. This study highlights a limitation of using reference values in wildlife medicine, where ideal collection and handling of samples may be compromised and prevent accurate interpretation under specific circumstances.

Introduction

Issues and techniques associated with the conservation of critically endangered wildlife are increasingly becoming more sophisticated and relying on the expertise of skilled specialists such as veterinarians.[1,2] In small populations, individual animals are vital to conservation efforts, thus, making it economically feasible as well as a conservation necessity to manage the health of individuals. When wildlife are identified as sick, often clinical signs are vague and the history is unknown, hence clinical pathology is a useful diagnostic tool. Unfortunately the interpretation of these data can be difficult, because hematological and biochemical reference values for many species are incomplete.

The kakapo *Strigops habroptilus* is a critically endangered large flightless nocturnal parrot endemic to New Zealand.[3] The New Zealand Department of Conservation's National Kakapo Team has been intensively managing the free-ranging population since 1995, increasing the number of birds from 50 to a current population of 83. The health management program for this species involves regular monitoring of individual adults and all chicks from the day of hatching. This level of supervision means that it is both possible and appropriate to detect and treat pathological conditions. To improve diagnostic evaluation of sick individuals, a set of hematological and biochemical reference ranges were established in 1996. These were the basis for comparison of all blood data from the birds until 2002 when another large set of data was collected from adults and for the first time, chicks.

Hematological and biochemical results can be significantly affected by many factors including: the age or sex of the individual,[4,5] the sampling site,[6] sample handling,[7] the time and temperature of sample storage [8,9] and laboratory processing methods.[10,11] Reference ranges and intervals calculated for common veterinary species are based on large sample sizes using standardized methods and calibrated for individual laboratory variation to produce accurate and reliable values, but this is not the case for the majority of wildlife species. In wildlife medicine, references intervals are often derived from inappropriately small sample sizes, and may not be collected, stored or processed under ideal or consistent conditions.[12] The aim of this study is to present hematological and biochemical data collected from kakapo over seven years and examine the effects of various sample handling and processing methods. The limitation of comparing results to

reference ranges where one or both sets of values have not been ideally generated is examined in the context of a field-based wildlife recovery program.

Materials and Methods

Kakapo

Adult kakapo weigh between 1.2 and 3.5 kg with females weighing generally less than males.[3] The introduction of exotic mammalian predators contributed to the extinction of the kakapo on mainland New Zealand by the 1970's. Its population is now restricted to 83 free-living individuals on three offshore islands, with no birds kept in captivity. The location of all birds is regularly monitored by radio-telemetry and each kakapo is hand-captured at least once yearly for a health examination. Kakapo breed irregularly and this usually occurs every few years, coinciding with heavy tree fruiting. Chicks remain in the nest until approximately 70 days of age, after which they begin to leave the nest and gain more independence (D Eason, unpublished data). The islands on which kakapo are managed are serviced by boat, light plane or helicopter. These services are infrequent (sometimes as little as once every two weeks) and thus if samples are to be transported to the laboratory, collection must coincide with scheduled transport.

Blood collection and sample processing

In 1996 and 2002, up to 4 milliliters of whole blood was collected from one of two veins. The cutaneous ulnar vein was the common venipuncture site for blood collections in 1996. A winged infusion set helped with holding the 25-gauge needle in place as blood was extracted. One difficulty in using this vein is that birds must have their wing opened and were restrained in a manner that is not usual when handling the birds. It was difficult to hold the needle in place if the bird struggled and the vein was prone to collapse. The medial metatarsal vein was used for venipuncture in 2002. The advantage to sampling from this vein was that the bird could be restrained more comfortably and with less struggling (with one person holding the bird in an upright position with the head under their arm and the bird's legs extended behind it). The vein was occluded by hand or with a light tourniquet above the hock allowing the phlebotomist to use both hands to restrain the distal leg and withdraw blood. The vein was visually detected or palpated and was immobile and less prone to collapse. Jugular veins were not useful for blood sampling

kakapo, as kakapo have large amounts of subcutaneous fat in the cervical region preventing visualization of the vein or jugular groove.

Blood was collected from 45 healthy adult kakapo (greater than 90% of the world's population) in June and July of 1996. The blood was divided between an EDTA tube for hematology and a clot separation tube for biochemistry. Blood smears were made at the time of collection without anticoagulant. Samples were stored on ice packs for 2-8 hours before the EDTA sample was refrigerated at 4 °C and the clotted sample was centrifuged and separated with the serum frozen at minus 20 °C. Blood was stored for 1-5 days until it could be removed from the island as a batch and sent on ice to the analytical laboratories.

In 2002, successful breeding occurred that enabled blood to be collected from 21 chicks at an average age of 61 ± 15 days (mean ± SD) and weight ranging from 1.2 to 2.2 kg. Blood was collected from the medial metatarsal vein and stored in EDTA microtainers for hematology and lithium heparin microtainers for biochemistry. Blood smears were made at the time of collection. Samples were collected over two nights and refrigerated at 4 °C for 12-36 hours before removal from the island. There was no access to a centrifuge and the biochemistry samples were submitted as heparinized blood for separation at the laboratory, but courier transport failed and the blood was unrefridgerated for an additional 48 hours before analysis, rendering these samples unsuitable for hematological and biochemical analysis.

To determine the effect on biochemical results of delayed separation of plasma from the cellular components of blood, samples were collected from 28 healthy adult birds in June and July of 2002 during routine health examinations and divided into two lithium heparinized microtainer tubes. One heparinized sample from each bird was stored at 4 °C as whole blood, and the other sample was centrifuged, plasma separated and frozen at minus 20 °C . The mean time from collection to centrifugation was 3.5 ± 1.5 hours (mean ± SD). The paired samples were submitted for biochemical analysis at the same time with the whole blood samples being centrifuged and separated at the laboratory, approximately 36 hours after collection. Additionally, blood samples (0.5 ml) were collected from this group and stored in EDTA microtainers to expand the hematology database. Blood smears were made at the time of collection and remaining blood was stored at 4 °C until courier shipment to the laboratory. During all collections, blood tubes were filled at least to the minimum required level.

Hematological and biochemical analyses

For all blood analyses, processing occurred within 12 hours of arrival at the analytical laboratory. In 1996, hematology was carried out at the Alpha Scientific laboratory in Hamilton, NZ, and the profile included a packed cell volume (PCV), hemoglobin concentration (Hb), an absolute white blood cell count (WBC) and both absolute and percentile white blood cell differentials (heterophil, lymphocyte, monocyte, eosinophil and basophil). Blood smears were examined for hemoparasites. The PCV was determined by the microhematocrit method and Hb determined using a manual cyanmethemoglobin conversion method.[13] An ammonium oxalate diluent was used to lyse RBC for the hemocytometer direct cell counts and a drop of eosin used to colour the WBC nuclei.[14] The white cell differential was determined from a 100-cell count from smears. Whole blood was not available from the chick samples therefore WBC counts were estimated from slide smears.

In 1996 the biochemistry analysis was performed at Massey University Institute of Veterinary and Biomedical Sciences laboratory using a Cobas Mira (Roche Diagnostic Systems, USA) commercial wet-chemistry reference analyzer. The biochemistry and electrolyte profile included measurement of the concentrations of sodium, potassium, phosphorous, urea, uric acid, creatinine, total protein, and selenium as well as the activity (at 30°C) of alkaline phosphatase (AP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT).

In 2002, both hematology and biochemistry was carried out at the Alpha Scientific laboratory. A Hitachi 717 (Roche Diagnostic Systems, USA) wet-chemistry reference analyzer was used for biochemistry analysis and the same techniques as 1996 were employed for hematology with the exception of hemoglobin. In 2002, Hb was measured on a Hemocue B-hemoglobin analyzer (HemoCue AB, Sweden), which bypasses the cyanmethemoglobin conversion step. Biochemistry tests differed from 1996 and consisted of glucose, sodium, potassium, calcium, phosphorous, uric acid, creatinine, total protein, creatine kinase (CK), ALP, AST, and lactate dehydrogenase (LDH). Biochemistry enzyme activity was evaluated at 30 °C. In some instances not all tests could be run due to insufficient blood volumes, thus the numbers of values for each test are not equal.

Statistical analysis

On the two occasions where birds had more than one sample taken during the sampling periods, these were averaged to produce a single value for each parameter. Means and standard deviations were calculated for all variables and all variables were tested for normality with the Shapiro-Wilk's W test. Parameter variances from 1996 were compared to the 2002 values by a Levene's homogeneity of variance test (HOV). Due to the non-normality of much of the data and a number of significantly different variances, the non-parametric Mann-Whitney *U* test was performed to compare medians (Tables 1, 2, 4). In Table 2, creatinine values < 20 were converted to 19 for statistical comparison. For the paired early and late separated plasma samples, variances were compared with a Levene's HOV test and medians with a Wilcoxon's test for matched pairs (Table 3). The mean percentage change in the parameter values that were significantly affected by storage from 3.5 to 36 hours was calculated by finding the difference between the means of the paired samples and dividing this by the mean value for the group separated after 3.5 hours.[6] For all biochemical and hematological parameters collected in both 1996 and 2002, the effect of sex on both medians and variances was assessed using a Mann-Whitney *U* test and a Levene's HOV test respectively. A correlation coefficient was calculated for all early separated plasma biochemistry values with respect to time from collection to separation (mean 3.5 hours, range 100-430 minutes). Whether this coefficient was positive or negative was compared to the change seen in the samples over 36 hours (+ or – change). Statistical analyses were performed on Statistica,[15] all tests were two-tailed and significance for all tests was assigned at the $P < .05$ level. To control for the group-wide type 1 error rate in tables with multiple statistical tests, a sequential Bonferroni correction was applied when judging the significance of each test's *P*-value.[16] In tables where one of the parameter's $N < 5$, statistical analyses were not done due to the low power of the test.

Results

Adults samples -1996 versus 2002

The means, standard deviations and reference intervals (maxima and minima) for all parameters measured in 1996 and 2002 are listed in Table 1 (hematology) and Table 2 (biochemistry). Parameter variances were found to differ significantly between the two

years for two of the five valid biochemical comparisons (total protein, AST). Parameter medians were found to differ significantly between the two years for four out of the eight valid biochemical comparisons (phosphorous, creatinine, AP, AST) and two of the thirteen valid hematological comparisons (PCV, Hb). None of the parameters collected in either 1996 or 2002 showed any sex effect on medians or variances (Mann-Whitney *U* test: Bonferroni adjusted *P*-values all = NS). No blood parasites were detected. Hemolysis was not reported for any of the samples.

Assay	N	Adult 1996	N	Adult 2002	Levene HOV *P*-value	Mann-Whitney *U* *P*-value
PCV (l/l)	40	0.39 ± 0.03 (0.32 – 0.43)	24	0.42 ± 0.05 (0.34 - 0.55)	0.07	**0.002****
Hemoglobin (g/l)	35	129.6 ± 9.5 (110 – 146)	24	151.3 ± 9.9 (130 - 168)	0.88	**<0.001****
WBC (x 10^9/l)	45	17.2 ± 6.5 (6.8 – 36.9)	28	19.6 ± 6.8 (8.2 - 40.5)	0.93	0.12
Heterophil %	45	78.7 ± 10.0 (40 – 93)	28	81.6 ± 7.1 (57 - 92)	0.67	0.06
Heterophil (x 10^9/l)	45	13.39 ± 4.98 (5.2 - 26.6)	28	16.05 ± 6.12 (7.2 - 36.9)	0.09	0.19
Lymphocyte %	45	17.1 ± 10.1 (4 - 56)	28	15.6 ± 6.9 (6 - 39)	0.06	0.39
Lymphocyte (x 10^9/l)	45	3.11 ± 2.68 (0.7 - 13.7)	28	3.06 ± 1.55 (0.7 - 7.2)	0.04	0.66
Monocyte %	45	1.08 ± 1.08 (0 – 4)	28	0.57 ± 0.69 (0 - 2)	0.37	0.18
Monocyte (x 10^9/l)	45	0.17 ± 0.20 (0 - 1)	28	0.11 ± 0.14 (0 – 0.4)	0.10	0.05
Eosinophil %	45	1.62 ± 1.57 (0 - 7)	28	1.60 ± 1.22 (0 - 4)	0.88	0.69
Eosinophil (x 10^9/l)	45	0.31 ± 0.34 (0-1.2)	28	0.32 ± 0.29 (0 - 1)	0.43	0.66
Basophil %	45	1.24 ± 1.24 (0 - 5)	28	0.67 ± 0.90 (0 – 3)	0.20	0.07
Basophil (x 10^9/l)	45	0.20 ± 0.23 (0 - 1.1)	28	0.11 ± 0.17 (0 – 0.6)	0.04	0.06
Fibrinogen (g/l)	-	N A	16	2.41 ± 1.28 (0.8 - 6.3)	Not compared	Not compared

** *P*-values significant after sequential Bonferroni correction

Table 1. Hematology reference value comparisons between samples collected in 1996 and 2002. Values are expressed as mean ± SD (minima to maxima range) for all samples where $N > 1$. Hematology values reported include packed cell volume (PCV) and total white blood cell count (WBC). *P*-values for variance (Levene HOV) and median (Mann Whitney *U*) comparisons between the two data sets are listed.

Assay	N	Adult 1996	N	Adult 2002	Levene HOV *P*-value	Mann-Whitney U *P*-value
Glucose (mmol/l)	-	Not performed	30	12.37 ± 1.55 (9.3 – 6.2)	N/A	N/A
Sodium (mmol/l)	28	147.7 ± 5.1 (137.4 – 155)	4	148.7 ± 2.2 (147 – 152)	Not tested	0.93
Potassium (mmol/l)	24	4.69 ± 0.98 (3.4 – 7.2)	4	4.32 ± 0.79 (3.8 – 5.5)	Not tested	0.42
Calcium (mmol/l)	-	Not performed	30	2.44 ± 0.17 (1.92 – 2.73)	N/A	N/A
Phosphorous (mmol/l)	31	0.79 ± 0.29 (0.39 – 1.42)	29	1.18 ± 0.31 (0.5 – 1.9)	0.85	**<0.001****
Urea (mmol/l)	33	0.9 ± 0.53 (0.14 – 2.17)	-	Not performed	N/A	N/A
Uric acid (mmol/l)	36	0.16 ± 0.07 (0.08 – 0.33)	29	0.18 ± 0.12 (0.03 – 0.6)	0.22	0.89
Creatinine (umol/l)	35	34.4 ± 27.1 (15 – 121)	26	<20	Not tested	**<0.001****
CK (U/l)	-	Not performed	30	114.3 ± 43.6 (55 – 234)	N/A	N/A
Total protein (g/l)	38	45.2 ± 16.4 (23.1 – 94.8)	30	46.5 ± 5.2 (36 – 57)	**0.01****	0.67
AP (U/l)	34	96.8 ± 47.3 (17 – 238)	26	134.3 ± 52.7 (36 – 303)	0.83	**0.005****
AST (U/l)	38	144.1 ± 34.6 (100 – 266)	30	82.8 ± 16.7 (60 – 121)	**0.009****	**<0.001****
ALT (U/l)	37	7.78 ± 6.81 (0 – 38)	-	Not performed	N/A	N/A
GGT (U/l)	12	5.08 ± 2.97 (1 – 11)	-	Not performed	N/A	N/A
LDH (U/l)	-	Not performed	29	197.5 ± 80.2 (89 – 414)	N/A	N/A
Selenium (umol/l)	38	2.51 ± 2.17 (0.45 – 9.1)	-	Not performed	N/A	N/A

** *P*-values significant after sequential Bonferroni correction

Table 2. Biochemical and electrolyte reference value comparisons between samples collected in 1996 and 2002. Values are expressed as mean ± SD (minima to maxima range) for all samples where $N > 1$. Biochemistry values reported include creatine kinase (CK), alkaline phosphatase (AP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT) and lactate dehydrogenase (LDH). *P*-values for variance (Levene HOV) and median (Mann Whitney *U*) comparisons between the two data sets are listed.

Adult paired samples - 2002

Paired sample ranges and their statistical comparisons are listed in Table 3. Only LDH variances differed significantly between the matched samples. Significant differences between the medians of separated and non-separated samples were found for glucose, calcium, CK, AP, AST and LDH. While the change seen in potassium were very large (71 % increase), the sample sizes were too small for interpretation. The magnitude of change between significantly different paired samples ranged from an average of 1.6 % for calcium to 77 % for LDH. Correlation coefficients comparing the early separated sample values and the time from collection to separation (between 100 and 430 minutes) found only weak correlations for all parameters (all $r^2 < 0.11$). In comparing the slopes of these correlations (+ or -) to the direction of change seen in values that were significantly different in the paired sample tests, four were in the same direction and three in the opposite direction. Of the highest three correlation coefficients for early separated samples ($0.23 < r < 0.33$), two were in the opposite direction to that predicted by the paired sample tests.

Chick samples - 2002

Hematological parameters determined using blood smears from kakapo chicks are listed in Table 4. There were no differences in variances between any of the chick and adult comparisons (Levene HOV test all $P > 0.17$). The Mann-Whitney U test indicated no significant differences between adult and chick values when P-value significance was assessed using a Bonferroni correction.

Assay	N	Plasma separated at 3.5 hours	Plasma separated at 36 hours	Levene HOV *P*-value	Wilcoxon matched pairs *P*-value	% Average change
Glucose (mmol/l)	21	12.5 ± 1.6 (9.5 – 16.2)	10.8 ± 1.9 (8 – 14.8)	0.17	**0.001****	- 13 %
Sodium (mmol/l)	3	149.3 ± 2.3 (148 – 152)	148.6 ± 2.3 (146 – 150)	Not tested	Not tested	- 0.4 %
Potassium (mmol/l)	3	3.9 ± 0.15 (3.8 – 4.1)	6.7 ± 0.17 (6.5 – 6.8)	Not tested	Not tested	+ 71 %
Calcium (mmol/l)	19	2.45 ± 0.11 (2.14 – 2.63)	2.41 ± 0.12 (2.15 – 2.64)	0.35	**0.01****	- 1.6 %
Phosphorous (mmol/l)	17	1.30 ± 0.31 (0.7 – 1.9)	1.34 ± 0.34 (0.9 – 2)	0.53	0.58	+ 3 %
Uric acid (mmol/l)	16	0.18 ± 0.1 (0.06 – 0.48)	0.18 ± 0.09 (0.06 – 0.44)	0.64	0.27	0 %
Creatinine (umol/l)	21	<20	<20	Not tested	Not tested	N/A
CK (U/l)	21	104.3 ± 25.7 (55 – 165)	122.8 ± 36.2 (63 – 205)	0.51	**0.001****	+ 18 %
Total protein (g/l)	20	45.5 ± 4.9 (36 – 53)	46.1 ± 5.3 (37 – 56)	0.78	0.02	+ 1.3 %
AP (U/l)	18	138.7 ± 60.1 (36 – 303)	109.4 ± 52.4 (34 – 277)	0.78	**<0.001****	- 21 %
AST (U/l)	21	84.9 ± 15.5 (62 – 114)	90 ± 13.0 (65 – 114)	0.22	**0.001****	+ 5.9 %
LDH (U/l)	16	185.3 ± 60.4 (117 – 378)	328.9 ± 82.4 (192 – 497)	0.03	**<0.001****	+ 77 %

** *P*-values significant after sequential Bonferroni correction

Table 3. Plasma biochemistry for paired adult samples separated at 3.5 hours and at 36 hours post collection. Values are expressed as mean ± SD (minima to maxima range) for all samples. Biochemistry values reported include creatine kinase (CK), alkaline phosphatase (AP), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH). P-values for variance (Levene HOV) and median (Wilcoxon matched pairs) comparisons between the two data sets are listed. The average change in parameter values between the paired samples was calculated by ((36 hour value – 3.5 hour value) / 3.5 hour value).[6]

Assay	N	Chick values	Comparison to 2002 adult (Mann-Whitney *U* *P*-value)*
WBC ($x 10^9$/l)	20	16.5 ± 8.3 (5.5 – 35)	0.08
Heterophils ($x 10^9$/l)	20	12.9 ± 6.9 (3.5 – 29.8)	0.07
Lymphocytes ($x 10^9$/l)	20	3.1 ± 2.1 (0.7 – 10.7)	0.68
Monocytes ($x 10^9$/l)	20	0.17 ± 0.16 (0 – 0.5)	0.23
Eosinophils ($x 10^9$/l)	20	0.18 ± 0.4 (0 – 1.8)	0.01
Basophils ($x 10^9$/l)	20	0.12 ± 0.15 (0 – 0.6)	0.81

* No *P*-values were significant after sequential Bonferroni correction

Table 4. Chick hematology values taken from blood smears and thus not affected by the delayed processing of the samples in 2002. Values are expressed as mean ± SD (minima to maxima range) for all samples. Hematology values reported include a total white cell count (WBC). P-values for median (Mann Whitney U) comparisons to the 2002 adult values are listed.

Discussion

Establishing an accurate set of reference values is critical for correct interpretation of clinical pathology results.[11,12] Reference interval calculation is affected by both the population mean and variation; population variability governs the spread of the reference values whether they are calculated as a reference range (the interval between the minimum and maximum values) or a reference interval (two standard deviations either side of the mean). Both variation and mean were included when comparing the data between 1996 and 2002 and the paired sample data. In a number of studies looking for significant differences between samples, only the means (or medians) are compared and the equally important variances are not discussed;[5,6] this approach will fail to detect the difference between two groups with identical means but different variability in their parameters. Reference values given in this paper include the means and standard deviations as well as the minimum to maximum range. Because many of the values are

not normally distributed and are calculated from less than 40 individuals, it is more appropriate to use the minimum to maximum range as a reference.[12,17]

The paired sample experiment in our study reinforces work in other species demonstrating that certain plasma biochemistry values will change significantly if the plasma is not separated from the cellular component of the blood.[8,18] Fudge [18] states that false elevations of bile acids, LDH, CK, AP, potassium and sometimes calcium are to be expected in such samples. We found consistent elevations in LDH, CK, AST and total protein in blood not separated before shipment. Because LDH and AST are found in erythrocytes, it is to be expected that their levels will increase over time due to leakage from the cells. Potassium was also markedly elevated, but the number of samples ($N = 3$) prevented statistical comparison. Contrary to Fudge [18], AP was consistently lower in late separated blood samples as was glucose and calcium.

Variations noted in a paired sample experiment can be very small and yet significant; however, change due to handling differences needs to be interpreted relative to the population reference range in order to determine its practical relevance. Obviously in the case of LDH, an average change of 77 % has a good chance of shifting the value outside of the reference range where the standard deviation is only 30% of the mean. Contrast this with calcium where an average 1.6 % decrease occurs if not separated. This change will be clinically negligible as the normal standard deviation is three times this amount. The correlation between biochemical values and the time taken to separate plasma from cells shows that any change occurring in the first few hours after collection (1.5 to 6.5 hours) cannot be easily detected due to swamping by the variation in the population and can be practically ignored in the field situation. However, samples should be separated as soon as is practical, as these changes are cumulative and likely to become significant with time.[18]

Protocols exist for the ideal generation of reference intervals[11,17] yet minimizing artifactual changes in blood samples due to handling, storage, or processing variation [9,12,19] may be difficult in some field situations. Because of the isolated conditions under which kakapo work must be undertaken, blood samples are usually collected several hours before plasma separation or refrigeration is possible, and a minimum of 12 hours in advance of transportation off the island. When a large number of birds are being sampled, a collection period of several days is necessary and plasma samples may require refrigeration on the island up to 72 hours before overnight shipment to the analytical

laboratory. One consequence of this is that either the reference intervals or the sample collected for comparison to the reference interval may be significantly affected by artifactual changes, making interpretation of findings difficult.

Just over one-third of the hematological and biochemical parameters were significantly different between 1996 and 2002, highlighting the impact of consistent sample handling, storage and processing on interpretation of results. The causes of the differences between the two years are likely to be multiple and can be broadly categorized into three areas. The first is that differences existed between the populations sampled. This effect is probably negligible because in 2002, of the 28 birds sampled, 22 were birds that were also sampled in 1996. Both sets of samples were collected in the winter (June and July) and there was a similar sex distribution. Effects of breeding season on females were minimal as no differences in median values could be attributed to sex in either 1996 or 2002.

Second is the effect of differences in handling and storage of the blood samples prior to processing. Of the two sampling years, differences could be found in the sampling site, sampling method, storage tubes (plasma or serum), and average time before processing; all of which have been shown in other studies to affect a number of hematological and biochemical parameters.[6,8,9,11,12,19,20] However, with the exception of ensuring consistency with regards to the types of blood tubes used, the methodological differences between the two years were minor or unavoidable and fail to explain the majority of the parameter variations.

The third and potentially most significant cause of variation between the two years was change of laboratory techniques. Different laboratory methods can produce significantly different measurements from the same blood sample, and thus each laboratory should generate their own set of reference values every time they alter their methodology.[8,10-12] Because of this, Raskin [12] cautions against strict adherence to reference ranges that have been calculated by a different laboratory or by using different techniques. Thus the results found in our study are not unexpected but serve to highlight a point often ignored in discussions regarding reference intervals. For many conservation projects, it might not be feasible to repeat the sampling effort required to generate a reference interval every time methods change or a new laboratory is used. This might be due to resource constraints, a lack of animals to sample from, or the risks associated with capture and handling outweighing the benefits of a more accurate dataset. The majority of

papers documenting hematological and biochemical values in non-mammalian animals are derived from captive populations where facilities are easy to access and sample processing is prompt.[4,21-26] In many free-ranging species, such as the kakapo, ideal collection, storage and processing of blood samples is often compromised. These constraints need to be recognized and factored into the interpretation of generated reference values.[10] Regardless of the cause of the differences noted between samples in this study, this should highlight some of the limitations of reference values. Interpreting reference values needs to be undertaken cautiously, especially in situations where methodological differences may exist between the individual sample and those collected to generate the reference interval.

Acknowledgements

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CHAPTER IV

Aspergillosis in a North Island robin (*Petroica longipes*)

A female North Island robin incubating her clutch on Tiritiri Matangi Island

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Chapter reference:
Low, M., Berggren, Å., Morgan, KJ & Alley, MR. *Clinical Communication*: Aspergillosis in a North Island robin (*Petroica longipes*). Submitted to the *New Zealand Veterinary Journal*.

Abstract

CASE HISTORY: A 3-year-old female North Island robin (*Petroica longipes*) was found dead on Tiritiri Matangi Island during the breeding season.

PATHOLOGICAL FINDINGS: The bird was in poor condition, with a 13 x 8 mm granulomatous mass in the thoracic cavity causing displacement of the heart and left lung. Histologically, the mass was a large granuloma infiltrated with fungal hyphae, and the liver contained multifocal aggregates of inflammatory cells.

DIAGNOSIS: Thoracic aspergillosis with multifocal hepatitis.

CLINICAL RELEVANCE: Determining the causes of death in wild bird populations is often hampered by a lack of carcass recovery, autolysis and poor clinical history. In this case, the life history of the bird was known and recovery of the body was relatively swift. This is the first published description of aspergillosis in a free-living North Island robin.

Introduction

Aspergillosis is an infection caused by the naturally occurring, ubiquitous fungal genus *Aspergillus*: with the species *A. fumigatus* most commonly implicated in avian disease (Bauck 1994). The spores from these fungi are widespread and clinical disease is thought to occur when stress or immunosuppression allows the growth of the fungi in the bird's lungs or air sacs (Bauck 1994; Perrott 2001). Aspergillosis has been reported from a wide range of bird species, and is most commonly considered to be a disease of captivity or close-confinement (Schultz et al 1996; Albicker-Rippinger and Hoop 1999). However, in the New Zealand stitchbird / hihi (*Notiomystis cincta*), free-living birds appear to be unusually susceptible to developing the clinical disease (Alley et al 1999). It has been suggested that such species-specific susceptibility to *Aspergillus* may be due to poor immunological adaptation to pathogens found in highly modified environments that wildlife are now forced to inhabit (Perrott 2001). However, in order to test this hypothesis, more data on the incidence and susceptibility of other avian species to aspergillosis are required.

In this paper we describe an *Aspergillus* granuloma in the thoracic cavity of an adult female North Island robin (*Petroica longipes*). This species is a forest passerine endemic to the North Island of New Zealand and nearby offshore islands (Higgins and Peter 2002). To our knowledge, aspergillosis has not been previously reported in this species.

Case history

In October 2000, the female North Island robin hatched on Tiritiri Matangi Island (36°36'S, 174°53'E): an island located in the Hauraki Gulf, 25 km north of Auckland, New Zealand. During the next two breeding seasons, this female successfully raised seven chicks to fledging age: a reproductive performance above the average in this population (mean 2.5 chicks per year) (Armstrong et al 2000). Her breeding territory was in one of the old forest remnants, which are thought to be of relatively high quality (Armstrong and Ewen 2002). During the summer breeding season of 2003/2004, this female produced one clutch of eggs that failed to hatch, and another where a single chick died before fledging. On the 19th December 2003, the female was found dead in her

territory, and it was thought she had died ~24 hours previously. The body was put in a freezer (-20° C) and kept for three months before it was thawed and examined on 19th March 2004. At this time the body cavity was opened and an abnormal mass was found in the thoracic cavity. The body was placed in 10 % neutral buffered formalin and stored for 11 days before a more detailed post mortem examination was undertaken.

Pathological Findings

On external examination, the keel bone was very prominent due to a reduced mass of pectoral muscle. On opening the thorax, a 13 x 8 mm pale-cream granulomatous consolidated lesion was found attached to the wall of the left chest and the right side of the heart, which was causing displacement of the left lung (Figure 1). The gastrointestinal tract was empty, and the crop was full of maggots. The brain was missing, presumably having been eaten by maggots. After removal, the lesion weighed 1.3 grams; approximately 5% of the normal body weight of a female robin. The lesion and other visceral tissues were fixed in 10% buffered formalin and processed routinely for histopathology. Sections were cut at 4 microns and stained with haematoxylin and eosin (H&E) and Young's (1969) fungal stains and examined using light microscopy.

Histologically, the thoracic mass consisted of a large central core of necrosis, surrounded by a periphery of large numbers of mixed inflammatory cells: mainly macrophages, lymphocytes and heterophils. On both H&E and Young's stained sections, fungal elements were evident. These branching and septate hyphae extensively infiltrated the entire lesion, with several areas of intense accumulation at the centre of the lesion being evident on sub-gross examination of the Young's stained sections (Figure 2). No conidiophores or spores were visible. The liver was autolysed but did contain numerous multifocal aggregates of inflammatory cells. In one lung lobe, a large infiltration of inflammatory cells was also evident. Although the affected tissues were not cultured because of their storage history, the nature of the lesions and morphology of the fungal hyphae were typical of aspergillosis seen in other avian species.

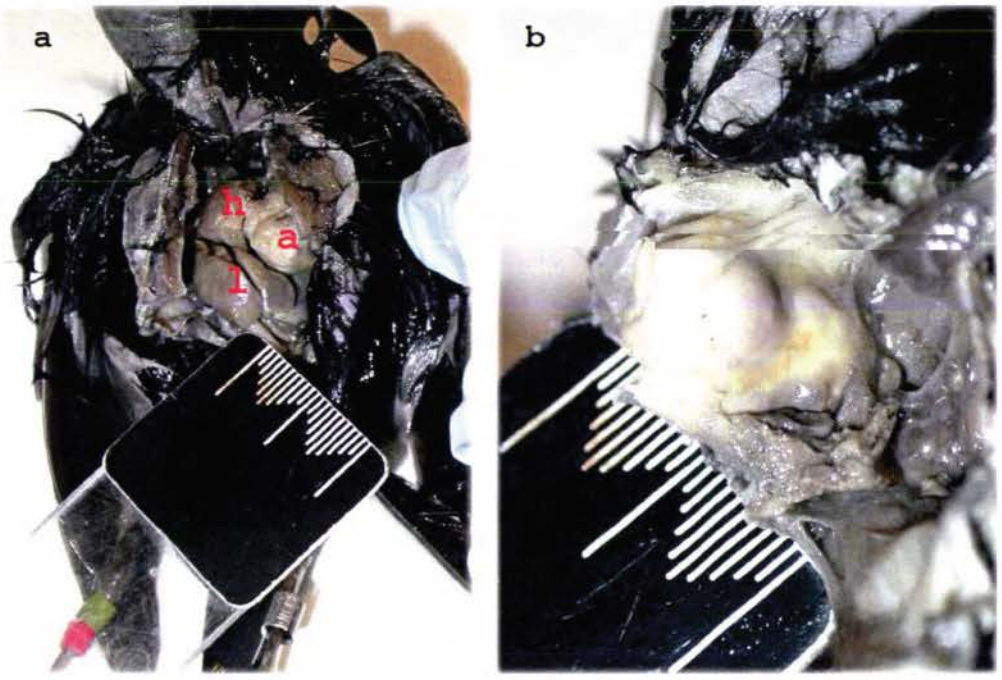

Figure 1. Aspergillus granuloma in a North Island robin. **(a)** The bird is on its back with the chest open; the heart (*h*) and liver (*l*) are displaced to the right by the granuloma (*a*) attached to the left chest wall. **(b)** Close-up of the granuloma, showing the fibrous capsule surrounding it. Each small gradation on the scale is 1mm.

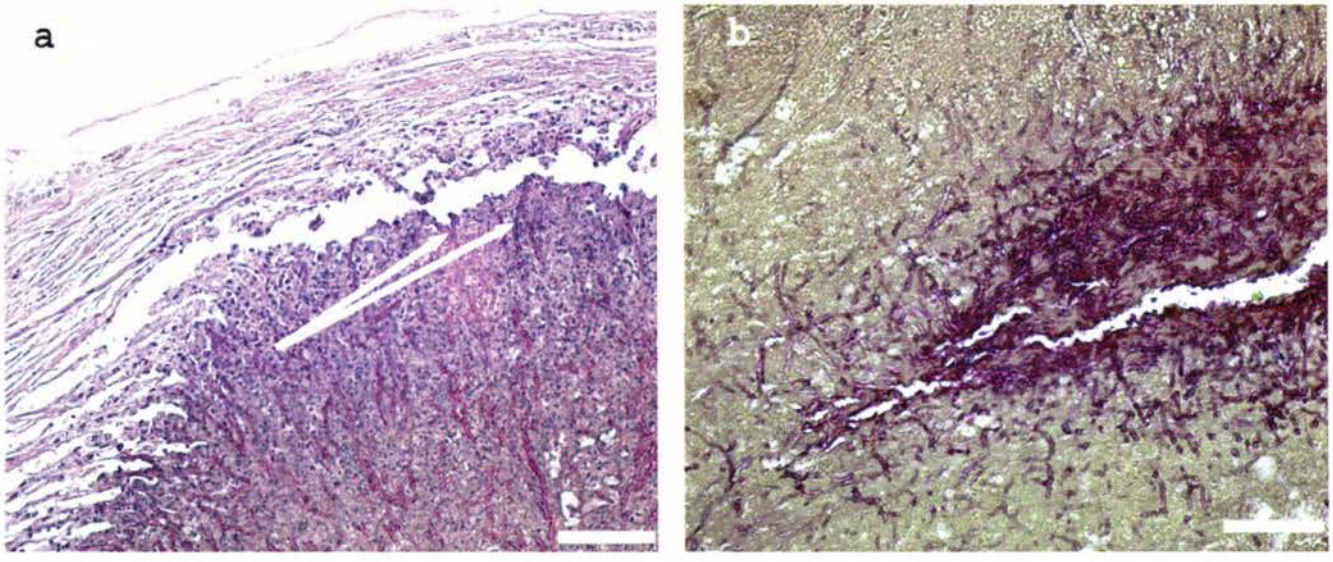

Figure 2. Photomicrograph of a haematoxylin and eosin stained section of the aspergillus granuloma. In **(a)** the fungal hyphae extend to the edge of the lesion (arrows), just under the fibrous capsule; they are the purple filaments within the tissue. While the hyphae could be found throughout the lesion, they were concentrated in the centre **(b)**. The white scale bar indicates 0.1mm.

Discussion

The development of clinical aspergillosis in this robin is likely to have been influenced by a combination of three factors affecting the bird's susceptibility to the disease. The first being a compromise to the host's immune function due to seasonal changes in hormone levels or stress associated with the breeding season. *Aspergillus* spp. is a ubiquitous fungus, with infection generally thought to be limited to individuals suffering immunosuppression, stress or a concurrent primary infection (Bauck 1994). In other species, seasonal immunosuppression associated with hormonal changes or reproductive effort has been found to increase disease susceptibility (e.g. increasing coccidial oocyst shedding, Duffy et al 2000; Duckworth et al 2001, and susceptibility to haematozoan infection, Norris et al 1994).

The second factor implicated in the development of respiratory aspergillosis is a high level of environmental *Aspergillus* spp. contamination (Campbell 1986). While this is typically associated with captive environments, Perrott (2001) presents evidence that free-living species in modified bush environments are potentially exposed to massive *Aspergillus* spore counts. On Tiritiri Matangi Island, there is a relatively high total mean density of 158,000 *A. fumigatus* colony-forming units (CFUs) for every gram of soil (Perrott 2001). The number of CFUs is positively correlated with the amount of environmental disturbance, leading to circumstances where free-living animals may be in contact with overwhelming numbers of spores (Perrott 2001): a situation usually restricted to some captive situations. This level of environmental contamination in the soil suggests a third factor potentially important in the development of clinical aspergillosis in the robin – its feeding behaviour. Robins spend 90% of their foraging time on or within two meters of the ground, where they search soil, litter and rotting logs for invertebrates by picking or digging up prey using their beak (Powlesland 1981). This feeding method is likely to promote contact with fungal spores in the soil and rotting vegetation; particularly, when one considers that *Aspergillus* colonises areas of disturbance (Perrott 2001), and robins preferentially feed in these areas (Higgins and Peter 2002).

Despite the intensive long-term monitoring program of robins on Tiritiri Matangi Island, the proportion of dead birds recovered for meaningful post-mortem examination was very small (~2%). Thus, the importance of aspergillosis as a mortality factor in this robin population still remains unclear. It is important for wildlife managers to place results such

as these into perspective. Carcass recovery is affected by numerous mechanisms such as: time of year, level of monitoring, habitat parameters, population density, and the cause of death. Thus, bodies that are recovered for post-mortem examination may not necessarily represent an unbiased sample as to the causes of death in that population. For example, predation by the native owl, the morepork (*Ninox novaeseelandiae*), may be a significant mortality factor in North Island robins, but because of its very nature, carcasses may never be recovered. Therefore, while reporting the causes of death from opportunistic carcass recovery is important for documenting the presence of diseases within populations, placing these findings in the wider context of their relative importance with other mortality factors presents a serious challenge to wildlife health managers.

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CHAPTER V

Knemidocoptes spp. implicated in the seasonal development of a facial dermatitis in a population of free-living stitchbirds

Lateral views of the heads of two male stitchbirds. The top picture shows a bird with a healthy black head-plumage. The lower picture shows a bird affected by *Knemidocoptes*-induced feather damage

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Chapter reference:
Low, M., Alley. M. R. & Scott, I. *Knemidocoptes* spp. implicated in the seasonal development of a facial dermatitis in a population of free-living stitchbirds. Submitted as a Short Communication to the *Journal of Wildlife Diseases*.

Abstract

From September 2001 to February 2005, observations of an island population of the New Zealand stitchbird (*Notiomystis cincta*) revealed a progressive feather-losing dermatitis that developed during the breeding season around the birds' eyes, base of the bill and ventral neck. The lesions were significantly more likely to develop in males (96%) than females (51%), with males experiencing a more severe form of the condition. Histology from a dead bird revealed the presence of burrowing mites within the lesions, and isolation of mites from skin crusts of a live bird allowed them to be identified as *Knemidocoptes* spp. These data are highly suggestive of *Knemidocoptes* spp being a causal factor in the development of the skin lesions in this population.

The stitchbird (or hihi: *Notiomystis cincta*) is an endangered passerine, which is restricted in its distribution to three islands off the coast of New Zealand (Higgins et al 2001). The New Zealand Department of Conservation has attempted to establish new populations to improve its conservation status; however, the majority of these translocations have been unsuccessful. Reasons proposed for its poor translocation performance have been: lack of food at crucial times of the year (Armstrong and Ewen, 2001), competition from other honeyeater species (Wilson, 1997) and the impact of disease (Alley et al., 1999). Stitchbirds appear unusually susceptible to aspergillosis, a systemic fungal infection often associated with poor immune function (Alley et al., 1999). A link between disease susceptibility and hormonal processes, as documented in other species (Casto et al., 2001), has been suggested as a possible explanation for disease patterns in the stitchbird (Alley et al., 1999).

During breeding observations of the stitchbird population on Tiritiri Matangi Island (36°36' S, 174° 53' E) (Low 2005), birds were observed with areas of feather loss around their face and neck (Fig. 1).

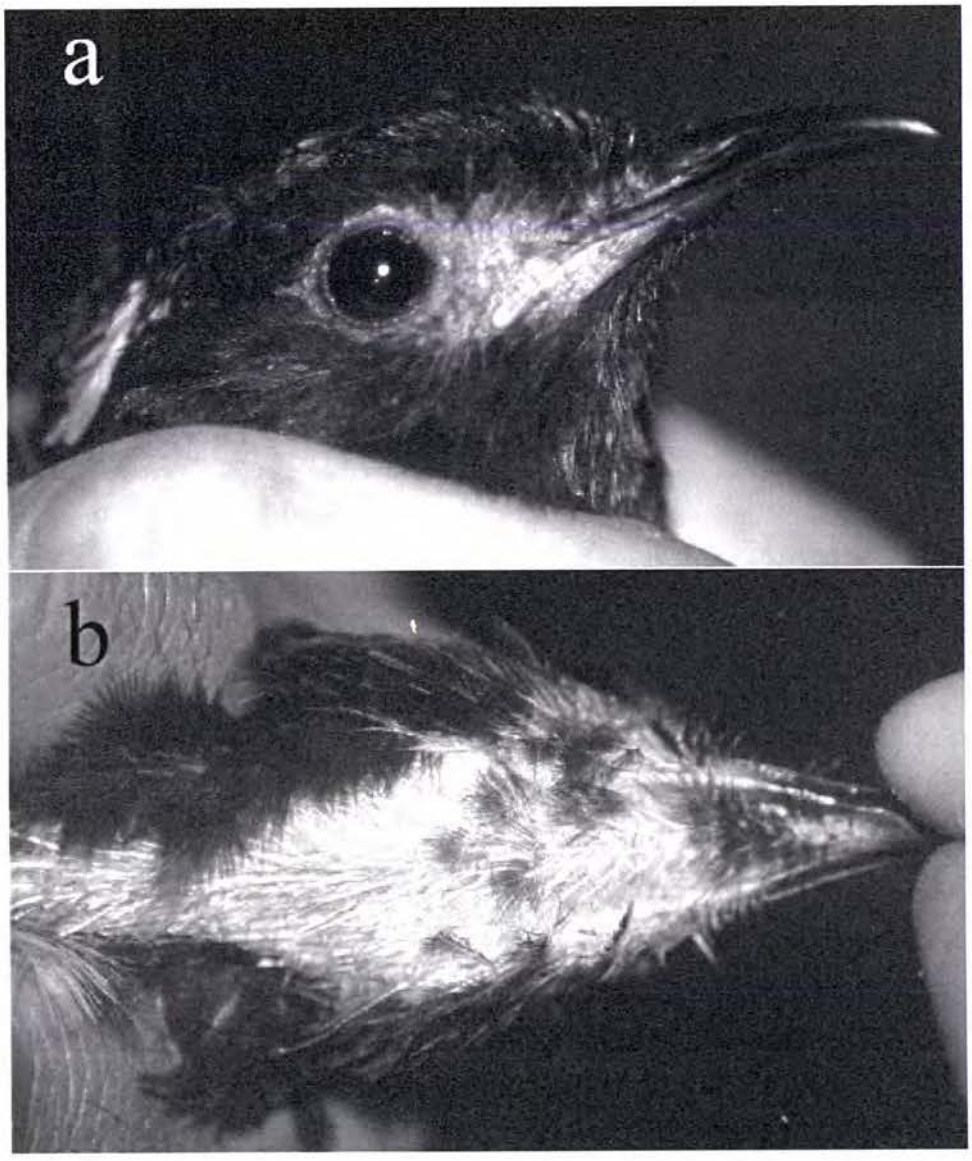

Figure 1: Mite-associated feather loss in a male stitchbird with a patchy feather distribution around the eyes **(a)** and the ventral neck **(b)**.

Few lesions were observed in September 2001, but during the next 5 months a steady increase in the prevalence and severity of the condition were noted. The lesions were usually associated with an obvious pruritis, which manifested as face rubbing and scratching. The lesions and pruritis were suggestive of a burrowing skin mite, such as *Knemidocoptes* spp – a common avian parasite (Ritchie et al., 1994). Because this condition had not been previously described, the aim of this study was to: (1) describe the lesions as they presented in individual birds and assess their prevalence within the

population, (2) determine the likely causative agent of the lesions, and (3) evaluate the implications of this disease relative to the current species' recovery program.

In November 2001, a 5-year-old male stitchbird showing the typical facial and ventral-neck distribution of feather loss was found dead inside a nest box. Skin samples from the affected area were preserved in 10% buffered formalin. These were then processed routinely for histology and stained with hematoxylin and eosin. Histological examination revealed a generalized loss of feather follicles, and extensive areas of epidermal orthokeratotic hyperkeratosis, which was most severe at the base of the skin folds. Several subcorneal pustules were present in the hyperkeratotic areas and approximately 6-8 cross-sections of individual burrowing mites were found beneath the stratum corneum in each skin section examined (Fig 2). The mites had a round or ovoid body, short legs and were often associated with irregular focal areas of acanthosis in the adjacent epidermis and occasional small aggregations of lymphocytes and plasma cells in the upper dermis.

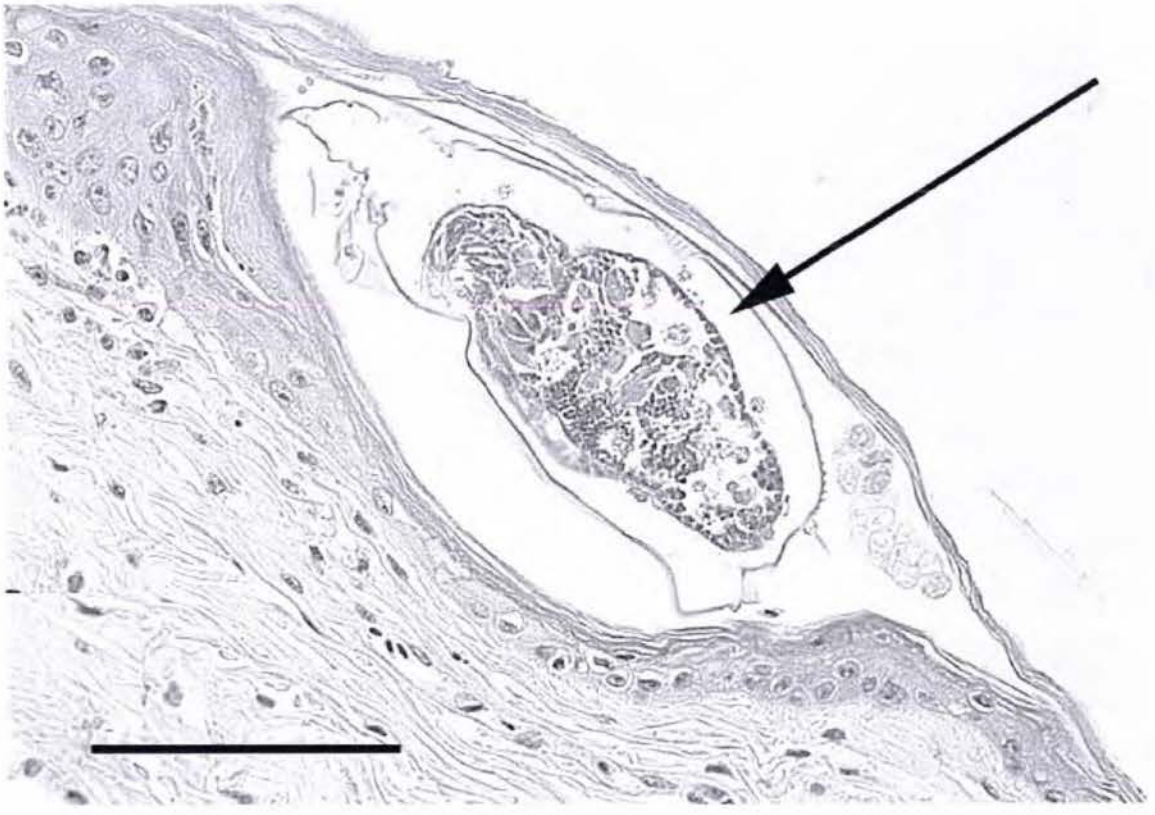

Figure 2: Hematoxylin and eosin stain of a skin section taken from the neck of a dead male stitchbird showing ventral neck feather loss. Within the stratum corneum the cross-section of a burrowing mite consistent with *Knemidocoptes* spp. is clearly visible (arrow). The scale bar equals 100 µm.

Subsequently, five male stitchbirds that had died of various causes, but lacked skin lesions, were examined histologically; none had any evidence of mites in the corresponding areas of their skin. This suggests that a relationship exists between the presence of mites and the feather-losing dermatitis seen in the wild birds.

In February 2002, at the end of the breeding season but before molting, all adult birds (males=25, females=29) were captured using cage traps at supplementary feeding stations. All areas of feather loss or skin crusting were described, and the extent of lesions scored on a half point scale from 0 – 3 (0=none, 3=extensive) for their face, ventral beak and ventral neck; lesions were not found in any other area. On close examination, the lesions were found to be a result of both feather loss and feather shortening (breakage) suggesting mechanical factors were involved, such as rubbing or scratching. In mildly affected individuals, lesions were confined to the head and consisted of patchy areas of feather loss around the eyes and between the eyes and beak (Fig. 1a). In severe cases, these lesions were more extensive and involved crusting around the base of the bill and feather loss extending ventrally from the tip of the lower bill to the top of the carina of the breast (Fig. 1b). The fact that there was a significant positive correlation between the presence and severity of head lesions and the appearance and extent of ventral neck lesions (Spearman rank order correlation: r_s=0.56, n=54, P<0.001) supported the idea that the lesions in the two areas were part of the same syndrome.

Males were significantly more likely to exhibit lesions than females (males=96%, females=51%, Fisher's exact test P<0.001), and of birds with lesions, males were more likely to have lesions extending to the ventral neck area (males=50%, females=13% Fisher's exact: P=0.03). While first year adults were as likely to exhibit some form of lesion as older birds (76% versus 72%), they were significantly more likely to exhibit neck lesions (1st year=40%, older=13%, Fisher's exact: P=0.03). Louseflies (Diptera: Hippoboscidae) were observed in the feathers of males significantly more often than in females (males=68%, females=31%, Fisher's exact: P=0.01), though they were not associated with the presence of mites (Fisher's exact: P=0.23).

For 25 of these birds, a deep skin scraping of the lesions was attempted, which involved applying a small drop of glycerol to the area to be sampled and scraping a scalpel blade across the skin. Scrapings were examined under magnification after being concentrated onto a microscope coverslip via suspension in 1M sucrose. More than one type of mite was detected from the skin scrapings; however, only feather mites, most

probably *Hemialges* spp. (Figs. 3a, b), were isolated. The lack of burrowing mites from these samples was probably due to two factors: (1) the mites are usually beneath the stratum corneum, which made them difficult to dislodge during a skin scraping, and (2) the lesions were small and often could not be properly sampled because of overlying denuded feather shafts; this resulted in the blade often scraping the feathers rather than the skin.

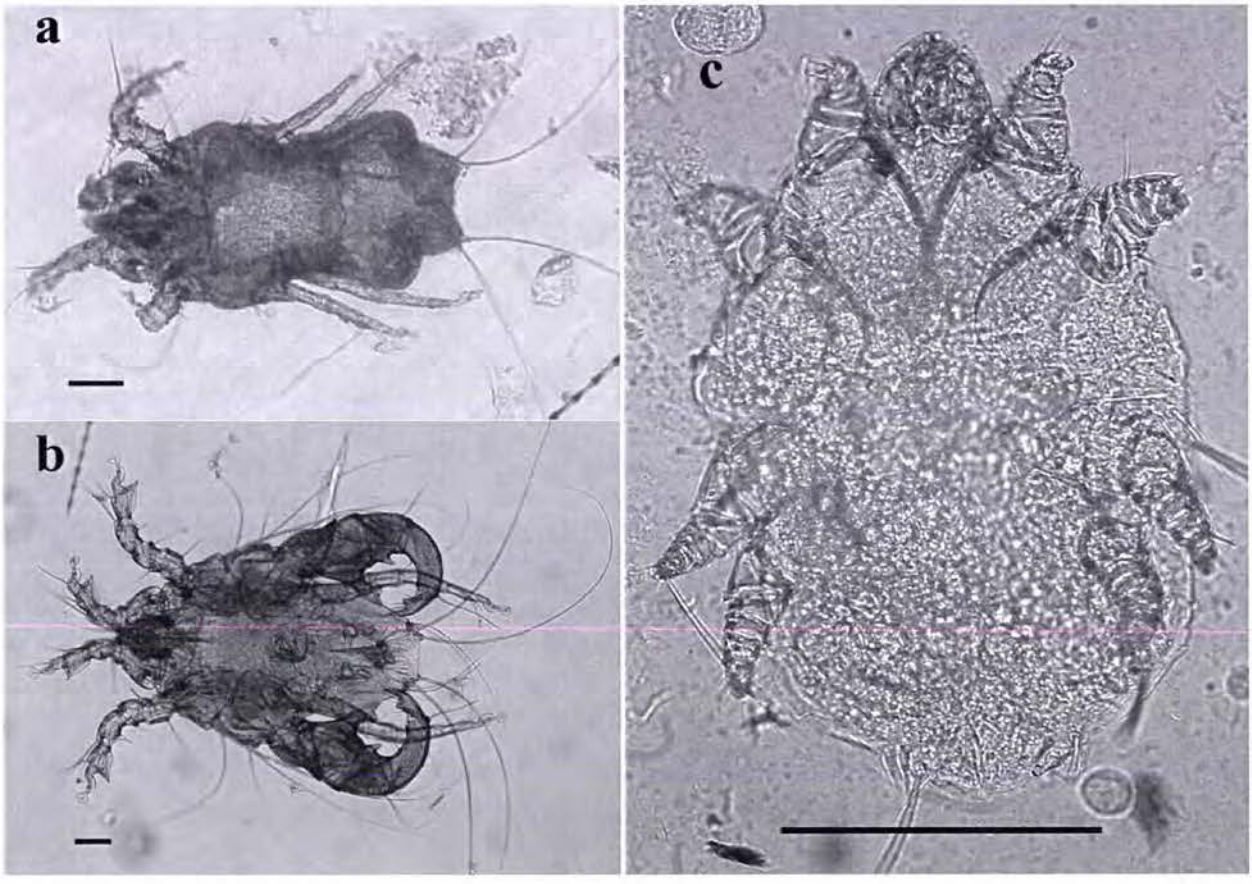

Figure 3: Various mites recovered from skin scrapings and crusts of adult male stitchbirds. Numerous feather mites were recovered as seen in **(a)** and **(b)**; these have not been definitively identified (see text for details). The burrowing mite in **(c)** is tentatively identified as *Knemidocoptes pilae* and is believed responsible for the feather-loss condition described. The scale bar in each section equals 100 μm.

In November 2002, a male with only one foot was caught and, because of his condition, he was unable to preen his head. He had extensive 3mm-thick, proliferative crusting around his eyes and head, with associated feather loss; this caused distortion of the eyelid margins and an inability to properly close his eyes (Fig. 4a). From the crusts, a large number of burrowing mites were recovered and identified from photomicrographs as *Knemidocoptes* spp (Fig. 3c). This mite has been recorded from the skin of at least two other free-living bird species in New Zealand (Bishop and Heath, 1998), and was consistent with those found in the histological skin sections described above. The one-legged bird was concurrently given an oral miticide that is known to be effective against *Knemidocoptes* (Ivermectin 200ug kg: Ivomec® liquid for sheep, 800 ug/ml, MSD-AgVet) and recaptured 5 weeks later for assessment of this therapeutic trial. At recapture, all crusting lesions around the eyes and under the feathers on his head had disappeared and his eyelids appeared to be functioning normally (Fig. 4b). This response is highly suggestive of the mites being the causative agent.

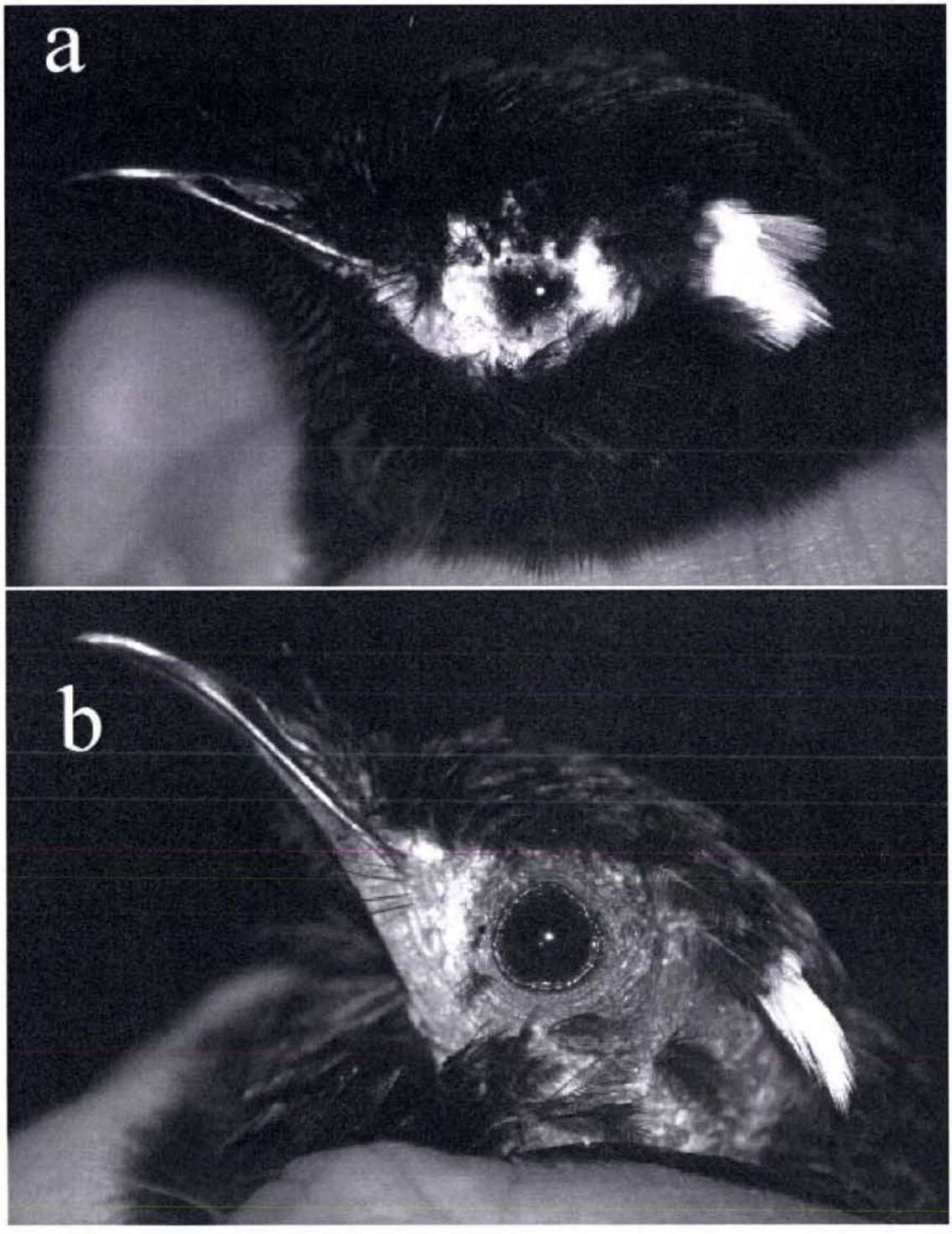

Figure 4: The male stitchbird used for a therapeutic trial of an oral miticide (Ivermectin 200 ug/kg). **(a)** shows the bird immediately pre-treatment with severe skin crusting and obvious deformation of the eyelid margins. Five weeks post-treatment the bird showed no crusting and normal function of the eyelid margins **(b)**. The skin around the eyes appeared healthy with the exception of some hyperpigmentation and feather loss.

In June 2003, 30 male, 28 female and 55 juvenile stitchbirds were caught and had their lesions described in order to provide a pre-breeding baseline to compare the post-breeding lesions collected in February 2002. Prior to breeding, lesions were relatively rare (females=10%, males=18%) and were restricted to the face and ventral beak; no ventral-neck lesions were observed. Lesion scores for each of the three areas examined (face, beak and neck) were summed and compared between the pre-breeding (June) and post-breeding (February) groups. The severity of lesions was significantly lower in June for males (June=0.18±0.06; February=3.86±0.51: Mann-Whitney *U*: Z=6.11, n_1=25, n_2=30, P<0.001) and females (June=0.12±0.07; February=1.29±0.29: Z=3.47, n_1=28, n_2 =29, P=0.003). There was no significant effect of age or sex on the distribution or severity of the lesions at this time (Mann-Whitney *U*: all P>0.39).

The weight of evidence points to knemidocoptic mites being the causative agent in the development of the lesions described in this stitchbird population; however, a wider-scale therapeutic trial, and samples from more dead specimens are needed to confirm this. The higher prevalence of lesions in males and the progression of lesions during the breeding season are suggestive of a compromised immune system as a result of seasonally high testosterone levels; there is an increasing body of work demonstrating a link between high testosterone levels and decreasing immunocompetance (Duffy et al., 2000, Duckworth et al., 2001). In stitchbirds, it is thought that their susceptibility to aspergillosis is at least in part due to a testosterone-mediated reduction in immune function (Alley et al., 1999). In other species it has been shown that there is an interaction between parasite fecundity and the host immune system that exhibits a seasonality directly related to male hormone profiles (reviewed in Folstad and Karter, 1992). Young birds appear predisposed to more extensive feather loss than their older conspecifics; this also was only evident during the breeding season. A more severe form of the disease in young birds may be because of a naïve immune system or reflect the greater stress endured by these birds during their first breeding season.

The distribution of lesions observed in the stitchbird is unusual for a burrowing mite. Symptoms of knemidocoptic mange are usually crusting lesions on the legs and around the beak and face; so called 'scaly-leg' or 'scaly-face' disease (Madill, 1987, Mason and Fain, 1988, Mainka et al., 1994, Pence et al., 1999). No leg lesions were observed during the study and while lesions did occur around the base of the beak, they

did not have the classic 'honeycomb' appearance reported in cage birds (Madill, 1987). In stitchbirds, the most common lesions were not proliferative crusts but feather loss and feather blunting (tips broken and barbs removed) with only minor skin crusting. Histology showed that although proliferative changes were present in the epidermis, only moderate inflammatory changes occurred in the underlying dermis suggesting that most of the feather loss was likely to be the result of rubbing and scratching by the bird rather than the direct effect of the mites on feather follicles. *Knemidocoptes*-induced alopecia has been reported in aviculturally raised red-crowned kakariki (*Cyanoramphus novaezelandiae*) – another New Zealand species – however, this mite was isolated from the feather calamus and not from within the skin (Shoshana and Uri, 1993).

The likelihood of stitchbirds surviving the winter was not significantly different for birds with lesions versus those without; with the trend being in the opposite direction to that expected (Fisher's exact: P=0.33). In both males and females, neither the presence nor severity of mite lesions was correlated with weight or condition of the birds at the time of examination in February 2002 (t-test: all P>0.3), suggesting that any need to treat or eradicate the mites is currently not justified. However, because the Tiritiri Matangi population is earmarked as a source population for future translocations of this species, conservation managers will need to carefully consider these results. It will be necessary to establish whether this condition exists outside of the study population prior to future stitchbird transfers, as limiting the spread of diseases is a focus of current translocation efforts in New Zealand.

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CHAPTER VI

Sub-lingual oral fistulas in a free-living population of stitchbirds (hihi: *Notiomystis cincta*)

Female ym/wy attempts to take nectar from a supplementary feeder despite having her tongue protruding from a sub-lingual oral fistula

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Chapter reference:
Low, M., Alley. M. R. & Minot, E. Sub-lingual oral fistulas in a free-living population of stitchbirds (hihi: *Notiomystis cincta*). In Prep.

Abstract

Sub-lingual oral fistulas (OF) are an uncommon, but consistently observed, pathological entity affecting the New Zealand stitchbird *Notiomystis cincta*. The lesion is typically described as a hole in the floor of the oral cavity from which the tongue protrudes below the mandible. In this study, the prevalence of OFs was measured in a free-living population of stitchbirds on Tiritiri Matangi Island during three population-surveys between 2002 and 2005. Between surveys, individuals with an OF were caught and the progress of their lesion was monitored. The majority of birds with an OF had a small localized lesion alongside the edge of the mandible without the tongue protrusion. Oral fistulas were not associated with any reduction in the bird's condition or productivity, but if the tongue consistently deviated through the fistula, it affected nectar-feeding efficiency. No fistulas were found in nestlings, but 9-10% of adult birds had some form of OF; this suggests that the fistulas develop after fledging. Repeated measures of birds show that the OFs do not progress beyond the formation of the initial hole unless the tongue protrudes from the OF; this results in continuous rubbing and erosion of the floor of the oral cavity and, ultimately, the mandible itself. Histopathology confirms that fistulas occur in the thinnest part of the oral-cavity floor, at the attachment point of the skin to the mandible. Pathology from a bird with an oral abscess at the same site suggests a possible aetiology for the condition.

Introduction

The New Zealand stitchbird (or hihi *Notiomystis cincta*) is a range-restricted endangered passerine for which past conservation efforts have primarily focussed on establishing new populations via translocation (Higgins et al. 2001). Currently, there are four free-living populations: the source population on Little Barrier Island and three small translocated populations – Kapiti Island, Tiritiri Matangi Island and Wellington's Karori Sanctuary. Previous studies have highlighted the stitchbird's susceptibility to aspergillosis and atoxoplasmosis: diseases that cause significant morbidity and mortality in both wild and captive populations (Cork et al. 1999; Alley et al. 1999). Because translocation provides a means by which disease agents can be transmitted between populations (Viggers et al. 1993), there is a requirement that diseases at the source and release locations be identified and their potential impacts understood (Jakob-Hoff et al. 2004). While much about the risks associated with aspergillosis and atoxoplasmosis is understood, there is one condition that has been widely observed in stitchbirds and yet almost nothing is known about its cause, prevalence, or effects on survival: sub-lingual oral fistulas.

Sub-lingual oral fistulas have been observed in stitchbirds since the early 1990s and were first described by Castro and Taylor (2001). In the five reported cases, they observed the bird's tongue hanging out of a large hole in the tissue comprising the floor of the oral cavity. Observations of similar tongue protrusions have now been reported in all recent translocated stitchbird populations – Mokoia Island, Kapiti Island, Tiritiri Matangi Island, Mt Bruce National Wildlife Centre (Castro and Taylor 2001; R.Collen Personal Communication) – as well as the natural population on Little Barrier Island (J. Ewen Personal communication). While Castro and Taylor (2001) used the terms 'bill abnormality' as a label for the condition, it is still uncertain whether the bill is involved at all; because of this, we have adopted the term 'sub-lingual oral fistula' (henceforth 'oral fistula' see also Castro and Taylor 2001) to describe any lesion in the floor of the oral cavity that creates an abnormal communication between the oral cavity and the external surface of the lower jaw.

Hypotheses proposed for the existence of these oral fistulas include: (1) genetic abnormality; possibly associated with small founder populations on islands where stitchbird translocations have taken place, (2) nutritional deficiencies, and (3) injury; either associated with their acrobatic feeding or aggression during the breeding season (Castro and Taylor 2001). A long-term study of the Tiritiri Matangi Island stitchbird

population provided an opportunity to evaluate predictions arising from these hypotheses and to collect data in order to better understand the prevalence, impact and progression of the abnormality over a period of several years.

Methods

Study Population

Birds in this study were observed during four breeding seasons between 2001/02 and 2004/05 on Tiritiri Matangi Island (36°36'S, 174°53'E); a scientific reserve located off the northeast coast of New Zealand's North Island. The island is ca. 220 ha in area, with stitchbirds restricted to remnant and regenerating closed-canopy forest patches totalling around 30 ha. All birds on the island were uniquely colour banded with their ages and social parentage known. Stitchbirds on Tiritiri Matangi Island breed during the spring and summer (September to February) and may lay up to three clutches of between two and six eggs (4.05 ± 0.06, $N = 32$). Stitchbirds were translocated to the island in 1995 as part of the ongoing management of the species by the New Zealand Department of Conservation where they now form a closed population. The population is small but expanding (32 breeding females in 2001/02, increasing to 53 breeding females in 2004/05) allowing all breeding attempts to be monitored. Stitchbirds feed on fruits, insects and nectar (Wilson 1997), and have a specialised tongue for extracting nectar from flowers (Higgins et al. 2001). Supplementary food in the form of a 20 % (w/v) sugar solution was provided year round at nine feeding stations and used by all birds on the island. These feeding stations were necessary due to a shortage of natural food and were situated at the forest edges and not contained within birds' territories. Stitchbirds usually require tree cavities for successful nesting; however these are not readily available on the island as the vegetation is predominantly young regenerating forest. Hence, small groups of two or three nest boxes were placed throughout potential nesting areas (78 in 2000 and 86 in 2001). Each nest box was attached to tree trunks approximately 1.5 metres off the ground and had a hinged lid which allowed easy monitoring of nesting.

Capture and measurement

During three periods of the study (Feb 2002, June 2003, Feb-April 2005), a large proportion of the stitchbird population on the island was caught and examined for the

presence of oral fistulas. In 2002, this represented 100% of the adults present; in 2003, this was 96% of all stitchbirds present; and in 2005, approximately 60% of the birds were caught and examined. Specific birds were not targeted for capture during these periods, and thus, the number of oral fistulas described is likely to represent their true prevalence in the population. Birds known to have oral fistulas were targeted for capture at other times to monitor their fistulas and note any changes; these capture periods coincided with captures for other studies (e.g. Low et al. 2005).

Stitchbirds were caught as they came to feed at supplementary feeding stations by quickly closing the door to the feeder and then extracting the bird by hand. The ability to target specific birds in this way prevented the same bird being caught more than once during each of the capture periods. Upon capture, all birds were weighed to within 0.5g using a Pesola spring balance and had their condition checked by palpation of the keel bone. During the 2002/03 breeding season, birds were weighed on a daily basis using a set of electronic scales (Weighing Systems Ltd., Nelson, NZ) to an accuracy of ± 0.5 grams (see Low 2004). The scales were attached to a hummingbird feeder containing artificial nectar so that when birds came to drink, they stood on a perch linked to the weighing mechanism. An electronic readout of the bird's weight was displayed allowing the identity and weight of each bird to be recorded by the observer. From this, changes in the weight of a male stitchbird who suffered from a tongue protrusion through his oral fistula mid-way through the breeding season were recorded.

Observations of oral fistulas

A free-ranging bird was recorded as suffering from an oral fistula if it was observed with its tongue protruding from a hole in its lower bill (the criterion used by Castro and Taylor 2001). In addition to this, we closely examined birds at capture that were not displaying any detectable tongue protrusion, and, thus, would otherwise be presumed to be 'normal'. These birds were held on their backs and the floor of the lower bill was examined in the following way: (1) a smooth-ended probe was used to lift feathers originating from the ventral feather tract – the line of feathers that extends from the tip of the mandible to the midline of the ventral neck – to expose the underlying skin, (2) the skin was then examined on both the left- and right-hand sides of the ventral feather tract, with the probe being used to stretch any skin folds that might be hiding lesions, (3) if a fistula was detected, it was determined whether it communicated with the oral cavity by looking for

the ventral surface of the tongue through the hole; only if the tissue deficit was complete was the bird recorded as having an oral fistula (4) the location of fistula was recorded and its size determined using vernier callipers ±0.05mm; any additional pathology associated with the beak was also noted, (5) if the tongue was protruding from the hole, the beak was opened and the probe was used to thread the tongue back into its proper position: exiting the oral cavity between the tips of the maxilla and mandible. From these data, birds were then assigned into one of five categories (Table 1).

Oral fistula score	Description of the lesion
OF 0	No lesion. All tissues associated with the oral cavity appeared normal.
OF 1	Full-thickness tissue deficit (fistula) in the skin of the mandible lateralized to the left or right of the midline ventral feather tract (VFT). Tongue located within the oral cavity and exited the beak as per normal.
OF 2	Fistula to the left or right of VFT. Tongue showed short or longer-term periods of protrusion from the hole; however, it spontaneously returned to its normal position within the mouth, or remained in its normal position when the tongue was manually replaced.
OF 3	Fistula sometimes restricted to the left or right of the VFT, but often the deficit incorporated the VFT or the entire floor of the oral cavity. The tongue permanently exited the oral cavity via the fistula and immediately returned to this position even if placed back inside the beak.
OF 4	Same as for OF 3, but the action of the tongue beneath the mandible had resulted in erosion of a segment of the mandible to one side of the midline.

Table 1. Categories for classifying oral fistulas as used in this study

Histological examination

The mandibles and oral cavities of five birds were examined histologically. First, the lower beaks from three male stitchbirds who died of natural causes and were not suffering from any fistulas or mandibular abnormalities; particular emphasis was paid to the area of skin and oral mucosa adjoining the mandible where fistulas appear to form. Second, the oral cavity of a female stitchbird from the Mt Bruce National Wildlife Centre that had a

chronic oral fistula incorporating the entire floor of the oral cavity. Third, the lower beak and pharynx of a juvenile male stitchbird from Kapiti Island who had died from complications arising from a 6 x 6 mm abscess in the floor of the oral cavity 12mm caudal to the symphysis of the mandible. All birds had been preserved in 10% buffered formalin soon after death. For the three males with no abnormalities, their mandible was removed, and tissue for histology in the form of a transverse section of the lower bill was cut 15mm from the anterior tip of the mandible; this is the approximate location of most oral fistulas. For the female with the oral fistula and the male with the oral abscess, a sagittal section of the head and beak was cut to provide a good cross-section of the point where the oral fistula or abscess began. All tissues were cut in 4 micron sections and these were stained with haematoxylin and eosin.

Data analysis

Data were not normally distributed; thus, non-parametric statistics were used for all analyses. Means are displayed ± 1 standard error unless otherwise indicated.

Results

During the study, 11 birds were discovered to have oral fistulas: five females and six males. Two young birds were observed with a tongue protrusion through a hole in their lower beak, but were unable to be caught for examination. Of the remaining nine birds, five had a small fistula along the medial edge of the left mandible, two along the medial edge of the right mandible, and two had lost the floor of their oral cavity at the time of first examination (Fig. 1). With one exception, there did not appear to be any active inflammation or infection associated with the fistulas: with the edges of the holes appearing to have completely reepithelialised. In the one bird with some minor pathology at the fistula, this was associated with rubbing of the tongue on the tissue of the ventral feather tract; rubbing occurred because of the angle the tongue was forced to exit the fistula.

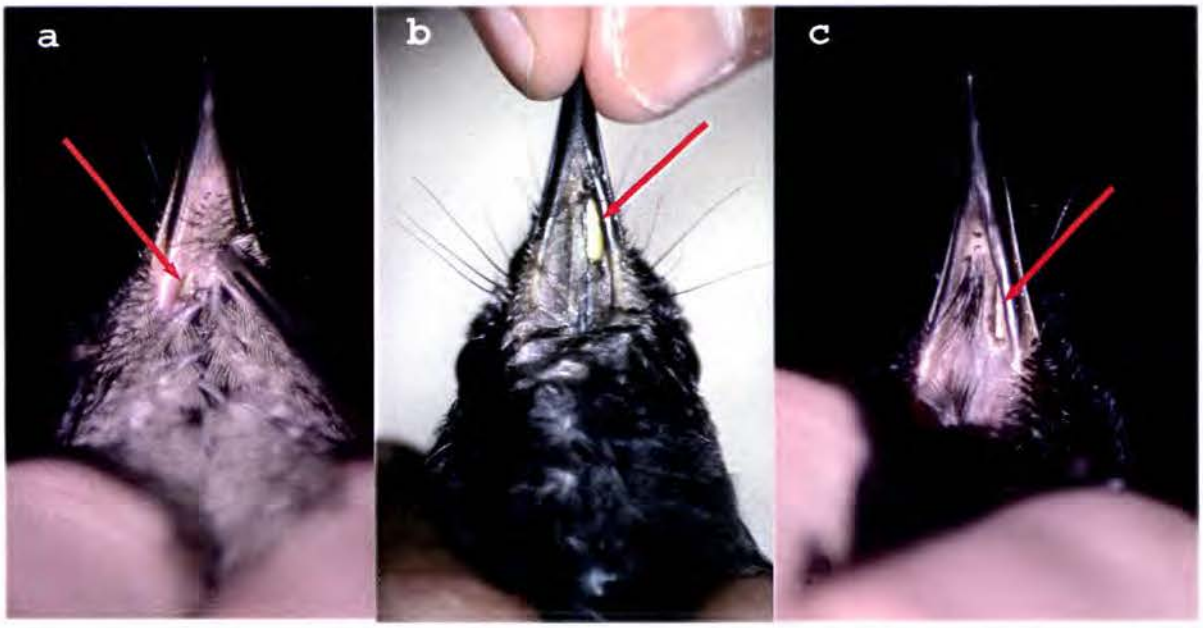

Figure 1. Ventral view of the mandibles of three stitchbirds with oral fistulas (arrows). In all cases the fistula lies alongside the bone of the mandible and does not extend beyond the midline ventral feather tract – unless the tongue protrudes and causes the fistula to open further.

Prevalence of oral fistulas

During the four years of this study, the prevalence of oral fistulas in the adult population remained relatively stable at between 9 and 10% (Table 2). While there were higher proportions of adult males with oral fistulas in all surveys when compared to females (Table 2), this difference was not significant (Fisher's exact: $P > 0.1$). Juveniles were less likely to be found with an oral fistula during these surveys, a difference which was on the margin of significance when compared to adults (2.5% versus 9.5%, Fisher's exact test: $P = 0.052$). In 2001/02, of the 103 chicks banded at 21 days of age, none had any oral fistulas at the time of examination. This was also the case for the 67 dead chicks that were recovered from inside and outside of nests. The lack of any detectable lesions between hatching and fledging was significantly different to that seen in adults (0% versus 9.5%, Fisher's exact test: $P < 0.001$).

Of the 11 birds with oral fistulas, six came from first clutch nests and five from second clutches. For 10 of the birds whose parents where still alive and were able to be caught and examined, none were ever recorded as suffering from an oral fistula during the period of the study. However, two birds were siblings from the same nest ('bm/rg' and 'bm/ry').

Population survey	Adults		Juveniles		Total	
	Male	Female	Male	Female	Adults	Juveniles
Feb 02	3/25 (12%)	2/29 (6.9%)	-	-	5/54 (9.3%)	-
June 03	4/33 (12.1%)	2/29 (6.9%)	0/22 (0%)	1/18 (5.5%)	6/62 (9.6%)	1/40 (2.5%)
Feb - April 05	3/30 (10%)	2/24 (8.3%)	0/14 (0%)	0/17 (0%)	5/54 (9.3%)	0/31 (0%)

Table 2. Prevalence of oral fistulas in adults and juveniles during the three population surveys

Temporal changes

During the study, seven of the oral-fistula birds were caught more than once; this allowed the progression of their lesions to be monitored over time (Table 3). Two of these birds were first examined at a young age when they did not have an oral fistula (21 days and 2 months). An additional four birds were first caught when their fistulas were small and not associated with any tongue protrusion. Two of these birds did not have any change in the appearance of their lesion for several years, while the other two enlarged slightly and this coincided with a stage 2 tongue protrusion (Table 3). In no cases did the fistula ever become smaller or heal over. Four of the 11 birds never displayed tongue protrusions at any time.

Bird ID	Age of the bird at examination (Oral fistula score: fistula dimensions in mm)				
Females					
gb/om	6 months (1: 1.5x1.5)				
or/m*	2 years (1)	3.5 years (1: 5.5x1.5)	5 years (2: 6x2)		
ro/gm	7 months (2)				
wm/(w)	8 months (4)				
ym/wy*	8 months (3)	1.5 years (4: 10x5)	3 years (4: 15x5)		
Males					
bg/wm	2 months (0)	1 year (2: 7x1.5)	1.5 years (1: 7x1.5)		
bm/rg*	1 year (1: 5x1)	1.5 years (1: 6x1.5)	2 years (2: 6x1.5)	2.5 years (2: 7x1.5)	3 years (3: 7x2.5)
bm/ry	1 year (1: 4x1)	2 years (1: 4x1)	2.5 years (1: 4x1)	3 years (1: 4x1)	
rm/g*	4 years (1: 4.5x1)	5.5 years (1: 4.5x1)	7 years (1: 4.5x1)		
wm/bk*	1 year (1: 3x1)				
wb/wm	21 days (0)	2 months (2)			

* Birds still alive at the end of the study

Table 3. Oral fistula scores (range 0-4) and dimensions (in mm) for the 11 birds in this study relative to their ages at examination.

Examinations of birds with tongue protrusions provided evidence for the mechanism by which the fistulas progress from stage 1 to stage 4. Birds with no reported tongue protrusion during the study had no measurable changes in their fistulas. Birds in stage 2 or 3 had wider and longer lesions; this change appeared to be because the tongue provided constant pressure and rubbing on the anterior and medial surfaces of the hole; thus, causing local tissue inflammation and necrosis and opening the hole even wider. For the birds with long-term tongue protrusions, the beak fractured along a weak point created by the action of the tongue on the underside of the mandible. In the female 'ym/wy', this continued until 11.8mm of the left anterior mandible had been eroded (Fig 2).

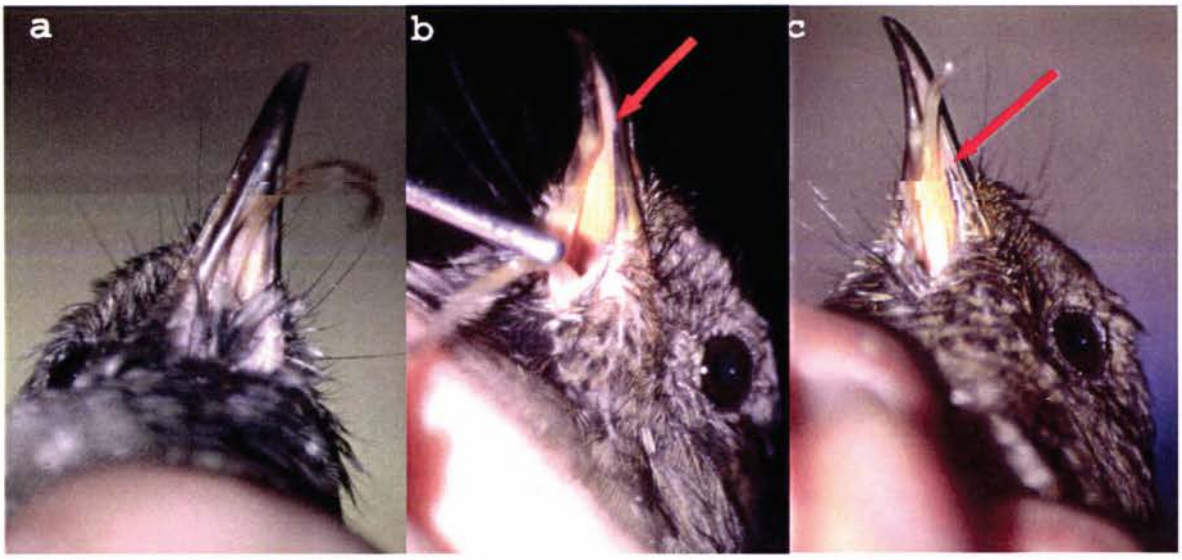

Figure 2. Gradual erosion of a section of the lower mandible due to action of the tongue in a female stitchbird (ym/wy); **(a)** ventral view of the lower bill with the tongue protruding from the fistula and creating a small fracture in the left anterior mandible (the bird is one year old); **(b)** same view 9 months later – the tongue is being held out of the way to show that a significant piece of the anterior left mandible is missing; **(c)** same view 2 year later – the erosion of the left mandible has progressed to the point where the anterior 12mm is missing: the tongue sits in this groove.

Impacts on foraging and productivity

The presence of an oral fistula did not have any measurable effect on the birds' condition at the end of the breeding season (Feb 2002; condition score 3.8 ± 0.1, n = 79 versus 3.7 ± 0.1, n = 3); immediately post-moult (April 2005; condition score 3.8 ± 0.1, n = 101 versus 4.0 ± 0.0, n = 7); or during the winter (June 2003; condition score 3.6 ± 0.1, n = 50 versus 3.4 ± 0.2, n = 5) (Man-Whitney U tests: all $P > 0.1$). All birds with oral fistulas were observed feeding on fruits, nectar and invertebrates. However, two birds with tongue protrusions (one stage 2 bird and one stage 4 bird) had markedly different supplementary feeder usage when compared to other birds in 2002/03. The female 'ym/wy' spent more than 50% of her time within 5m of the supplementary feeding station during October and November 2002, in contrast with an average of 6% for other birds. In 2005, this female was caught for examination and her faeces showed that she was capable of taking fruit: they were full of *Coprosma robusta* seeds. The male 'bg/wm' changed his feeding pattern when he progressed from stage 1 to stage 2; once his tongue protruded, the time he spent at the feeder during each visit increased threefold. During this same period his weight dropped markedly; this did not recover until he was caught and his tongue was replaced (Fig 3).

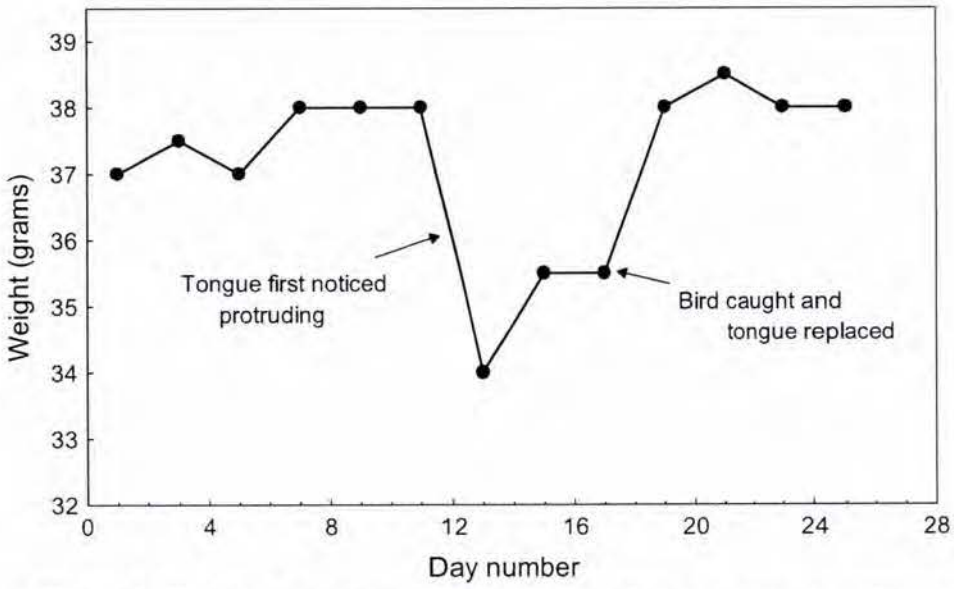

Figure 3. Weight fluctuations of male 'bg/wm' relative to a tongue protrusion.

For the six pairs in 2001/02 where one member had a stage 1 fistula, there were no differences between their productivity and that of the general population: first clutches (eggs laid, 4 ± 0.4 versus 4.02 ± 0.1; chicks fledged, 2.3 ± 0.9 versus 2.2 ± 0.3; chick weight at 21 days, 41.5 ± 1.4g versus 41.5 ± 0.5g); second clutches (eggs laid, 4 ± 0.4 versus 4.2 ± 0.2; chicks fledged, 1.2 ± 0.7 versus 1.3 ± 0.3; chick weight at 21 days, 40.8 ± 0.3 versus 40.3 ± 1.0) (Mann-Whitney U test: all $P > 0.5$). For the two females with stage 3/4 oral fistulas, they had very poor breeding success (Table 4); however, this was partly confounded by their late nesting and being the secondary female to a polygynous male.

Year & clutch	Reproductive success (eggs laid : chicks hatched : chicks fledged)	
	wm/w	Ym/wy
Year 1 clutch 1	4 : 3 : 0	-
clutch 2	2 : 0 : 0	-
Year 2 clutch 1	-	3 : 0 : 0
clutch 2	-	-
Year 3 clutch 1	-	4 : 0 : 0
clutch 2	-	3 : 0 : 0

Table 4. Reproductive success of two females with stage 4 oral fistulas

Histology

Histological examination of the mandibles of the three 'normal' males confirmed that the area where the fistula forms corresponds to where the floor of the oral cavity is its thinnest: as little as 0.2mm thick (Fig 4). These thin attachment points to the mandible are in contrast to the mid-way point between the two bones of the mandible; this area of tissue is significantly thicker as it provides the anchor point for the feathers and bristles of the ventral feather tract.

For the female stitchbird with the chronic oral fistula, the ventral skin beneath the oral cavity was continuous with the ventral oral mucosa at the caudal margin of the oral fistula (Fig. 5). Approximately 5mm caudal to the fistula opening, the skin epithelium of the throat became acanthotic (from 2-4 cells in thickness to 10-20 cells) and showed extensive hyperkeratosis. This thickening continued into the oral mucosa where the ventral oral epithelium was approximately 30 cells in thickness (normal thickness ~10 cells). The submucosa showed no significant changes. As the ventral oral mucosa extended towards the mid-line it increased further in thickness and formed extended irregular epithelial pegs; a few lymphocytes had accumulated around the submucosal blood vessels in this area. A section near the mid-line showed a small submucosal cyst (approximately 0.5 mm diameter) present in the ventral oral mucosa, 3 mm caudal to the fistula opening. No inflammatory changes were present in the submucosal tissues.

The ventral pharyngeal region of the juvenile male contained a large abscess which extended from the base of the tongue into the lower mandible and sublingual salivary glands (Fig. 6). It consisted of a central accumulation of necrotic exudate containing numerous colonies of bacteria (both Gram positive and negative coccobacilli). This was surrounded by a thick zone of fibrous granulation tissue which extended into the lingual muscle and adjacent mandibular bone. The affected bone showed varying degrees of lysis of the cortex, remodelling and periosteal proliferation of reactive bone. A more lateral section of the oral cavity contained a large nodule of ulcerating granulation tissue attached to the ventral oral mucosa. This had a small central area of ossification and cartilage formation.

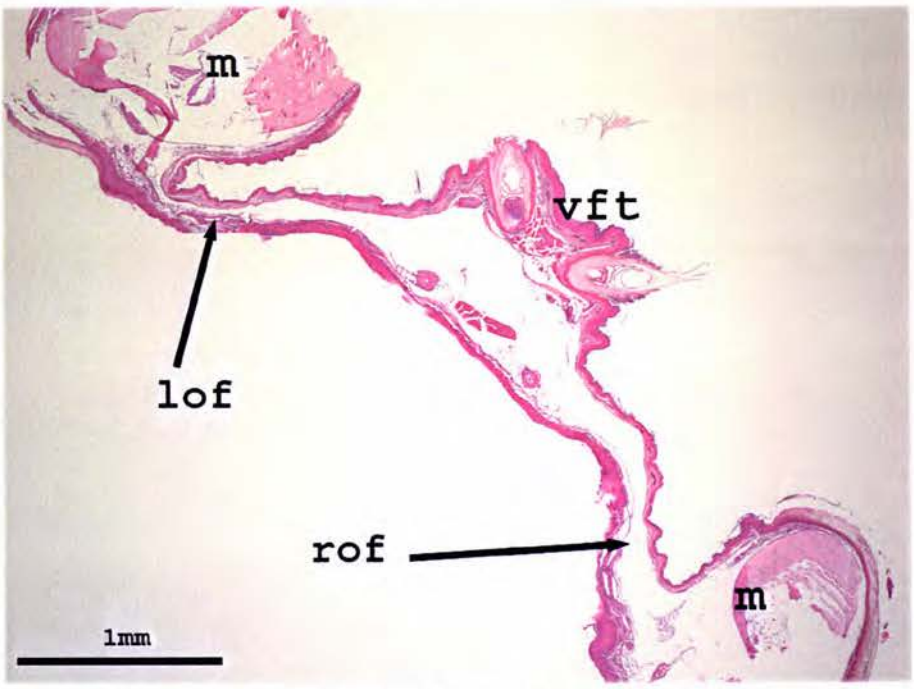

Figure 4. Photomicrograph of a haematoxylin and eosin stained transverse section of the lower jaw of a male stitchbird. The left and right mandibular bones **(m)** are joined by a thin section of tissue that comprises the floor of the oral cavity. The ventral feather tract **(vft)** runs along the midline and here the tissue is significantly thicker than at the margins of the mandible where left- **(lof)** and right-sided oral fistulas **(rof)** are found to develop.

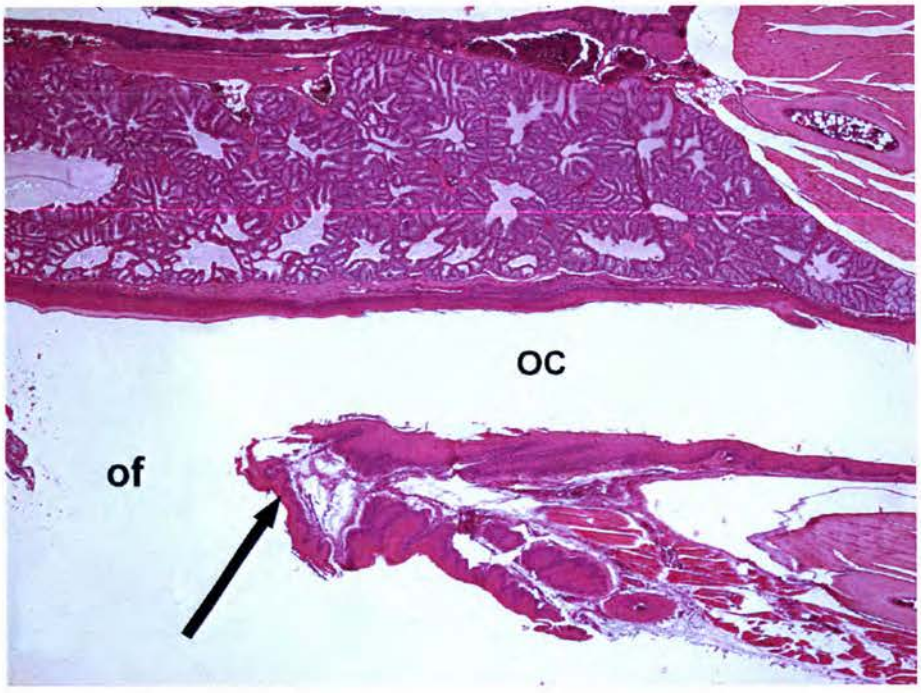

Figure 5. Photomicrograph of a haematoxylin and eosin stained sagittal section of the oral cavity **(oc)** of a female stitchbird with an oral fistula **(of)**. The arrow indicates the caudal edge of the oral fistula and the transition from the external skin of the mandible to the ventral oral mucosa.

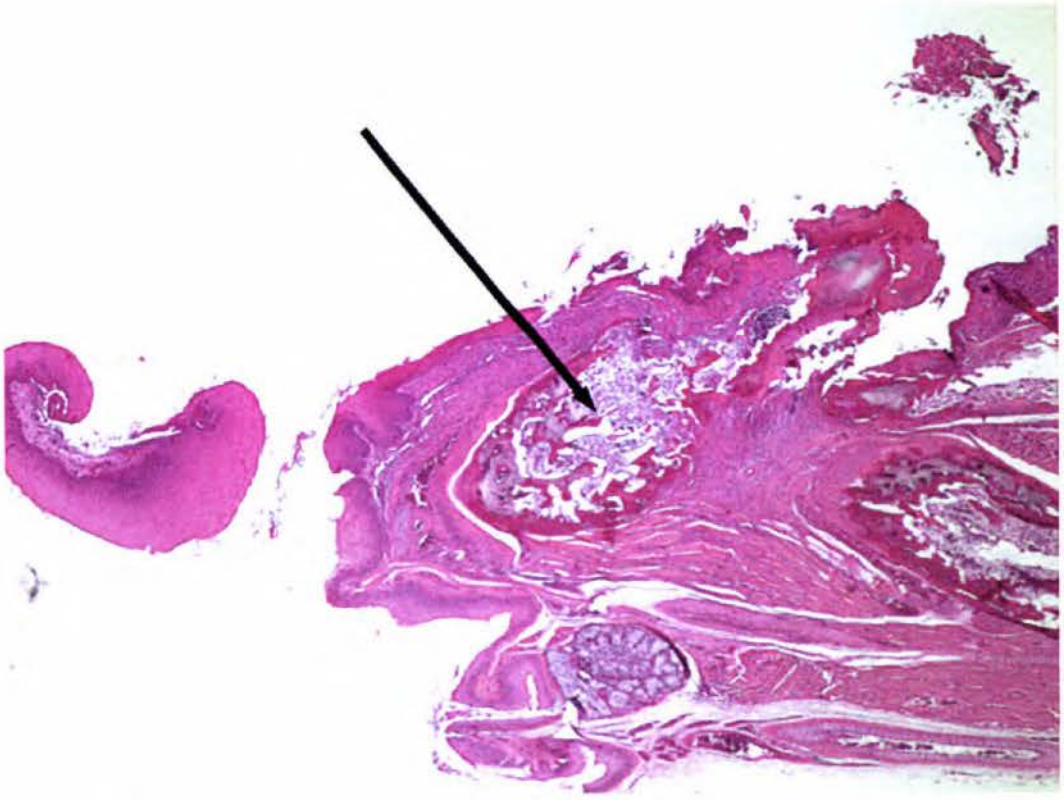

Figure 6. Photomicrograph of a bacterial abscess on the floor of the oral cavity of a juvenile male stitchbird. The arrow shows the accumulation of necrotic exudates in the centre of the abscess.

Discussion

To date, it has been generally assumed that sub-lingual oral fistulas in stitchbirds are characterised by a visible deviation of the bird's tongue through the hole in the lower bill (Castro and Taylor 2001). From our study, it is apparent that birds with a tongue protrusion represent a minority of the birds affected by oral fistulas; thus, estimates of prevalence based on sightings of birds with tongue protrusions will significantly underreport this condition.

Despite the causal factors responsible for the development of oral fistulas remaining elusive, data collected from this study has helped evaluate the three non-exclusive hypotheses proposed by Castro and Taylor (2001) in explaining the existence of oral fistulas in stitchbird populations. The first hypothesis supposes that reduced genetic variability due to inbreeding has fixed detrimental alleles within a limited gene pool; small founder populations at translocation sites were suggested as being a factor because of the observation that tongue protrusions had only been observed at translocation sites.

However, the fact that a stitchbird with an oral fistula and tongue protrusion has recently been observed at the source population on Little Barrier Island (J. Ewen personal communication) and that all four of the intensively monitored translocation sites have reported oral fistulas, are highly suggestive that the prevalence of oral fistulas in the source population is similar to that in translocated populations. Chicks and juveniles are significantly less likely to exhibit oral fistulas when compared to adults, and this, combined with the data from two birds who developed their fistulas after two months of age, demonstrate that the condition is not congenital; it develops after birds leave the nest. While this does not necessarily mean that there is not a genetic component to the disease, it does suggest that if inbreeding plays a role, a second factor is probably needed to induce the pathology. This interpretation is further supported from the lack of any genealogical association between birds with oral fistulas in this study.

The second hypothesis is that translocation sites are nutritionally deficient and that oral fistulas are a clinical manifestation of a nutritional deficiency. With the observation of the condition in the Little Barrier population – a self-sustaining population in a diverse forest habitat – and at the Mt Bruce National Wildlife Centre, where the birds are given a nutritionally balanced diet, this explanation seems unlikely. Additionally, in none of the birds examined in this study were there any clinical signs indicating a nutritional deficiency (Ritchie et al. 1994).

The third hypothesis proposed by Castro and Taylor (2001), was that oral fistulas were a result of an injury or infection associated with feeding or aggression during the breeding season. This still remains a possibility, but the mechanism by which this could occur is unclear. No adult birds were ever found to develop fistulas from one season to the next; the six birds that developed fistulas during the study were young, and the fistulas had most likely formed prior to their first breeding season. One possibility that has not been previously considered is a relationship between *Knemidocoptes* infections and the development of the fistulas. However, there is currently no evidence that birds displaying mite lesions are any more likely to suffer from an oral fistula (M. Low unpublished data). Histopathology showed that the fistulas form in the area of the oral cavity where the tissue is at its thinnest and that, once formed, there is little active inflammation / infection associated with it. The juvenile male from Kapiti with the abscess on the floor of his oral cavity, suggests a possible aetiology for the fistulas. Necrosis associated with an abscess

or insect sting, may be produce a fistula where the floor of the oral cavity is thin; however, this hypothesis is yet to be confirmed.

While we still do not know how or why the fistulas form, we do have a much clearer indication of when and where they occur as well as the progression of the disease over time. All fistulas were found to originate in the same place: against the medial surface of the mandible (on the left or right), approximately mid-way along its length (see Fig. 1). In a number of cases the fistula did not develop beyond this stage and did not appear to inconvenience the bird in any way. However, occasionally, the tongue managed to exit the oral cavity through the fistula rather than through the opening of the beak. If this occurred, the fistula began to grow in size due to the action of the tongue on the surrounding tissue. The result of this being that the fistula became so large, the tongue could not be held inside the beak and it permanently protruded through the fistula; once this occurred, a section of the mandible was eroded through the continual movement of the tongue on the underside of the beak.

The impact of this change in tongue direction – and presumably feeding efficiency – was dramatic (in the short-term, at least) for the male who progressed from a stage 1 to a stage 2 oral fistula during the 2002/03 breeding season. This was most likely because of his inability to efficiently ingest nectar from the supplementary feeders. Despite this, there was no evidence that birds with oral fistulas or tongue protrusions could not maintain their condition or body weight in the long-term. Even with half of the left side of her mandible missing, the stage-4 female stitchbird in this study was in good condition and managing to take supplementary nectar as well as fruit (as evidenced by the seeds in her faeces). However, there was some evidence in support of these stage 4 birds having difficulty in successfully hatching and raising offspring. Despite this, evolutionary selection pressures against this disease may be weak; especially when one considers that most birds will breed a number of times before a tongue protrusion has any impact on their ability to forage and regurgitate food to offspring.

The main focus of conservation medicine is to limit the spread or impact of diseases in vulnerable animal populations (Deem et al. 2002). Currently there is no evidence to suggest that sub-lingual oral fistulas are the result of an infectious disease, and thus, birds with this condition do not need to be isolated. Also, the idea that this is a disease exacerbated by inbreeding is not supported by current evidence, and, thus, additional translocations of birds as recommended by Castro and Taylor (2001) to

improve genetic diversity are currently not justified. Because a number of birds eventually do progress from a stage 1 fistula to one with a tongue protrusion – and this might impact on their ability to forage for offspring – it would be wise to inspect the underside of the bill for any bird targeted for translocation, and remove them from the group of birds to be moved. More work is still required to understand the pathogenesis of this condition in the stitchbird, and to answer the question of why it has not been reported in other species.

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Thesis Discussion

Will wildlife-health initiatives deliver what they promise?

'Conservation medicine' or 'wildlife health' is increasingly being recognised by conservation biologists as an important tool in the conservation and management of wildlife populations (Viggers et al. 1993; Daszak et al. 2000; Deem et al. 2001). Population health issues are now being included with traditional ecological measures such as biogeographical patterns, community structure, population dynamics and individual behaviour, as a vital area to be understood for conservation efforts to be effectively employed (Deem et al. 2001). National databases have been created around the world to better facilitate the communication of wildlife-health data between agencies and to allow the spread of diseases within wild populations to be better monitored (Leighton et al. 1997; Sainsbury et al. 2001; Anon 2002). Disease screening and reporting is now becoming mandatory in some countries' conservation programmes (e.g. New Zealand, Department of Conservation 2004). These initiatives indicate a positive change in attitude to the significance of diseases as a threat to the conservation of wildlife; however, there are still many issues that require clarification before veterinary expertise can be viewed as being effectively integrated into conservation biology.

A reading of the current literature on conservation medicine shows that it is almost exclusively preoccupied with limiting the spread of infectious diseases (Reed & Stockdale 1994; Daszak et al. 2000; Deem et al. 2001). While this is a laudable goal, it threatens to overshadow the role of non-infectious diseases as well as failing to address the possibility that interventions by conservation managers or researchers themselves, may create significant health issues. A case in point is the impact of using individually identifiable marks on wildlife: such as transponders or leg bands (see chapters 1 and 2). Considering the number of animals marked for study purposes each year, the number of studies that critically evaluate their impacts is vanishingly small. Identifying and quantifying risks with management techniques needs to be undertaken proactively and should be encouraged by the research community and management organisations (Calvo & Furness 1992). It is possible that in some situations, currently accepted management practices have a greater negative impact than any benefits derived from them; it is up to adherents

and practitioners of conservation medicine to look beyond the threat of disease, and broaden their approach to this field.

One upshot of this is the idea that conservation medicine itself may incur costs that outweigh its well-publicised benefits; one way this may come about is through channelling scarce funding or resources into inappropriate or non-cost-effective disease testing programmes. Are projects better off searching for unlikely, but potentially disastrous disease agents prior to translocation, or should they limit their search to probable diseases? It is generally accepted that translocations of animals should not proceed if there is a risk of disease transmission to a naïve population (Viggers et al. 1993; Jakob-Hoff et al. 2004). But if the status of a disease is unknown at the release location, what benefit is derived from testing for it at the source location; how are the results to be interpreted? While there may be a good justification for doing so, conservation biologists need to be wary of the *a priori* assumption that disease testing is always valuable. Indeed, there may be many instances where disease testing cannot be properly interpreted and thus represents a waste of time and money that could be better invested in some other aspect of that species' conservation.

Another key aspect to wildlife health programmes is the creation of 'normal' or baseline haematological and biochemical parameters to allow interpretation of data collected from sick individuals. Two questions need to be asked of these programmes; (1) what is the value of the generated reference ranges if the sampling methods and laboratory processing methods are not consistent between generation of the baseline data and the collection of data from sick animals in the field? and (2) is there a need to have a bank of these data available for all species, or is this only necessary for species where individuals are likely to be treated on a routine basis? It needs to be considered whether for some species it would be more cost-effective and efficient to generate a set of 'normal' values only if a disease outbreak occurred (if ever). In chapter 3, a number of the blood parameters generated in 1996 are effectively useless; was this exercise a waste of money?

Conservation medicine is seen as an integration of conservation biology and veterinary science; however, the two approaches may not always be in agreement over the significance of diseases. There is often an assumption in veterinary medicine that all diseases are bad, therefore, if possible, they should be eliminated from individuals or populations. In the New Zealand Department of Conservation's Wildlife Health Standard

Operating Procedures Manual (Department of Conservation, 2004), it states that animals shall not be released or transferred if they are sick or have abnormalities (p21). However, the definition of illness includes the presence of external parasites, which, as biologists know, are not necessarily pathogenic. Such wording in this document infers that there is a need to eradicate all internal and external parasites prior to translocation or release. This view of parasites is at odds with two views currently held in conservation biology. The first is that not all parasites (at least at low levels) are detrimental; indeed, in some cases ongoing low-level parasite exposure may be important in maintaining immunity against the parasite (Viggers et al. 1993). Many feather mites are non-pathogenic, and even the facial dermatitis described in chapter 5 as a result of *Knemidocoptes* has not been shown to have any significant impact on morbidity or mortality. The second concern raised by an 'eradicate all parasites' mentality, is that it does not take into account the endemic parasitic biodiversity of that vertebrate species. It has been argued that rare wildlife species should not be routinely treated with parasiticides prior to translocation as this may result in the extinction of unique parasitic fauna and a reduction in global biodiversity (Horak, 2001).

The final challenge to the fledgling field of conservation medicine is a hierarchical one. In situations where veterinarians are consulted for advice on the treatment or testing of diseases in conservation programmes, exactly who should make the final decision regarding the medical approach (McInnes & Low 2001)? Obviously the veterinarian understands the testing protocols and clinical manifestations and consequences of diseases, and may understand the epidemiology, but it is unlikely that she / he will have a good working knowledge of the specific conservation programme they are advising on. The conservation manager will better understand the logistical and financial compromises that the project will have to endure because of disease screening, but lacks the background to compare these to each step in disease testing. In such situations, who decides whether it is better to test for a rare disease or buy a new data-logger? While such a concern may appear trivial, it is a fundamental problem that will continue to plague decision-making until veterinarians with conservation and research backgrounds are incorporated into conservation programmes.

Wildlife health initiatives have the potential to deliver much to conservation biology; however conservation medicine is not an end in itself. As scientists, we must not only focus on the potential benefits, but also its limitations and costs. Viewing current

applications of conservation medicine as a panacea to be uncritically applied in all circumstances may have unintended detrimental consequences for wildlife conservation in the long-term.

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