Regent Honeyeater Disease Risk Analysis

February, 2015

Acknowledgements

The following individuals and organisations contributed to the compilation of information on which this risk analysis is based: Larry Vogelnest, Michael Shiels, Paul Andrew (Taronga Conservation Society Australia), Dean Ingwersen (BirdLife Australia), Karrie Rose (Australian Registry of Wildlife Health) and Tiggy Grillo (Wildlife Health Australia).

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Cover photo: Regent Honeyeater, *Anthochaera phrygia* by Dean Ingwersen.

Given that this process is iterative and involves incorporating the views of workshop participants it is also important to note that final hazard lists and risk levels may not reflect the views of the authors of this report.

Jakob-Hoff R, Carraro C, Sainsbury AW, Ewen J, and Canessa S, (Eds) (2014) *Regent Honeyeater Disease Risk Analysis*. IUCN SSC Conservation Breeding Specialist Group: Apple Valley, MN.

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Executive Summary

The Regent Honeyeater, *Anthochaera phrygia*, is a critically endangered species endemic to Australia that has been the subject of an intensive recovery program for some twenty years. Since 2007 a captive breed-for-release program, led by Taronga Zoo and involving up to ten other zoos and wildlife parks, has been used to reinforce remaining wild populations in south-east Australia. This captive breeding program is seen to be critical to the success of the overall recovery program. However, it is recognized by all involved that the movement of birds between captive and wild sites carries some risk of the transfer of, or exposure of birds to, infectious agents which may endanger Regent Honeyeater and other bird species at the destination sites. To mitigate these risks disease risk management practices were established prior to this Disease Risk Analysis (DRA) and are included as Appendix IV.

In general captive Regent Honeyeaters have enjoyed good health and breed well. However, in the 2013 breeding season, the finding of previously un-recorded internal and external parasites prompted concern for the potential of these organisms to threaten the health of wild populations of Regent Honeyeaters and other species at the destination site should they be transferred as a result of release of captive-bred birds. This finding prompted the commissioning of this wildlife Disease Risk Analysis (DRA), a structured, evidence-based process to identify disease hazards and, through an assessment of the likelihood of occurrence and the magnitude of potential disease consequences, identify risk management options for hazards identified as a high priority.

The process endorsed by the IUCN Species Survival Commission and World Organisation for Animal Health (OIE) was applied. This included clarifying the goal of the DRA and carefully exploring and defining the group's objectives around the management of disease risks. A wide source of information was used to inform decisions including information on current species management and husbandry practices, published and unpublished sources of information on disease susceptibilities of Australasian honeyeaters and consultation with, and elicitation of expert opinion from, a representative group of experts and stakeholders in the Regent Honeyeater recovery program - both jointly (in a workshop setting) and individually.

Key findings

Five fundamental concerns were expressed by the workshop attendees:

- The impact of disease on wild Regent Honeyeaters
- The impact of disease on captive Regent Honeyeaters
- The impact of disease on other species in the destination ecosystem
- The cost of health and disease management
- The welfare impacts of individual Regent Honeyeaters from the process of health and disease management

The group established the minimisation of these as their fundamental objectives. Consequently these should be kept in mind when evaluating hazard risk levels and management options.

Through a review of published and available unpublished sources 44 potential disease hazards that may negatively impact the Regent Honeyeater breed-for-release program were identified: 32 infectious, 8 non-infectious and 4 of undetermined cause (table 2, p.19). Of these a detailed risk analysis of five infectious hazards was completed and overall risk to the program assessed as follows:

Hazard	**Overall Risk**
Aspergillus fumigatus	*MEDIUM*
Isospora sp. coccidian	*MEDIUM*
Trypanosoma sp.	*LOW*
Salmonella sp	*LOW*
Feather lice	LOW

On the basis of this review a range of disease risk management options are identified and provide a basis for review of existing risk management protocols. The review clearly could not assess the risk from unknown hazards, for example parasites harboured by captive Regent Honeyeaters which have not yet been described - and yet evidence shows that such agents can represent a high risk of disease following translocation.

Acceptable Risk

As discussed in this report, a vital step in the disease risk analysis process is communication with stakeholders – the people with the most knowledge and experience in the management of the recovery program. Because, in most practical situations, zero risk is rarely, if ever, attainable, one of the decisions to be made by this group is the level of risk that is acceptable when weighing up the application of ideal disease risk management options against other risks to the program (see p.9). This was initially mooted at the DRA workshop and discussed more fully following the completion of the analysis. Specifically we recognised a common risk factor being accidental introduction of a novel disease with release of captive Regent Honeyeaters and the possibility that this may have already occurred. Ideally, to minimise this risk all Regent Honeyeaters in the breed for release program would be held in permanent quarantine, separate from exotic[1] birds, because contact between Regent Honeyeaters and exotic birds in zoos represents the greatest risk of disease to this program.

[1] From a disease risk perspective, if the reintroduced birds do not cross (or interact across) ecological, evolutionary or geographical barriers the number and severity of potential hazards is reduced substantially. We therefore define “exotic” in this sense: i.e. as those birds that Regent Honeyeaters do not naturally co-occur with in the wild, including birds held with captive Regent Honeyeaters who were previously held with birds from outside the natural range of this species. This definition therefore includes Australian native species, where they have been in contact with non-native species, and species non-native to Australia."

However, program managers voiced concern that the full application of such a protocol would not necessarily be in the best interests of the program overall. The managers expressed the view that the application of full quarantine conditions for Regent Honeyeaters housed in multi-species aviaries was challenging but that such multi-species environments provided the benefit of enabling the honeyeaters to interact and compete with other species as they would need to once released. The health of these birds is closely monitored by the zoos' specialist wildlife veterinarians and all illnesses and deaths fully investigated with corrective actions taken to protect the remaining birds as appropriate[2].

We recognise and acknowledge these concerns and that risk is proportional and dependent on the tolerance of those making decisions. Our recommendations should therefore be viewed in that light and considered in the context of the entire recovery program and its objectives as outlined in this report.

Recommendations

We suggest the Recovery Group consider the following three major recommendations as key to the minimisation of disease risk to this program:

1. A long-term plan to place all Regent Honeyeaters in the breed for release program, in permanent quarantine, separate from exotic birds.

2. Place increased resources into health surveillance of the free-living population of Regent Honeyeaters and monitoring the causes of morbidity and mortality.

3. Complete disease risk analyses for all the hazards identified in Table 2 to provide a comprehensive evidence-basis for all risk management decisions.

[2] Note that one of us (Sainsbury) works with several zoological collections which place animals in permanent quarantine to reduce the risk from disease in translocation. For example, fishers estuarine moth at Colchester zoo, fen raft spider at ten zoological collections across the UK, mountain chickens at ZSL, cirl buntings at a facility run by Paignton zoo staff, dormice at ZSL and Paignton zoo. Further information on these can be provided on request.

Introduction

Background to this Disease Risk Analysis

The iconic Regent Honeyeater, *Anthochaera phrygia*, is a critically endangered endemic passerine with a current estimated wild population of less than 400 birds across south-east Australia. In an effort to prevent extinction, the species has been the subject of an intensive recovery program for over 20 years. Following a small-scale trial in 2000, a captive breed-for-release program was added to recovery efforts in 2007. From a founder base of nine male and nine females, some 312 chicks had been produced by 2013 and, of these, 117 were released (Liu *et al*., 2014).

While disease does not appear to have been a significant factor in the decline of the species, resistance to disease is frequently compromised in populations with a narrow founder base. In addition, the translocation of birds between captive and wild sites always carries the risk of the inadvertent transfer of disease-causing organisms (pathogens[3]) between these sites, thereby potentially exposing translocated and resident birds and other species at the recipient site to novel organisms to which they have no innate resistance. Birds that appear perfectly healthy may also be carriers of pathogens that, under the stress of translocation, may manifest and cause overt disease (Jakob-Hoff *et al*., 2014; Sainsbury *et al*., 2012).

In recognition of these disease risks all translocated birds are given a veterinary health screen prior to transfer. As a result a number of external and internal parasites have been identified in Regent Honeyeaters although their significance is not, as yet, well understood. In common with most other critically endangered species, knowledge of the diseases of free-living Regent Honeyeaters is extremely limited. Consequently, given the critical status of the species and the on-going importance of the breed-for-release program a systematic, evidence-based disease risk analysis (DRA) was instigated by the Taronga Conservation Society Australia in collaboration with BirdLife Australia and other stakeholders.

Key Concepts of Disease Risk Analysis

(Extracted from Jakob-Hoff et al., 2014)

Risk

Risk is usually defined as the chance of encountering some form of harm, loss or damage. For this reason it has two components:

1. the likelihood[4], or probability, of something happening and, if it does happen,
2. the magnitude of consequences of the deleterious activity.

[3] The term 'pathogen' in this report includes all infectious and parasitic agents

[4] Throughout the report, we use the terms 'likelihood' and 'probability' interchangeably.

Because of the element of chance, we can never predict exactly what will happen but, through an appropriate process, we can estimate the probability of any particular outcome occurring (Brückner *et al*., 2010).

Risk Analysis

"*Risk analysis* is a formal procedure for estimating the likelihood and consequences of adverse effects occurring in a specific population, taking into consideration exposure to potential *hazard*s and the nature of their effects" (Thrusfield, 2007). It is a tool for decision makers to combine science and policy.

Disease

At the most basic level, disease is defined as any impairment of the normal structural or physiological state of an organism. The manifestation of disease is often complex and may include responses to environmental factors such as food availability, exposure to toxins, climate change, infectious agents, inherent or congenital defects, or a combination of these factors (Wobeser, 1997).

Infectious microbes are a normal part of the *ecosystem* and thus disease plays an important role in maintaining the genetic health of populations and in regulating population numbers (Smith *et al*., 2009). However, in a highly disturbed environment, where significant and relatively permanent changes from earlier ecological states have occurred, disease may threaten the survival of an entire population.

Objectivity

Risk analysis, as for all rational treatments of decision problems, combines subjective and objective elements. On the one hand, the definition of risk is inevitably subjective as risk is always defined relative to the observer (for example, to the stakeholders of a recovery program and their objectives). This subjectivity is natural and, in fact, represents the reason for conducting the DRA. On the other hand, the analysis of risk and the evaluation of the consequences represent the "science" component of a DRA, and should seek to minimise its subjectivity through rigorous estimation. Both the subjective and objective components of the DRA are essential for rational decision-making. The important aspect being the preservation of their independence ensuring, for example, objectives and estimates of consequences are not confused. For this reason, *transparency* in declaring all assumptions made is essential (MacDiarmid, 2001).

Acceptable risk

The risk communication process is essential in helping decision makers to deal with one of the most difficult problems encountered during the risk analysis process; namely, determining what constitutes an 'acceptable risk' (MacDiarmid and Pharo, 2003). Deciding whether or not a particular risk is acceptable depends on the objectives and risk attitudes of the involved stakeholders (MacDiarmid and Pharo, 2003; Thrusfield, 2007).

Assumptions

A risk assessment may sometimes be criticised because some of its inputs are based on assumptions. However, all decision-making is based on assumptions, and uncertainty and subjectivity do not mean that valid conclusions cannot be drawn. Even though many of the inputs of a risk assessment are surrounded by uncertainty, one may be able to have confidence that the 'true risk' is unlikely to

exceed the estimate resulting from a careful and conservative analysis (MacDiarmid, 2001).

Uncertainty

As in all complex situations not all the relevant facts are available when dealing with wildlife disease. As noted above, more often than not, available data are scant. Consequently, qualitative analysis is the most common approach used. A comprehensive literature review, the use of appropriate analytical and decision making tools and the explicit recording of assumptions and limitations will ensure the best use of available information, identification of significant data gaps for further research and the level of uncertainty decision makers should take into consideration.

Method Used to Conduct this DRA

The process followed for this wildlife DRA was a combination of that described by Jakob-Hoff *et al*. (2014) and Sainsbury and Vaughan-Higgins (2012). This is summarised in Figure 1 and forms the structure of the analysis that follows.

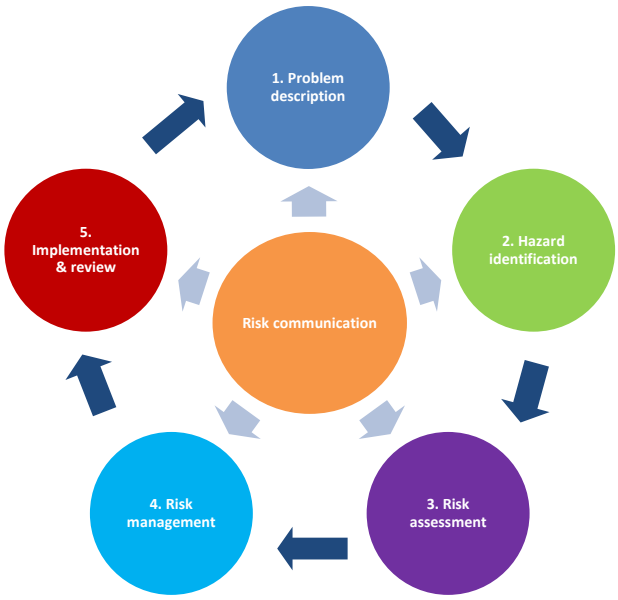


Figure 1: DRA process steps.

The risk analysis was developed between September 2014 and February 2015 using the staged, consultative process DELWPcted in figure 2.

Key stakeholders and experts were identified at the beginning of the process and provided the source of much of the information needed to complete this DRA. Initial desktop data gathering comprised a review of published and unpublished information on the diseases of Australian honeyeaters (Meliphagidae) and the management of captive (*ex situ*) and free-living (*in situ*) Regent Honeyeaters. Detailed preliminary risk assessments were also developed for three pathogens identified by the experts as of particular concern: *Aspergillus fumigatus*, *Isospora lesouefi* and *Trypanosoma* spp. Following discussion with project stakeholders, to these were added feather lice, an unidentified coccidian parasite

observed circulating in white blood cells and *Salmonella* spp. (see Hazard Identification below).

Figure 2: Staged consultative process used to conduct this risk analysis

This information was distributed to thirteen stakeholders who subsequently met with the authors to review and extend this information in a formal two-day workshop held to solicit expert opinion. This event was hosted on 15 – 16 October 2014 by Taronga Zoo and facilitated by the authors.

Regent Honeyeater workshop participants at Taronga Zoo

L to R (back): Stefano Canessa, Dean Ingwersen, John Ewen [behind] Rupert Baker, Claudia Carraro, Glen Johnson, Larry Vogelnest, Peter Menkhorst, Richard Jakob-Hoff; (front): Tony Sainsbury, Tiggy Grillo, Cheryl Sangster, Andrea Reiss, Frances Hulst, Michael Shiels. (Missing from photo: Peter Christie, David Geering, Jan Slapeta, Judith Gillespie)

The workshop enabled stakeholders to clarify their concerns as clear objectives to help drive hazard identification and assess management decisions, pool their knowledge, further identify possible hazards, consider options for risk management and identify information gaps.

The authors subsequently extended the initial review by incorporating the information generated at the workshop and further exploring the literature to complete the risk analysis as thoroughly as possible within the constraints of available resources.

A draft was circulated to workshop participants and their feedback taken into consideration in completion of this final report. Given that this process is iterative and involves incorporating the views of workshop participants' final hazard lists and risk levels may not reflect the views of the authors of this report.

DRA goal, scope, focus and question

The parameters of the disease risk analysis were discussed with stakeholders during the workshop and were defined as follows.

DRA Goal:

Using the knowledge and specialist expertise of key stakeholders, develop a disease risk management strategy for the Regent Honeyeater recovery program based on a structured, evidence-based analysis of currently available information.

DRA Objectives:

Throughout this DRA, we focus on the relevance of disease risks to five key aspects of the recovery program:

- The impact of disease on wild Regent Honeyeaters
- The impact of disease on other species in the destination ecosystem
- The cost of health and disease management
- The welfare impacts of individual Regent Honeyeaters from the process of health and disease management

It is assumed that risk management actions aim to minimise the above.

DRA Scope:

The scope, within the constraints of time and other resources, is confined to a qualitative analysis of relevant published and unpublished information on the susceptibilities of Regent Honeyeaters (and other honeyeaters of the family *Meliphagidae*) to infectious and non-infectious disease hazards taking into consideration the species' population biology, threats to survival and current conservation management practices and the impacts of these hazards on the objectives listed above.

DRA Focus:

The focus is the identification, assessment and mitigation of health risks associated with the captive-to-wild component of the breed-for-release program for this species. This includes consideration of all Regent Honeyeaters across all institutions.

DRA Question:

What is the risk from disease arising from identified health hazards, as a consequence of captive-to-wild translocations, that constitute a threat to the recovery of free-living populations of the Regent Honeyeater and how can this risk from disease be minimised?

Assumptions and Limitations

Given the scarcity of data on wildlife disease a wildlife DRA should always be considered a work in progress, particularly where management decisions are required continuously over a prolonged timeframe. As noted above, all decision-making involves some assumptions and is limited by various constraints. Making these assumptions explicit provides the level of transparency needed to identify further research and enable future refinements of the DRA as more information and resources come to hand.

Assumptions

- Regent Honeyeaters are susceptible to the full range of health hazards recorded to date in the *Meliphagidae*.
- The available data combined with the analytical and decision-making processes used by the experts involved in this DRA will enable reasonable decisions to be made to minimise health risks to the Regent Honeyeater program.

Limitations

- Due to time and other resource restrictions, the scope of this DRA is limited to consideration of disease risks associated with captive-to-wild translocations only.
- There is relatively little information on disease susceptibilities of Regent Honeyeaters and other honeyeaters.
- The individual, population and ecosystem level consequences of many of the diseases to be considered are also unknown.
- No comprehensive health/pathogen screening has been done on free-living Regent Honeyeaters.
- Not all potentially pathogenic organisms can be identified using currently available diagnostic techniques.
- The reference data used to evaluate health (e.g. normal range of blood and biochemistry values) for Regent Honeyeaters is quite limited.
- Some diagnostic tests (e.g. serology) have not been scientifically validated for this species and the level of uncertainty in their results is often unknown.
- The pharmacokinetics and pharmacodynamics of drugs that may be used for disease treatment has not been conducted for this species and extrapolation from other species is necessary.

Problem Description

Species biology

Regent Honeyeaters are a medium-sized (20 – 24cm), critically endangered endemic passerine (total population 350 – 400). Formerly distributed throughout temperate woodlands and forests in south-eastern Australia, from the Adelaide region (South Australia) to 100 km north of Brisbane (Queensland), there has been a continuing contraction in the Regent Honeyeater's range with breeding currently confined to four known sites in Victoria and New South Wales (Franklin *et al.*, 1989; Garnett *et al.,* 2011; Regent Honeyeater Recovery Team unpublished data).

Photo: Dean Ingwersen

Feeding on a diet of nectar and insects and generally nesting in the mid-upper canopy, the birds are dependent on box and ironbark eucalypts inland of the Great Dividing Range, and wetter coastal and riparian forests comprised of she-oak, swamp mahogany, and spotted gum. Reaching sexual maturity within one year the birds form monogamous pairs making cup-shaped nests from dry bark, grass and spider webs and aggressively defending their breeding sites. Lifespan of wild birds is estimated at 10 years while captive birds have been recorded to live up to 17 years. The species is nomadic and ranges widely outside the breeding season, with captive-bred birds having travelled 40 - 100km from the release sites.

Causes of decline

The major cause for the species' decline has been the clearing and fragmentation of woodland and forest containing the birds' preferred tree species. Whilst clearing directly reduces the amount of habitat available, it can also make remaining remnants unsuitable as they become too small or isolated. The major continuing threat is further degradation of habitat, particularly on-going insidious reductions in habitat quality and lack of regeneration. Noisy Miners (*Manorina melanocephala*) and other aggressive species become more common in fragmented and degraded habitat (due to their preference for open areas adjoining woodland) and exclude birds, including Regent Honeyeaters, from many native vegetation remnants.

Recovery strategy (Ingwersen *et.al.* in prep.).

A recovery program was established in 1994 adopting a strategy focussed on:

- Protection and restoration of habitat
- Population monitoring
- Research to support the recovery strategy
- Captive breeding as insurance and for release
- Raising community awareness and support

Captive breeding

The species breeds well in captivity often laying two to three (or more) clutches per season. Of the 62 pairs established at Taronga Zoo since the start of the recovery program, 42 have bred successfully, producing 205 chicks (Gillespie, 2013). Some 350 chicks have been raised and 117 birds released from the program up to 2013.

Table 1: Captive institutions involved in Regent Honeyeater management

	ADELAIDE	BEERWAH	CUDLEE	CURRUMBIN	GOSFORD	HEALESVILLE	MELBOURNE	PEARCEDALE	SUMMERTOWN	TARONGA	TIDBINBILLA
HELD	98	6	4	9	10	2	28	2	18	325	6
BRED	76	0	0	2	0	0	16	0	3	256	3
Start date	1 0ct 95	16-Sep-03	4-Apr-00	16-Oct-02	16-Nov-00	19-Nov-13	23-Apr-03	6-Aug-13	5-May-03	28-Sep-95	11-Sep-99
End date		4-Mar-12	31-Dec-04	28-Aug-08	17-Oct-13						19-Jan-03

Led by Taronga Zoo, the captive breeding program was initiated in 2007 and has involved 11 captive breeding sites in Australia under the Australasian Species Management Program (Table 1). The founders for the captive population were nine males and nine females sourced from the Chiltern area of Victoria and Capertee Valley and Cessnock in New South Wales. The captive birds are managed as metapopulations of the wild population requiring periodic supplementation of free-living birds for genetic management.

The 53 dedicated spaces available through the collaborating zoos in 2013 (Gillespie, 2013) is insufficient to enable the recovery plan goals to retain 90% wild heterozygocity and a maximum inbreeding coefficient of 0·125. Consequently the population will require occasional introduction of additional founders sourced from the wild for the duration of the recovery program. [This is an important distinction between an 'insurance' program, with an implied closed population, and an *in situ–ex situ* meta-population where the role of zoos is to amplify the wild population, not to preserve it indefinitely (Lees & Wilcken, 2009)].

Captive Management

Extracts from Liu et al (2014)

The aviaries (at Taronga Zoo where the majority of breeding occurs) are fitted with removable partitions that enable birds to be flocked in the non-breeding season and separated for the spring pairing. Rodent-proof mesh of 12·5mm × 25mm, with a gauge of 1·6 mm, is used to construct the enclosures. Most interiors have a natural substrate and are provisioned with dense foliage,

primarily tea-tree brush Melaleuca spp. Breeding has been successful in a variety of aviaries including those with concrete floors covered in mulch and pot plants. Breeding has also occurred in a multi-species walk-through enclosure housing a number of honeyeaters but, in general, success is greater when pairs are provided with dedicated aviaries.

Captive birds are provided with a balanced frugivore and insectivore diet (Wombaroo© nectar), sliced oranges and a variety of live insects, including Mealworms (*Tenebrio molitor)* and crickets (*Acheta domesticus*) supplied by Pisces Enterprises©, House-fly (*Musca domestica)* maggots bred on site and moths. During the breeding season a calcium supplement (Rep-Cal©) is dusted onto the insects. To encourage natural foraging, favoured flowering trees and shrubs are planted in the aviaries and provided as cut browse when available. Foraging studies at Taronga have shown that body mass and fat deposition in both sexes increase through autumn, which is consistent with other Australian honeyeater species (Munro & McFadden, 2005a).

Chicks fledge at 2 weeks, gaining independence at just over a month, at which point the male will resume calling and drive the hen back to the nest. In the wild at this time, the male chases fledglings further away from the nest site (Higgins *et al*., 2001). Spatial constraints in captivity mean that, once the first nest fledglings are feeding independently, they must be relocated into a large juvenile crèche to protect them from the male's aggression. Re-clutching may occur 30 days after chicks fledge. Given the opportunity to form a pair early in the season, females can lay three or, rarely, four successful clutches.

Acclimatization and Release

Current practice is to maintain birds at the release site in soft-sided dome tents for between 24 and 72 hours to allow them to acclimatize to the area. The period before release, which involves catching the birds for quarantine, health screening and confinement during travel of up to 9 hours, is stressful and can result in weight loss. Therefore, the pre-release tents provide a good environment for re-establishing weight prior to release, because substantial free food is supplied while the birds are being acclimatized. Browse and perches are easily sourced from nearby local flowering eucalypts to assist honeyeaters to adapt to the 'local' food plants and adjust to local temperature fluctuations. (The main release site at Chiltern is usually 4–6°C cooler during the day, and can be up to 10°C cooler at night, than the temperature in Sydney, which is where all birds are marshalled and quarantined). During the pre-release period keepers and release staff

Photo: Chris Tzaros

monitor the birds to ensure locomotion and natural behaviour are not affected after transmitter harnesses are fitted.

Post-release monitoring since 2008 has not revealed any mortalities occurring in the first 72 hours after release. In all releases since 2008 survival 10 weeks post-release has been conservatively estimated to be above 70%, which is much higher than releases of other avian species in Australia (Regent Honeyeater Recovery Team, unpublished data). Monitoring of birds released in 2008, 2010 and 2013 established that post-release survival was not influenced by age or sex and that birds showed no significant change in condition post-release, indicating a rapid acclimatisation to life in the wild.

At the time of writing, five captive-bred individuals have been observed three years post-release and one four years after release. There have been several captive-captive and captive-wild pairings that have resulted in nest building, egg laying and hatching although few fledglings from these pairings have been recorded to date (Regent Honeyeater Recovery Team, unpublished data).

Current Disease Risk Management

"Taronga provides a central marshalling point prior to release. Screening for, and evaluation of, potential infectious agents in the Regent Honeyeaters are carried out as part of the pre-release strategy" (Liu *et al*., 2014). Current disease risk management protocols for Taronga Zoo are described in Appendix IV.


Photos: Taronga Zoo

For the 2013 breed-for-release cohort, all captive birds selected for release were dusted for lice before transport for release (a two-step process, where birds were dusted two weeks apart), and similarly all birds were treated for cestodes (another two stage, two week process). No treatment for the identified blood parasites was administered.

Free-living Regent Honeyeaters are captured using mist nets and, during this process, by-catch (other honeyeaters and woodland bird species) are routinely captured and handled during extraction from nets. Faecal material is often expelled onto the extractor's hands during this process. Regent Honeyeaters are placed in calico holding bags (to reduce stress and risk of injury) and carried to the processing station (usually not further than 200m from the nets). Individuals of by-catch species are released immediately. Calico bags are not re-used in a banding session - they are removed from each field session once used and not reintroduced until they have been washed. This involves soaking the bags overnight in a solution of NapiSan (active ingredient sodium percarbonate) followed by a full cycle in a washing machine using standard detergent mixed with NapiSan. During fieldwork extractors' hands are washed if faecal material

is encountered while handling birds although, at times, this is not possible until after birds have been handled with dirty hands (i.e. removal is required before hands are cleaned).


Photo: Mick Roderick

Following banding and morphometric measurement, Regent Honeyeaters have blood samples collected for DNA analysis. This is performed by field personnel. As only a small volume of blood is required the sample is collected by stabbing the brachial vein with a sterile 27 gauge hypodermic needle. A dab of Vasoline is placed on the venepuncture site prior to sampling to assist in droplet formation, making collection easier. Following venepuncture blood is withdrawn into microhaematocrit tubes by capillary action. After sampling, light pressure with fresh cotton wool or tissue is applied to the puncture site to assist clotting. Sampled blood is immediately transferred to an Eppendorf tube containing 99% ethanol. Once the puncture site has clotted an antiseptic wipe is used to clean the sampling site, the bird is assessed in the hand for any negative impacts of handling and, if none are observed, released.

Weighing cones and rulers, callipers and associated equipment are regularly wiped with 90% ethanol during field work. All captured birds are released at the site of capture within 1 hour of capture, but usually within 15 minutes.

Hazard Identification

Sources of Information

Information on the potential infectious and non-infectious disease susceptibilities of Regent Honeyeaters was limited to diseases of Australasian honeyeaters, principally members of the family Meliphagidae. Information was sourced from:

- A review of published literature sourced through the Web of Science database
- The Australian Registry of Wildlife Health (ARWH)
- Wildlife Health Australia electronic Wildlife Health Information System (WHA eWHIS)
- A survey of captive holders of Regent Honeyeaters[5]
- The expert knowledge of participants in the stakeholder workshop held at Taronga Zoo 15-16 October, 2014

The current list is provided in Table 2. Prioritization of hazards was based on a systematic review of a preliminary list of 40 identified hazards by an expert panel at the Taronga Zoo workshop. This panel was asked to use a rank of low, medium or high for each of these hazards in terms of

- the likelihood of exposure and
- the impact or magnitude of consequences if exposed

Hazards were considered in relation to three potential 'at-risk' populations:

- Captive Regent Honeyeaters
- Wild Regent Honeyeaters exposed to captive-bred Regent Honeyeaters
- Other wild birds at the destination sites exposed to captive-bred Regent Honeyeaters.

On this basis, the following disease hazards were selected for detailed risk analysis:

Aspergillus fumigatus	The most common infectious cause of mortality in captive Regent Honeyeaters.
Coccidia	*Isospora lesouefi* commonly found in faeces of healthy Regent Honeyeaters. A coccidian also recently detected in white blood cells in a small number of captive Regent Honeyeaters; not known to occur in wild Regent Honeyeaters or other birds at release sites was also considered. Intestinal coccidia are pathogenic in some honeyeater species; the clinical significance of the systemic form is currently unknown.
Trypanosoma spp.	Also recently detected parasite in captive Regent Honeyeaters. Current uncertainty around species identity or if this species occurs in wild Regent Honeyeaters or other birds at release sites. Clinical significance unknown.
Feather lice	A recently detected external parasite in captive Regent Honeyeaters. Although clinical significance is generally low in other species these parasites have not been fully identified or observed in wild Regent Honeyeaters to date.

[5] Majority of data from Taronga Zoo courtesy Dr. Larry Vogelnest and Zoos South Australia (ZSA), courtesy Dr. David McLelland

Salmonella spp.	A known pathogen of a wide range of species, including honeyeaters, and recent mortalities recorded in other species sharing an aviary with Regent Honeyeaters at Taronga Zoo.

The expert panel hazard rankings - and the rationale on which they were based - are provided in Appendix II.

Table 2: Infectious and non-infectious hazards recorded in Australian honeyeaters

Hazard	References	Hazard	References
Infectious hazards		**Non-infectious hazards**	
Feather mites* (*Trouessartia* spp., *Anharpyrhynchus* spp.)	Bochkov and Klompen (2014); Vogelnest L. *pers.comm.*	Metabolic bone disease*	ARWH (2014)
Nasal mites (Rhinonyssidae?)	Vogelnest L. *pers. comm.*?	Malnutrition (includes vitamin D toxicity & thiamine deficiency)	O'Sullivan (2007), Holz *et al.* (2002)
Feather lice* (*Menacanthus* and *Brueelia* spp.)	Vogelnest L. *pers. comm.*	Neoplasia*	ARWH (2014), ZSA (2014)
Ticks (*Ixodes* spp.)	O'Sullivan (2007)	Transmitter harness injury	Liu *et al.* (2014)
Proventricular nematodes (ID unknown)	ARWH (2014)	Trauma/Predation/Misadventure	ARWH (2014); ZSA (2014), WHA eWHIS (2014)
Cestodes* (ID unknown)	Vogelnest L. *pers. comm.*	Foreign body/obstruction	ARWH (2014)
Trypanosoma spp.*	Vogelnest L. *pers. comm.*	Toxins (including snake bite)	ARWH (2014), WHA eWHIS (2014)
Isospora spp.*[6]	Morin-Adeline *et al.* (2011); Yang *et al.* (2014); ZSA (2014)	Developmental (not specified)	ARWH (2014)
		Diseases of Unknown Cause	
Other systemic protozoa (ID unknown)	ARWH (2014)	Embryonic death*	ARWH (2014)
Microsporidia	ARWH (2014)	Cardiovascular (cardiomyopathy/avascular necrosis)*	ARWH (2014)
Encephalitozoon hellum	ARWH (2014)	*Dermatitis/hyperkeratosis /acanthosis**	*ARWH (2014),* WHA eWHIS (2014)
Plasmodium spp.	Peirce *et al.* (2004); ARWH (2014); Baillie and Brunton (2011)	Egg peritonitis	ZSA (2014)
Haemoproteus spp.	Bennett *et al.* (1994a)		
Leucocytozoon **spp**.	Bennett *et al.* (1994)		
Avipox virus	ARWH (2014); WHA eWHIS (2014)		
Salmonella spp.	Vogelnest L. *pers. comm.*; Ewen *et al.* (2007)		
Yersinia spp.	ZSA (2014)		

[6] "Atoxoplasmosis" is caused by some species of *Isospora* which have a phase that is extra-intestinal, circulating in monocytes. This genus has been synonymized with *Isospora* (Barta *et al.*, 2005), although a great deal of confusion still surrounds its taxonomy. However, the transmission pathway remains faecal-oral and, consequently the two forms of the parasite are considered together in this risk analysis.

Non-haemolytic E.coli	ZSA (2014)
Pasteurella multocida	ZSA (2014)
Mycobacterium avium	Vogelnest L. *pers. comm.*
Klebsiella pneumoniae	Vogelnest L. *pers. comm.*
Klebsiella oxytoca	ARWH (2014); ZSA (2014)
Aeromonas hydrophila	ARWH (2014)
Proteus mirabilis	ARWH (2014)
Streptococcus sp.	ARWH (2014)
Enterococcus sp.	ARWH (2014)
Aspergillus spp.*	Cork *et al.* (1999); Perrot and Armstrong (2011), ARWH (2014) ZSA (2014)
Candida spp.	ARWH (2014); ZSA (2014)
Candida albicans	ARWH (2014)
Mucor spp.	ARWH (2014)
Penicillium spp.	ARWH (2014)
Pantoea aglommerans	ARWH (2014)

*(*identified in Regent Honeyeaters)*

ARWH = Australian Registry of Wildlife Health; ZSA = Zoos South Australia mortality records

Risk Assessment for Priority Hazards

Disease risk assessments were conducted on hazards of particular concern to the recovery group on the basis that they were either a common cause of mortality (*Aspergillus fumigatus*), a frequent diagnostic finding (*Isospora lesouefi*) or recently identified organisms in Regent Honeyeaters that might present a risk, through novelty, to the free-living wild populations of birds at destination sites (*Trypanosoma* spp., systemic coccidia, feather lice and *Salmonella* spp.).

In all translocations there is a risk that animals to be moved may carry infectious organisms from the source environment that are novel (and therefore potentially hazardous) to animals at the destination site or may encounter hazards *en route* to, or at, the destination site to which they have not been previously exposed. The stress involved in the translocation process may also compromise the animal's immune status such that organisms that are normally present in or around the animals can cause disease. Hazards are classified by Sainsbury *et al*., (2012) as:

Source hazards: Those infectious agents for whom the animal to be reintroduced is a potential vehicle to the destination. These hazards are non-native to the destination or, if native, are of a different strain.

Transport hazards: Infectious agents present on the journey from the source to the destination site which may be novel to Regent Honeyeaters. Translocated animals can be a potential vehicle for introduction of these hazards to the destination site. Transport hazards are also those infectious agents moved with materials such as transport boxes, equipment, food and water.

Carrier hazards: These are commensal infectious agents to which the source population has co-adapted and co-evolved but which, when the host is subjected to stressors, such as those associated with translocation, or factors which affect parasite dynamics, such as alterations in host density, cause disease in animals at the destination site or during transit. Such hazards are effectively "carried" from the source to the destination population.

Destination hazards: Those infectious/non-infectious agents present at the destination site but not known to be present at the source.

Figure 3 identifies the points on the translocation pathway at which the host and disease hazard may interact and are referenced in the risk assessments that follow.

We assessed the risk of disease from hazards based on release, exposure and consequence assessment.

In the release assessment we determined the likelihood that Regent Honeyeaters from the captive breeding program will be exposed to, and infected with a hazard and described the pathway necessary for the hazard to be released into the destination environment.

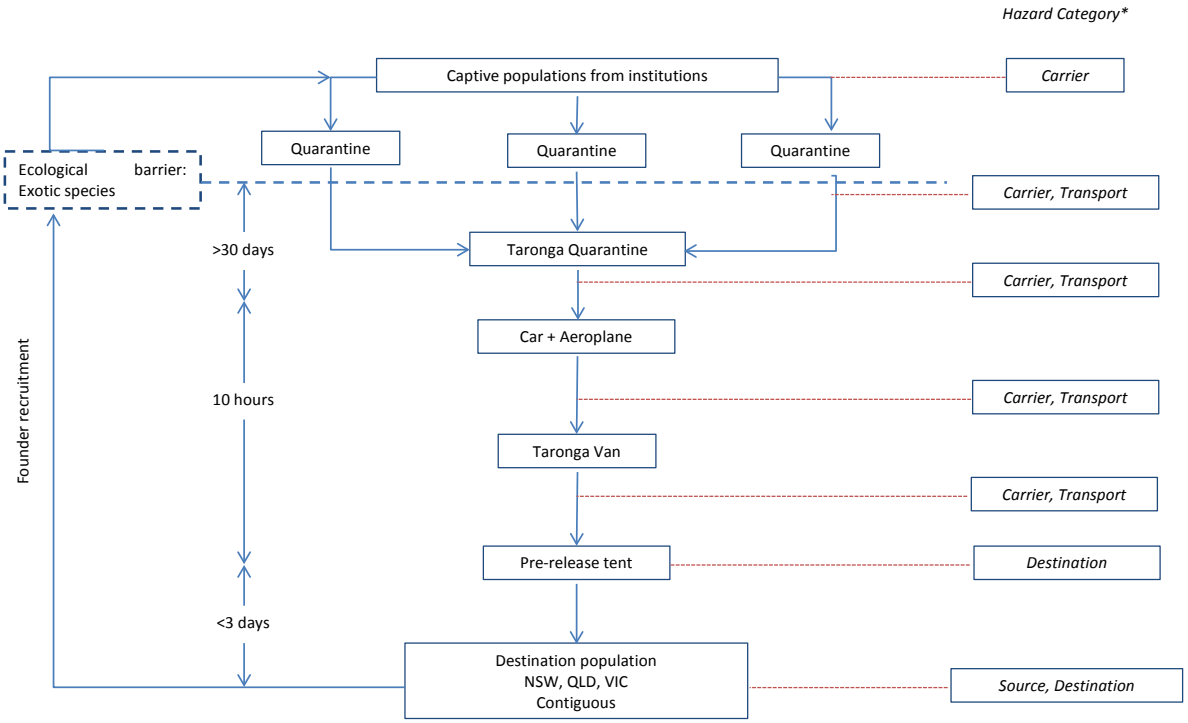
In the exposure assessment we described the biological pathway that might permit an animal at the destination to be exposed to the hazard, and for the hazard to be disseminated in the destination environment, and we estimated the likelihood of this occurring.

In the consequence assessment we assessed the likelihood and severity of biological, environmental or economic consequences associated with the entry (source, carrier and transport hazards), establishment and spread of the hazard.

Finally, risk estimation, based on the method of Murray *et al*. (2004), used the combined results of the exposure, release and consequence assessments to qualitatively assess the risk of disease associated with the hazard (negligible, low, medium or high).

As a final step, in the Risk Management part of our process we communicated potential ways to reduce the risk associated with the hazard using reasoned, referenced and logical discussion as specified by Murray *et al*. (2004).

Figure 3: Regent Honeyeater captive to wild translocation pathway



Hazard categories: Carrier;Transport; Destination;Source(see text for definitions)

Aspergillus fumigatus in Regent Honeyeaters

Transport Hazard

Justification for Hazard Status

Aspergillosis has been a commonly documented disease in captive Regent Honeyeaters (ARWH, Gillespie, 2013).

The causative organism, *Aspergillus fumigatus* is a naturally occurring cosmopolitan fungus and common avian pathogen (Bauck, 1994). Its spores can be distributed by global wind currents (Smith *et al.*, 1996) and therefore can be found almost everywhere. However, *A. fumigatus* may be less abundant in mature (undisturbed) forests compared to modified environments (Perrott and Armstrong, 2011). Many birds probably carry the fungal spores in their lungs and air sacs until immune suppression, possibly as a consequence of stress, triggers clinical disease (Bauck, 1994). Since the translocation is likely to act as a stressor to the honeyeaters the disease, aspergillosis, could occur. There are multiple strains of *A. fumigatus* and the strains present *en route* from the source to the destination site may differ (Chazalet *et al.*, 1998).

Risk Assessment

Release Assessment

Inhalation is considered the main infectious route for *A. fumigatus* in birds (Oglesbee, 1997) and, because of their small size, the spores are not trapped

completely in the nasal cavity or trachea and some are able to reach the lungs and air sacs (Fedde, 1998). In the lung parenchyma the spores are engulfed by phagocytic epithelial cells (Maina, 2002) as part of the innate defence mechanisms.

A. fumigatus is ubiquitous and therefore infection via inhalation of the spores is highly likely to occur, especially if there is a high density of birds during quarantine and transport, and mouldy or contaminated substrate is used in the transport boxes. Humidity, warm environment, poor ventilation (Phalen, 2000; Tell, 2005), poor sanitation (Oglesbee, 1997) and long-term storage of feed (Khosravi *et al.*, 2008) are factors capable of increasing the amount of spores in the air. The probability of infection is increased if the immune status of Regent Honeyeaters is compromised through stressors such as transport and reintroduction. Juvenile birds are particularly susceptible.

Since many birds probably carry this fungus (Bauck, 1994) the probability of a Regent Honeyeater being infected on release is **high**.

Exposure Assessment

Regent Honeyeaters can be exposed to *A. fumigatus* at any stage of the reintroduction process before release, and are likely to retain the infection. *A. fumigatus*, even if primarily found in the environment, is one of the few fungal species which has shown the ability to grow in the respiratory systems of animals (Glimp and Bayer, 1983; Vincken and Roels, 1984).

The likelihood of at least one bird being exposed to *A. fumigatus* is estimated as **high**.

Aspergillosis is an infectious but not contagious fungal disease, which spreads neither by horizontal (bird to bird) nor by vertical (dam to egg) transmission (Kearns, 2014). The likelihood that a fungal infection will infect and disseminate amongst Regent Honeyeaters and other birds at the destination site is **very low if not negligible**. However, frequently more than one bird in a group is affected as a result of exposure to the same stressors or other environmental conditions.

Consequence Assessment

An increased concentration of spores in the environment and a compromised immune response in individual animals can result in unsuccessful elimination of the infection and may predispose a bird to aspergillosis (Beernaert *et al.*, 2010). Numerous factors compromising a bird's immunity can also make individuals more susceptible to infection and disease. Examples of factors increasing the risk of developing aspergillosis, once infected, include overcrowding (MCMillan and Petrak, 1989), shipping (Tshai *et al.*, 1992), quarantine or capture of wild birds (Abrams *et al.*, 2001), metabolic bone disease (Vanderheyden, 1993) and traumatic injuries (Xavier, 2008).

Avian aspergillosis is often classified as acute or chronic. Acute aspergillosis primarily occurs in young and recently translocated birds (Woodford and Rossiter, 1994) and is thought to be the result of inhaling an overwhelming number of spores (Vanderheyden, 1993). Chronic aspergillosis is more likely to occur in older birds that have been in captivity (Locke, 1987) and is generally associated with immune suppression (Vanderheyden, 1993).

There is a **high** likelihood of transported birds being infected with *Aspergillus* in the aviaries at the source site or during transport, and, given the stress

involved, a **high** likelihood that at least one of these birds will develop aspergillosis. Although aspergillosis is predominantly a disease of the respiratory tract, other organs can be involved leading to a variety of clinical manifestations. Breathing difficulty (dyspnoea, gasping, polypnoea), sleepiness (somnolence) and other signs of nervous system involvement, inappetence, emaciation, and increased thirst may be seen (Kearns, 2014).

The stress associated with translocation and the involvement of young birds would suggest a **medium** likelihood of disease occurring and significant biological consequences of disease during transport or at the destination site through multiple deaths and consequently failure of the reintroduction.

The likelihood of significant environmental consequences of infection are assessed as **negligible** due to the fact that *A. fumigatus* is found worldwide.

Economic consequences could be significant considering the high monetary costs associated with both the recovery program as a whole and the treatment of sick birds, which is prolonged and often ineffective.

Risk Estimation

There is a **high** likelihood of transported Regent Honeyeaters being infected with *A. fumigatus*; but there is a **very low** likelihood of further exposure of birds and dissemination of the hazard amongst Regent Honeyeaters and other bird species at the destination site. As a consequence of the stressors placed in translocated Regent Honeyeaters there is a **medium** likelihood of significant biological consequences and a **negligible** likelihood of significant environmental consequences. The overall risk for this hazard is, therefore, **MEDIUM**.

Risk Evaluation

Based on the risk assessment above, preventative measures should be employed to reduce the risks from *A. fumigatus* as a transport hazard.

Risk Management Options

The best ways to reduce the risks from *A. fumigatus* is to: a) translocate healthy birds, b) reduce stress during translocation and c) lower the environmental spore count both at the breeding aviaries, quarantine facilities and during transport. In order to meet these goals, the following actions should be considered:

a) estimated total white blood cell (WBC) count is an important screening method because elevated counts are frequently associated with aspergillosis (Ewen *et al*., 2012). Preventative treatment of birds during holding (a single oral dose of itraconazole plus a daily dose of itraconazole presented in sugar water supplementary feeders) has been chosen as a hazard management option in the translocations of a threatened New Zealand bird, the hihi *Notiomystis cincta* (Ewen *et al*., 2012);

b) preventing overcrowding and minimizing any potential stressors such as large temperature fluctuations which, combined with stress due to the transport, may increase the likelihood of infection;

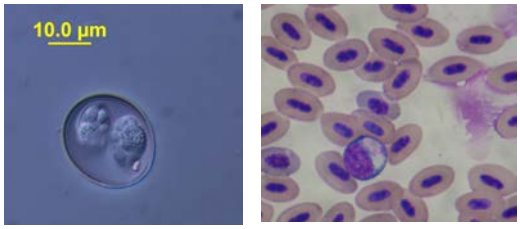
c) avoiding the use of mouldy or contaminated substrates in the transport boxes and ensuring these are clean and dry and not susceptible to developing damp areas during transport. Ensuring transport boxes are well ventilated. The hygiene at the breeding aviaries and quarantine facilities should be kept at high

standard, regularly replacing leaf litter substrates and nesting branches to avoid the development of fungus promoting environments. Enilconazole may be used to spray or fumigate clean enclosures, and all equipment should be clean and disinfected. Ideally, annual spore counts of the permanent quarantine aviaries should be performed, preferably in winter as the development of aspergillosis has shown a potential seasonal bias towards the winter months (Cork *et al*., 1999). There is a recommendation to select release sites with low spore counts of *Aspergillus* sp. within soil (< 100,000 Colony Forming Units per gram of soil) (Ewen *et al*., 2012) given previous evidence for poor population viability in habitats with high spore counts (Perrott and Armstrong, 2011).

Intestinal coccidia (*Isospora lesouefi*) in Regent Honeyeaters

Carrier hazard

Justification for Hazard Status



Coccidia from Regent Honeyeaters. a) sporulated oocyst from faeces; b) systemic coccidian in white blood cell. Photos: a) Jan Slapeta b) Taronga Zoo

Isospora lesouefi is the first *Isospora* species described in the Regent Honeyeater (Morin-Adeline *et al*., 2011). This parasite tends to be found in a large proportion of the population at the source (captive populations) and we assume this parasite will also be present at the destination site because *Isospora* spp. are host-specific and present at high prevalence in infected populations (Schrenzel *et al*., 2005) and have been conserved during other passerine translocations (McGill *et al*., 2010). Although intestinal coccidia are frequently present in healthy animals, they may become virulent under the influence of stressors. Since the translocation is likely to act as a stressor to the honeyeaters the disease, coccidiosis, could occur.

Risk Assessment

Release Assessment

A 91% prevalence rate of *I. lesouefi* oocysts in captive Regent Honeyeaters faecal samples (n = 90) has been reported (Morin-Adeline *et al*., 2011). Infection results from ingestion of infective oocysts (faecal-oral route). Unsporulated (non-infective) *Isospora* oocysts enter the environment in the faeces of an infected host. Under favourable conditions of oxygen, humidity, and temperature, oocysts sporulate and become infective in several days. A Regent Honeyeater must then ingest these oocysts when feeding or drinking and the oocysts can then invade and develop in the intestinal mucosa or epithelial cells in other body locations such as the respiratory tract. There is a high likelihood of exposure and infection of Regent Honeyeaters at Taronga Zoo given that *I. lesouefi* is already present at a high prevalence (Morin-Adeline *et al*., 2011). Coccidian oocysts in the environment are practically ubiquitous in highly populated confined areas and mechanical transfer (through rodent vermin, flying insects, other invertebrate pests, humans, contaminated feed, old litter) can also

occur (Fayer & Reid 1982). Moreover, oocysts in the environment are resistant to most disinfectants and can remain viable for up to two years (Pence, 2009).

Exposure Assessment

Regent Honeyeaters that become infected with *Isospora* at the source site (captive populations) will carry the parasite to the destination site. Coccidia are known to be strictly host, tissue and cell specific (Schrenzel *et al*., 2005). Therefore, while there is a high likelihood of dissemination of this hazard amongst Regent Honeyeaters, there is a low likelihood of dissemination amongst other birds. *I. lesouefi* is also unlikely to infect other bird species at the reintroduction site.

There is limited data available on the parasites and diseases of wild Regent Honeyeaters. Faecal samples collected from a small number of wild Regent Honeyeaters that were trapped for banding screened positive for coccidian oocysts. The exact number of individuals screened is unknown and the species of coccidia has not been identified (L.Vogelnest, *pers. comm.*).

Consequence Assessment

There is a **high** likelihood of birds becoming infected with *Isospora* at the aviaries at the source site and a **high** likelihood that at least one of these birds will develop coccidiosis. Clinical signs include diarrhoea, fever, inappetence, weight loss, emaciation, and in extreme cases, death. However, in the absence of chronic stressors, most infections are subclinical. To date, despite frequent demonstration of the presence of this organism via faecal screens or during necropsy, clinical signs of coccidiosis have not been seen in Regent Honeyeaters to date. It is invariably an incidental necropsy (often just on histopathology) finding. Some diseases such as Metabolic Bone Disease could have been secondary to coccidiosis in the few birds seen with this condition (C.Sangster, *pers. comm.*)

In general, clinically healthy, mature animals can be sources of infection to young, susceptible animals. The immature immune system of young birds makes them more susceptible to develop clinical coccidiosis which can also be precipitated by stress.

Marked necrosis of the intestinal villi associated with endogenous *Isospora* development have been revealed through microscopic (histopathological) examination of tissues from Regent Honeyeaters (n=6) (Morin-Adeline *et al*., 2011). There is no evidence of *I. lesouefi* becoming systemic in Regent Honeyeaters but pre-release quarantine screening in 2013 has revealed an intracellular blood parasite (found in white blood cells) which has yet to be identified. It may be an intermediate stage in the life cycle of *I. lesouefi* (J. Slapeta and R. Adland, *pers. comm.*). *Isospora* spp are pathogenic in passerine birds (Adkesson *et al*., 2005; Cushing *et al*., 2011; Partington *et al*., 1989). The stress associated with translocation and the involvement of young birds would suggest a **medium** likelihood of clinical coccidiosis causing death in some individual birds at the destination site.

Risk Estimation

The likelihood of exposure, infection, release and dissemination in Regent Honeyeaters is **high** but there is a **low** likelihood of dissemination amongst

other bird species. There is a **medium** likelihood of significant biological consequences to some birds.

The overall risk for this hazard is, therefore, **MEDIUM**.

Risk Evaluation

Preventative measures should be employed to reduce the risks from *I. lesouefi*.

Risk Management Options

Prevention is based on the goal of limiting the intake of sporulated (i.e. infective) oocysts by young animals so that an infection is established to induce immunity but not clinical disease. While, for this reason it is desirable that birds be exposed to small doses of coccidia, parasite numbers can rapidly accumulate in the relatively confined space of an aviary, thereby exposing captive birds to potentially high numbers of oocysts.

Good husbandry practices therefore play an extremely important part in the management of this parasite and contribute to the aforementioned goal. Exhibits or enclosures should be designed so that they can be cleaned or stripped on a routine basis. Disinfection with a 10% hypochlorite solution will destroy oocysts and so minimise environmental contamination. Hygiene to minimize faecal contamination of food and water should be maintained at a high standard. Captive rearing-associated stressors (handling, sudden changes in feed, shipping) should be minimized wherever possible. Young birds should be housed in an enclosure that is as dry as possible to prevent coccidia oocysts becoming infective.

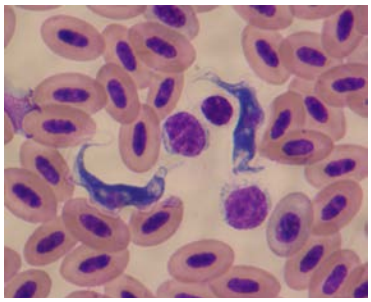
Administration of a coccidiostat should be considered to prevent a significant build-up of coccidia and, associated with good husbandry, prevent outbreaks while allowing immunity to develop (e.g. see McGill *et.al.* 2010). The use of coccidiostats should be strategic and based on a) regular faecal monitoring of the captive birds (with samples collected in the afternoon to coincide with maximum oocyst shedding) and b) prior to translocation (e.g. during quarantine) to minimise coccidian burdens during this period of intense stress.

It is important to note that maintaining infection in released birds while preventing disease offers significant advantages in maintaining immunity. By contrast, release of non-infected birds might jeopardize their health should they be exposed to coccidia through contacts with infected wild Regent Honeyeaters. Lowering aviary stocking densities and good husbandry practices play a key role in reducing stressors to a minimum.

Trypanosoma spp. in Regent Honeyeaters

Source and Destination Hazard

Justification for Hazard Status



Trypanosoma sp. in blood smear from Regent Honeyeater. Photo: Taronga Zoo

Avian trypanosomes, transmitted by various bloodsucking arthropods (black flies, hippoboscids, mosquitoes, biting midges or mites), infect populations of birds world-wide (Apanius, 1991).

Up to the present, *Trypanosoma* spp. have been detected during pre-release quarantine screenings in five captive Regent Honeyeaters of the 2013 release cohort and in two wild Regent Honeyeaters sampled before being brought into captivity from the Capertee Valley in 1997. Also, there may have been a single trypanosome in a blood sample collected from a bird in the Hunter Valley (L. Vogelnest, *pers.comm*.). It has not been confirmed if the *Trypanosoma* sp. found in the captive population of Regent Honeyeaters is the same as that seen in wild birds. To date, at least 96 *Trypanosoma* spp. have been identified in birds (Bennett *et al.*, 1982). However the identification method used by Bennett *et al*. (1982) assumes that trypanosomes are host-specific, something that has more recently been questioned (Bennett *et al.*, 1994b; Zídková *et al.*, 2012) and hence this method could have overestimated the number of known parasite species.

Because *Trypanosoma* spp. may not be host specific there is uncertainty about whether parasites differ between source and destination sites and to what extent they may cause disease.

Risk Assessment

Release Assessment

Transmission of avian *Trypanosoma* spp. is still poorly understood and, depending on the type of parasite/vector involved, it can occur via several routes. Notably, infection in birds can result from ingestion of infected vectors, or via contamination of host abraded skin and/or conjunctiva with parasites present in the vectors' faeces (Apanius, 1991; Votýpka and Svobodova, 2004; Votýpka *et al*., 2012). The likelihood of captive Regent Honeyeaters being exposed to and infected with avian trypanosomes when translocated is **medium** considering the apparently low prevalence of infection (5 of 45 Regent Honeyeaters in 2013 only) found in the captive population at Taronga Zoo.

To date, all parasitized Regent Honeyeaters (captive, n=5; wild, n=2) had shown extremely low levels of parasitaemia (2-3 parasites per blood smear). However, it should be noted that trypanosomes are relatively rare in peripheral blood and therefore the method used for determining the prevalence of trypanosomes can strongly influence the results of prevalence assays (Apanius, 1991). For example, examination of blood smears from a range of passerine species showed a prevalence of 18% which increased to 35% when microhaematocrit tubes were used for centrifugation prior to analysis (Bennett, 1962). In fact, because the latter technique allows concentration of trypanosomes a much greater prevalence of infection may be revealed (Apanius,

1991). Absence of evidence of parasitaemia is also a questionable indicator of infection due to the fact that trypanosomes can persist in the bone marrow of birds in which no parasites are observed in the blood (Apanius, 1991). Bone marrow culture has proven to be a more valuable prevalence assay compared to both blood smears and blood culture in different bird species (Diamond and Herman, 1954; Stabler *et al*., 1966).

Exposure Assessment

Wild Regent Honeyeaters and other bird species at destination could be indirectly exposed to *Trypanosoma* spp. through vectors that have fed on infected reintroduced Regent Honeyeaters, through contact with infected vector's faeces, or via oral ingestion of infected vectors. Trypanosome distribution within wild bird communities is strongly influenced by vector distribution and feeding preferences (Apanius, 1991).

Given the prevalence of infection in reintroduced Regent Honeyeaters is predicted to be low, there is a **low** likelihood of exposure and dissemination of the parasite amongst wild Regent Honeyeaters and other bird species at the destination site.

Consequence Assessment

On current evidence, there is a **low** likelihood that at least one Regent Honeyeater at the destination will be infected with *Trypanosoma* spp.

The generally held belief that these parasites are non-pathogenic is based on the generally low intensity of parasitaemia and the absence of obvious clinical signs in association with avian trypanosome infections (Apanius, 1991). However, numerous studies have indicated that trypanosomiasis does affect the growth and fitness of individuals infected with many parasites (Molyneux *et al*., 1983; Apanius, 1991; Tarello, 2005).

Given the apparent low prevalence of infection in free-living Regent Honeyeaters and apparent absence of clinical signs of disease in infected Regent Honeyeaters[7], the likelihood of significant biological and environmental effects as a consequence of dissemination of *Trypanosoma* spp. amongst Regent Honeyeaters and other birds is assessed as **low**, with low levels of mortality and a more subtle effect on individual's weight and breeding success possible.

Risk Estimation

There is a **medium** likelihood of Regent Honeyeaters being infected with *Trypanosoma* spp. when translocated; but there is a **low** likelihood of further exposure of birds and dissemination of the hazard amongst Regent Honeyeaters and other bird species at the destination site.

There is a **low** likelihood of significant biological and environmental consequences due to the introduction of the hazard at the destination site.

The overall risk for this hazard is, therefore, **LOW**.

Risk Evaluation

Risk estimation is low but not negligible, therefore preventative measures are justified to reduce the risks from *Trypanosoma* spp. as a source hazards.

[7] Two birds released in 2013 with positive blood smears were known to be alive 15 and 29 days post release respectively (L.Vogelnest, *pers.comm*).

On-going health screening of captive and free-living birds could help characterise the presence and prevalence of infection and identify the *Trypanosoma* spp.

Risk Management Options

Screening methods could be employed to identify infected birds which may then not be included in the translocated population. Current standard detection is by blood smear evaluation of the parasite, however the sensitivity of this time consuming assay is very low (Apanius, 1991). The detection levels of trypanosomes can increase significantly when microhaematocrit tubes are used (Bennett, 1962) and also using blood culture methods (Kirkpatrick and Suthers, 1987). Advances in molecular detection techniques have developed a new trypanosome-specific PCR diagnostic test for use on avian blood samples which shows a considerably lower error rate than the one associated with traditional blood smear analysis; and a sensitivity closer to that shown by blood culture (Sehgal *et al*., 2001). Since one bird host species can be infected by several trypanosome species, and one parasite species can infect birds of different orders (Zídková *et al*., 2012), PCR assays could be a very useful tool to help identify *Trypanosoma* spp. harboured by Regent Honeyeaters (both in the wild and in captivity).

Since *Trypanosoma* spp. is a possible source hazard (dependent on the results of identification tests) there is an argument that infected birds should not be released because the species present in captive birds might be novel and alien. If it is alien, the parasite might give rise to disease in free-living birds. However, given the limited evidence of pathogenicity and the worldwide distribution of *Trypanosoma* spp. there is insufficient evidence to justify taking such strict management actions.

Salmonella spp. in Regent Honeyeaters

Carrier Hazard

Justification of Hazard Status

Salmonella species have a worldwide distribution, and have been associated with disease and mortality in many bird species. Disease due to *Salmonella* spp. may occur in passerine birds precipitated by stress (which can lead to shedding of bacteria, thereby contaminating the environment). The reintroduction is likely to be a stressful process and it is probable that Regent Honeyeaters are susceptible to infection with *Salmonella* spp. and that these bacteria would cause disease in association with stress.

Risk Assessment

Release Assessment

A Regent Honeyeater becomes infected by ingesting food or water contaminated with the bacteria which have been shed in faeces by an infected host (mammal, bird or reptile), as *Salmonella* spp. are transmitted via the faecal-oral route (Tizard, 2004). The likelihood of Regent Honeyeaters being infected when translocated is **very low** since, to date, infection in this species has not been detected.

Exposure Assessment

Infected Regent Honeyeaters will contaminate the environment by shedding the bacteria in faeces. Many reptiles, birds and mammals may become exposed at the destination site, through the ingestion of faecal-contaminated food or water, as *Salmonella* spp. are often generalist pathogens (Daoust & Prescott 2007). Any bird, mammal or reptile which comes into contact with and ingests *Salmonella* spp. could potentially become infected, although exposure dose and host immunocompetence will play a role in the intensity of infection.

There is **low** likelihood of *Salmonella* spp. being disseminated at the destination site. The likelihood of this occurring will depend on the behavioural interactions of these species in the wild (for example feeding associations of multiple honeyeater species).

Consequence Assessment

There is a **medium to high** likelihood of at least one animal at the destination site becoming infected. Depending on the species and strain of *Salmonella* contracted, consequences range from no effect to potential population effects (Ewen *et al*., 2007) which would threaten the success of the translocation.

The translocation procedure itself could act as a stressor and lead to an increased susceptibility of the released Regent Honeyeaters to disease. There is a **low** likelihood that stress may precipitate salmonellosis in a large proportion of released Regent Honeyeaters and thereby leading to failure of the translocation.

Risk Estimation

The likelihood of Regent Honeyeaters harbouring *Salmonella* spp. is **very low**.

The likelihood of exposure is **high** and dissemination is **low**.

The biological consequences of translocation failure are predicted to be **low**.

As the consequences are predicted to be low, the overall risk estimation is also **LOW**.

Risk Evaluation

Preventative measures should be employed to reduce the risks from *Salmonella* spp. as a carrier hazard.

Risk Management Options

Methods to reduce the level of stress in translocated birds (through good management) and reduce the probability of faecal-oral transmission of infectious agents, may be effective in reducing the probability of an outbreak of disease at the destination site.

Feeding stations should be used with strict hygiene controls.

Treatment to prevent salmonellosis, or treat individuals which are showing signs is not usually recommended as it may encourage the development of a carrier state and would be difficult in free-living birds (Daoust & Prescott, 2007).

Salmonella swabs should be considered as a routine part of the pre-translocation health screens. As the organism is shed intermittently one can never assume a bird is not a carrier based on a negative faecal or cloacal swab. However the probability of this being so can be increased by taking multiple swabs during the

quarantine period. *Salmonella*-positive birds could be excluded from translocation and monitored for signs of disease.

Feather lice spp. in Regent Honeyeaters

Carrier and Source Hazard

Justification of Hazard Status

Avian lice are small, wingless, 6-legged, flat-bodied insects that parasitize birds and belong to one of two suborders within the Phthiraptera order: Amblycera, which occurs on feathers and skin, or Ischnocera, which are more restricted to feathers and therefore often referred as "feather lice".

Feather lice spp. were detected during pre-release quarantine checks in 3 captive Regent Honeyeaters of the 2013 release cohort. One bird was severely infested while the other two birds seemed to have a very low lice load (only one parasite each was detected). Lice were sent to Dr. Ricardo Palma, Te Papa Museum of New Zealand) on 8 December 2014. Three adult lice were sent: two females of the genus *Brueelia*, and one female of the genus *Menacanthus*.

The *Brueelia* females will be unidentifiable to species for two reasons: a male is needed and, also, they are likely to belong to an undescribed and unnamed species. *Brueelia* contains several hundreds of species from all passerine families that have had lice collected from them. There is no *Brueelia* described and named from any of Australian Meliphagidae species. There are a number of records as "*Brueelia* sp." but without species names. Dr. Palma is conducting further examinations of slide mounts from these specimens in January 2015. (L. Vogelnest, *pers.comm.*).

The captive non-native or naturally co-occurring bird species housed in the multi-species facilities (e.g. Wollemi exhibit) at Taronga Zoo may harbour non-native lice species although, given the host-specificity shown by many avian lice species, the parasite found in Regent Honeyeaters is unlikely to have been contracted from a non-native bird.

Although healthy birds keep their louse populations in check, lice can quickly increase in debilitated animals leading to feather damage, irritation, blood loss with effects on both an individual and a population level. Two sick Regent Honeyeaters at Taronga Zoo had heavy lice infestation. Both were anaemic but it was unclear if the lice were the cause of the anaemia or numbers increased due to the bird's debilitated condition. In neither case was there underlying evidence of concurrent disease (L. Vogelnest, *pers.comm.*). Since the translocation is likely to act as a stressor to the honeyeaters the disease, pediculosis, could occur.

Risk Assessment

Release Assessment

Carrier hazard:

The likelihood of Regent Honeyeaters at Taronga Zoo being exposed to and infested with feather lice prior to translocation is assessed as **medium** considering the apparently low prevalence of infestation found in the captive population at the source site (3 of 45 Regent Honeyeaters in 2013).

Source hazard:

Exotic lice species from exotic birds may be transmitted to Regent Honeyeaters indirectly via phoresis (see below). The likelihood of Regent Honeyeaters acting as a vehicle for release of exotic lice from captive exotic birds is assessed as **low**.

Exposure Assessment

Even though many avian lice are extremely host specific, host specificity should never be assumed, as demonstrated by some lice spp. occurring on multiple host genera, families or even orders (Clayton *et al*., 2008).

Bird lice transmission among hosts often requires physical contact between birds, such as between mates or between parents and their offspring in the nest (Hillgarth, 1996; Tompkins *et al*., 1996). However, movement between hosts by phoresis, or "hitchhiking" on hippoboscid flies, has been demonstrated in ischnoceran (feather) lice (Keirans, 1975). Hippoboscid flies are not as host specific as lice and therefore phoresis could explain the taxonomically diverse avian host range shown by some lice spp.

There is a **low** likelihood that animals at the destination will be exposed to feather lice spp.

There is a **low** likelihood of feather lice spp. being disseminated at the destination because of the predicted parasite host-specificity.

Consequence Assessment

There is a **low** likelihood that at least one Regent Honeyeater at the destination will be infested with feather lice spp.

Lice have shown the potential of both direct negative effects on wild birds (in American white pelicans (*Pelecanus erythrorhynchos*), Samuel *et al*., 1982; Dik, 2006; in Rock Pigeons (*Columba livia*), Booth *et al*., 1993), and indirect effects by acting as vectors or intermediate hosts of other parasites (Seegar *et al*., 1976; Bartlett, 1993).

Although heavy infestations can have effects at both an individual and a population level, we have not come across reports of lice infections in free-living wild birds resulting in disease outbreaks.

Immune-suppression is probably necessary if clinical disease is to occur. Clinical signs include pruritus (itching), dermal irritation, excessive preening and scratching. Although heavy infestations with sucking lice can cause anaemia in their hosts, this has rarely been reported in the case of avian lice. Sucking lice cause small wounds that may become infected. The translocation, acting as a stressor, may precipitate disease in Regent Honeyeater. However, diseases due to lice spp. appear to be sporadic suggesting that the likelihood of significant biological, environmental or economic consequences is **low**.

Risk Estimation

There is a **medium** likelihood of Regent Honeyeaters being infested with feather lice spp. when translocated, and a **low** likelihood of Regent Honeyeaters acting as a vehicle for releasing a non-native lice spp.

There is a **low** likelihood of exposure and dissemination of the hazard at the destination site.

There is a **low** likelihood of significant biological, environmental or economic consequences.

The overall risk for this hazard is, therefore, **LOW**.

Risk Evaluation

Risk estimation is low, not negligible; therefore preventative measures are justified to reduce the risks from feather lice spp. as a carrier and source hazard.

On-going health screening of captive and free-living birds could help characterise the presence and prevalence of infestation and identify the feather lice spp., in order to update this DRA in the future.

Risk Management Options

Lice spp. identification should be a priority as, if found to be a host-specific parasite, then the source hazard can be removed.

Overcrowding of birds should be avoided because it facilitates transmission of lice, with an increase in average louse load (Clayton, 1991).

Avoiding placement of Regent Honeyeaters in multi-host multi-origin aviary would reduce risk of novel host generalist lice species from being co-introduced.

Reducing stress from handling and quarantine by keeping handling to a minimum, and holding time for disease screening as short as possible.

Visual examination for parasite detection. This, in principal, should be easy because the parasite life cycle is restricted to the body of the host. However, some lice spp. are small and difficult to see; some spp. are restricted to microhabitats difficult to examine (interior of quill feathers) or can be hidden in the shafts of developing pin-feathers during moult (Moyer *et al.*, 2002).

Using pesticides for controlling louse load on infested captive Regent Honeyeaters. Pyrethrum dust or spray is probably the safest choice, having no side effects on animals. However, its killing rate is not 100% (Clayton *et al*., 2008). If this is found to be host-specific native parasite, efforts should be made to conserve the louse species, perhaps by treating heavy infestations only.

Making Risk Management Decisions

A number of possible risk management options for each priority hazard are presented in an option evaluation matrix of the type described by Jakob-Hoff *et al* (2014) (Appendix III). These provide a basis for review of existing protocols and recommendations for any variations suggested as a result of this risk analysis. The tables were developed during the workshop on the basis of a review of the literature and the risk assessments outlined above. Each table indicates the feasibility of individual actions and their effectiveness in meeting the five fundamental objectives of risk management.

An alternative DRA methodology was used to explore the risk management options for *Trypanosoma* spp. (included in Appendix III). This method provides a more formal rational decision-making approach. However, it requires more time and a structured treatment of expert opinion and uncertainty; therefore, its application to every individual hazard was beyond the scope of the workshop. It highlights a rational treatment of both opinion and uncertainty and is provided to demonstrate an alternative approach to selecting appropriate risk management actions.

When assessing disease risk management actions we recommended that the group reflected on their fundamental concerns as identified at the DRA workshop:

- The impact of disease on wild Regent Honeyeaters
- The impact of disease on captive Regent Honeyeaters
- The impact of disease on other species in the destination ecosystem
- The cost of health and disease management
- The welfare impacts of individual Regent Honeyeaters from the process of health and disease management

Any decision made should attempt to account and balance across these. As is normal in multi-disciplinary groups, a diversity of opinion was expressed by stakeholders at the workshop in regard to risks and their relative importance. It will help to keep the above objectives in mind when selecting management actions from the list of alternatives provided in the detailed risk assessments within this report and others to be completed.

Knowledge gaps and potential research opportunities

The following knowledge gaps were identified. Research into these would enhance the ability to make informed disease risk decisions. They are not listed in priority order.

Baseline health and disease data

Targeted surveillance of wild honeyeaters within the geographic range of Regent Honeyeaters - to provide baseline prevalence data for selected priority disease hazards.

Scanning surveillance for diseases in free-living wild honeyeaters which might lead to the discovery of currently unknown hazards, or the re-evaluation of the risk from disease of existing hazards.

Baseline health evaluation reference ranges for Regent Honeyeaters

Infectious Hazards

External parasites

Feather lice – identification and impact. (Note Dr Ricardo Palma at Te Papa New Zealand Museum, Wellington, a world authority on avian lice, has completed a preliminary identification of the lice found on Regent Honeyeaters – see pp 31-32).

Blood parasites

Trypanosoma species identification and prevalence in wild and captive Regent Honeyeaters

Systemic coccidia identification and prevalence in wild and captive Regent Honeyeaters

Other haemoprotozoa (e.g. *Plasmodium*/avian malaria) - blood parasite species present in honeyeaters and prevalence in wild and captive honeyeaters.

Fungi

Range of fungal organisms inhabiting the GI tract of healthy vs sick honeyeaters (e.g. *Candida*, *Penicillium*, *Pantoea*)

Non-infectious hazards

Metabolic bone disease – prevalence in wild Regent Honeyeaters – possible genetic predisposition?

Diseases of Unknown Cause

Facial dermatitis in Regent Honeyeaters – diagnosis of cause(s) and predisposing factors associated with this condition.

Causes of embryo mortality

Cardiomyopathy – prevalence in wild Regent Honeyeaters; causal or contributing factors

Conclusions and Recommendations

It is important to remember that epidemic diseases associated with translocations have been caused by previously unknown infectious agents (Sainsbury and Vaughan-Higgins, 2012). Given that our understanding of infectious agents harboured by Regent Honeyeaters is poor and the birds are in contact with numerous exotic species of birds in zoological collections, the release of a species of parasite novel to the destination represents the greatest risk of disease to this reintroduction program. Given the reintroduction program has been continuing for seven years, novel agents may already have been introduced to the free-living population. Evidence from other translocations shows that epidemic diseases may take many years to express themselves in affected free-living populations (Sainsbury *et al.*, 2008). This may be due to epidemiological characteristics of the disease, and therefore novel effects in Regent Honeyeaters may not be seen for many years. Given these circumstances, disease monitoring of the reintroduced population and related species of passerine birds should be given a high priority and should be conducted in close association with the monitoring of these species' population dynamics.

Recommendations

We suggest the Recovery Group consider the following three major recommendations as key to the minimisation of disease risk to this program:

1. A long-term plan to place all Regent Honeyeaters in the breed for release program, in permanent quarantine, separate from exotic[8] birds.

2. Place increased resources into health surveillance of the free-living population of Regent Honeyeaters and monitoring the causes of morbidity and mortality.

3. Complete disease risk analyses for all the hazards identified in Table 2 to provide a comprehensive evidence-basis for all risk management decisions.

[8] See p.6 for a DRA-focussed definition of the term 'exotic'.

Regent Honeyeater DRA Implementation and Review Action Plan

This plan was developed at the DRA workshop and provides a template for further planning and review by stakeholders.

Objective	Actions	Obstacles	Responsibility	Collaborators	Deadline	Cost	Measure
Complete DRA report	Draft report updating briefing notes and incorporating workshop outputs and including RA for feather lice and systemic coccidia; also actions and estimate of costs for any further validation	Time	Claudia Carraro	Richard Jakob-Hoff Tony Sainsbury John Ewen Stefano Canessa	7/12/14	Gratis	Circulated by agreed deadline
	Review draft and provide edits and feedback to CC	Time	Workshop participants		20/1/15	Gratis	Feedback returned by agreed deadline
	Finalise DRA report	Time	Claudia Carraro	Richard Jakob-Hoff Tony Sainsbury John Ewen Stefano Canessa	7/2/15	Gratis	Report finalised by agreed deadline

Objective	Actions	Obstacles	Responsibility	Collaborators	Deadline	Cost	Measure
Implement communications plan	Circulate report to selected stakeholders		Paul Andrew?	Dean Ingwersen	TBD		Report circulated to selected stakeholders
Establish a research plan	Review and prioritise knowledge gaps identified through the DRA process	Time Availability of collaborators	Larry Vogelnest	TZ vet and bird team , Dean Ingwersen + recovery team, Tiggy Grillo	TBD		Knowledge gaps reviewed and prioritised with collaborator input
	Develop research questions for identified knowledge gaps	Time Availability of collaborators	Larry Vogelnest	TZ vet team , Dean Ingwersen, Jan Slapeta	TBD		Research questions developed to deadline
	Implement Research Plan	Availability of people, funds and other resources to conduct the research	Rebecca Spindler (TBD)?	Caroline Hogg Dean Ingwersen Recovery Team Rupert Baker	TBD		Research projects funded and initiated

Objective	Actions	Obstacles	Responsibility	Collaborators	Deadline	Cost	Measure
Revise current disease risk management protocols for the RH breed-for-release program based on DRA recommendations	Revise disease risk management protocols for captive to wild translocations	Time	Larry Vogelnest	TZ vet and bird team and curators; other institutions	TBD		Disease risk management protocols revised
	Revise disease risk management protocols for captive to captive translocations after applying DRA methodology to these	Time	Larry Vogelnest	TZ vet and bird team and curators; other institutions; BirdLife	TBD		Disease risk management protocols revised
	Revise disease risk management protocols for wild to captive translocations after applying DRA methodology to these	Time	Larry Vogelnest		TBD		Disease risk management protocols revised

Objective	Actions	Obstacles	Responsibility	Collaborators	Deadline	Cost	Measure
Establish a decision tree for responding to the discovery of a novel organism **See Appendix V**	Develop a decisions tree (see draft below)	Nil	Larry Vogelnest	Paul Andrew Relevant wildlife agencies Recovery Team TZ vet team; other institution vets Relevant diagnosticians e.g. Jan Slapeta	30/11/14		Being able to include it in the DRA report
	Identify relevant decision makers and ensure they are aware and supportive of this protocol	Nil	Larry Vogelnest	Paul Andrew Relevant wildlife agencies Recovery Team TZ vet team; other institution vets Relevant diagnosticians e.g. Jan Slapeta	30/11/14		Relevant decision makers identified, consulted and endorse decision tree protocol

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Appendix I: Communications Plan

As with all endangered species recovery programs there are many people who share an interest in, have knowledge of value to, or can influence the implementation of, risk management recommendations. As a result a crucial component of a successful DRA is to identify these stakeholders and formulate a *Risk Communications* plan. As in this table, this should capture the name, contact details, what information they can provide, what information they need (and when) and their preferred communication method. The table can be modified to suit and should be regularly updated to maintain its currency.

Name*	Affiliation	E-mail address	Information source for:	Information Needs	Communication Preferences	
					E-mail	Telephone
Regent Honeyeater Recovery Team						
Dean Ingwersen	BirdLife Australia	dean.ingwersen@birdlife.org.au	Species ecology, data, recovery team info, release process, recovery plan	All updates	✓	✓
Glen Johnson	Victorian Government	Glen.Johnson@DELWP.vic.gov.au	release process and data, recovery team info, govt policy (Vic)	All updates	✓	✓
Peter Menkhorst	Victorian Government	Peter.Menkhorst@DELWP.vic.gov.au; pmenk@bigpond.net.au	Species ecology, recovery team info, govt policy (Vic),	All updates	✓	✓

Name*	Affiliation	E-mail address	Information source for:	Information Needs	Communication Preferences	
			recovery plan			
Peter Christie	NSW Government	Peter.Christie@environment.nsw.gov.au	Recovery team info, govt policy (NSW)	All updates	✓	✓
David Geering	NSW government	David.geering@environment.nsw.gov.au	Species ecology, recovery team info, govt policy (NSW), recovery plan	All updates	✓	✓
Hugh Ford	Uni of New England	hford@une.edu.au	Species ecology, recovery team info	Major achievements or changes	✓	✓
Alan Morris	Community Group	a.morris42@optusnet.com.au	Species ecology, recovery team info	Major achievements or changes	✓	
Beth Williams	Community Group	bethwillms@optusnet.com.au	Species ecology, recovery team info	Major achievements or changes	✓	
Eileen Collins	Community Group	nulgerong@westnet.com.au	Species ecology, recovery team info	Major achievements or changes	✓	
Iain Paterson	Independent Consultant	iain_paterson@live.com.au	Species ecology, recovery team	Major achievements	✓	✓

Name*	Affiliation	E-mail address	Information source for:	Information Needs	Communication Preferences	
			info	or changes		
Jim Shields	Community Group	jim.shields@bigpond.com	Species ecology, recovery team info	Major achievements or changes	✓	
Mike Clarke	La Trobe University	M.Clarke@latrobe.edu.au	Species ecology, recovery team info	Major achievements or changes		✓
Ray Thomas	Community Group	ray@regenthoneyeater.org.au	Species ecology, recovery team info	Major achievements or changes	✓	
Stephen Debus	Independent Consultant	sdebus@une.edu.au	Species ecology, recovery team info	Major achievements or changes	✓	
Ian Davidson	Independent Consultant	ian@regenerationsolutions.com.au	Species ecology, recovery team info	Major achievements or changes	✓	
Project veterinarians/pathologists						
Larry Vogelnest	Taronga Zoo	LVogelnest@zoo.nsw.gov.au				
Frances Hulst	Taronga Zoo	FHulst@zoo.nsw.gov.au				
Cheryl Sangster	Taronga Zoo	CSangster@zoo.nsw.gov.au				
Andrea Reiss	Zoo and	andrea@zooaquarium.org.au	ZAA	Regional	✓	✓

Name*	Affiliation	E-mail address	Information source for:	Information Needs	Communication Preferences	
	Aquarium Association			ZAA matters, regional captive animal health concerns		
Captive breeding collaborators						
Judith Gillespie	Taronga Zoo	JGillespie@zoo.nsw.gov.au	Regional management for release	Captive management and release planning	✓	02 9978 4669 0419 410 772
Michael Shiels	Taronga Zoo	mshiels@zoo.nsw.gov.au	Husbandry & release	as above	✓	02 9978 4374 0412 226 637
Paul Andrew	Taronga Zoo	pandrew@zoo.nsw.gov.au	Regional management	as above	✓	02 9978 4724 0409 036 063
Simon Duffy	Taronga Zoo	sduffy@zoo.nsw.gov.au	Taronga management		✓	02 9978 4604 0438 471 343
Mark Williams	Taronga Western Plains	mwilliams@zoo.nsw.gov.au	Media relations	All media & comms planning	✓	02 9978 4607 0417 293 507
Nick Boyle	Taronga Zoo	nboyle@zoo.nsw.gov.au	Taronga management		✓	02 9978 4388 0459 811 024
Rupert Baker	Healesville Sanctuary	RBaker@zoo.org.au				
Carla Srb	Healesville Sanctuary	csrb@zoo.org.au				

Name*	Affiliation	E-mail address	Information source for:	Information Needs	Communication Preferences	
James Biggs	Cairns Tropical Zoo	james@cairnstropicalzoo.com				
Gert Skipper	Adelaide Zoo	gskipper@zoossa.com.au				
Kelsey Engle	Australia Zoo	kelsey@australiazoo.com.au				
Tim Faulkner	Australian Reptile Park	tfaulkner@reptilepark.com.au				
Byron Manning	Cleland Wildlife Park	byron.mannning@sa.gov.au				
Clancy Hall	Currumbin Wildlife Sanctuary	chall@cws.org.au				
Steve Robinson	Darling Downs Zoo	admin@darlingdownszoo.com.au				
Hans van Weerd	Melbourne Zoo	hvanweerd@zoo.org.au				
Michael Johnson	Moonlit Sanctuary	michael@pearcedale.com				
Carolyn Hogg	Manager ZAA Science and Policy	carolyn@zooaquarium.org.au	ZAA			02 9978 4634
Australian Zoo & Wildlife Health Collaborators						
Tiggy Grillo	Wildlife Health Australia	tgrillo@wildlifehealthaustralia.com.au	WHA	National wildlife health surveillance coordinator	✓	02 9960 744 0406 383 582
Claire Ford	Australasian Species Management	Claire@zooaquarium.org.au	ASMP/ZAA			

Name*	Affiliation	E-mail address	Information source for:	Information Needs	Communication Preferences	
	Program					
Karrie Rose	Australian Registry of Wildlife Health	krose@zoo.nsw.gov.au				
Researchers						
Jan Slapeta	Sydney University	jan.slapeta@sydney.edu.au				
Administrators						
CEO	BirdLife Australia	ceo@birdlife.org.au	Recovery project implementation	Infrequent updates	✓	

Appendix II: Hazard Prioritisation by Elicitation of Expert Opinion

Working Group: Larry Vogelnest, Frances Hulst, Andrea Reiss, Cheryl Sangster, Rupert Baker, Michael Sheils

Facilitator: Richard Jakob-Hoff

The group reviewed the list of hazards circulated in the workshop briefing notes. This list was derived from pathology data on 127 honeyeaters (Meliphagidae) in the Australian Registry of Wildlife Health (courtesy Dr. Karrie Rose) of which 96 (75.5%) were derived from the necropsy of Regent Honeyeaters. To this the group added the following from their personal experience of health hazards relevant to honeyeaters: *Salmonella* spp., *Mycobacterium avium*, *Plasmodium* spp, *Trypanosoma* spp., harness injuries, systemic coccidia (as the taxonomic status of the genus *Atoxoplasma* is currently subject to debate the coccidia recently identified circulating in the blood of Regent Honeyeaters were referred to by the generic term 'systemic coccidia').

Limitations of the review process:

1. The expert panel is always limited to the expertise of the people able to participate, albeit, in this case, this represented a group with a significant body of relevant knowledge and expertise.
2. The hazards identified and reviewed in this exercise were limited to those for which data was available prior to the workshop. It was recognised as a reasonable, evidence-based starting point on which further research of honeyeater disease susceptibilities could be built.
3. The expert panel review at the workshop was confined to two hours.
4. Given the time available, the hazard review was confined to the following scenarios:
    a. The likelihood of exposure to, and degree of impact on the captive Regent Honeyeater population (primarily at Taronga Zoo but taking into consideration other captive locations in Australia)
    b. The likelihood of exposure of Regent Honeyeaters in the wild from captive released Regent Honeyeaters and the degree of impact on the wild Regent Honeyeater population
    c. The likelihood of exposure of other bird species in the wild from captive released Regent Honeyeaters and the degree of impact on the wild bird populations.
5. Given limitations of available data and expert knowledge, 'Likelihood' and 'Impact' were qualitatively expressed as Low, Medium, or High. A fourth category 'Unknown' was used to indicate the lack of any information.

Table 3: Hazard assessments by expert opinion at DRA stakeholder workshop

Hazard *(*identified in Regent Honeyeaters)*	**Captive Regent Honeyeaters**		**Captive to Wild Regent Honeyeater Transmission**		**Captive Regent Honeyeater to Other Wild Bird Transmission**	
	Likelihood	Impact	Likelihood	Impact	Likelihood	Impact
Infectious Hazards						
A. Macroparasites						
Feather mites*	H	L	H	L	L	L
Nasal mites	L	L	U	L	L	L
Feather lice*	H	L	H U	U	L	U
Ticks	L	L	NA	NA	NA	NA
Proventricular nematodes	L	L	L	L	L	L
Cestodes*	L	L	L	L	L	L
B. Microparasites						
Trypanosoma spp.*	H U	L	U	L	U	L
Isospora spp. – intestinal*	H	L	L	L	L	U
Coccidia – systemic*	U	L	U	U	U	U
Other protozoa – systemic	U	L	U	L	L	U
Microsporidia	L	L	L	L	L	U
Encephalitozoon hellum	L	U	L	U	L	U
Plasmodium spp.	L	U	L	U	L	U
Other haemoprotozoa	L	U	L	U	L	U
C. Viruses						
Avipox virus	M	L	L	L	L	L
D. Bacteria						
Salmonella spp.	M	M	L	L	L	L
Mycobacterium avium	H	L	L	L	L	L
Klebsiella pneumoniae	L	L	L	L	L	L
Klebsiella oxytoca	L	L	L	L	L	L
Aeromonas hydrophila	L	L	L	L	L	L
Proteus mirabilis	L	L	L	L	L	L
Streptococcus sp.	L	L	L	L	L	L
Enterococcus sp.	L	L	L	L	L	L
E. Fungi						
Aspergillus spp.*	H	L	L	L	L	L
Candida spp.	L	L	L	L	L	L
Candida albicans	L	L	L	L	L	L
Mucor spp.	L	L	L	L	L	L
Penicillium spp.	U	L	U	L	U	L
Pantoea aglommerans	U	L	U	L	U	L
Non-Infectious Hazards						
Metabolic bone disease*	L	L	U	L	NA	NA
Malnutrition (includes vitamin D toxicity &	L	H	NA	NA	NA	NA

thiamine deficiency)						
Neoplasia*	L	L	NA	NA	NA	NA
Transmitter harness injury	L	L	NA	NA	NA	NA
Trauma/Predation/Misadventure	M	L	NA	NA	NA	NA
Foreign body/obstruction	L	L	NA	NA	NA	NA
Toxins (including snake bite)	L	L	NA	NA	NA	NA
Developmental	L	L	NA	NA	NA	NA
Hazards of Unknown Cause						
Dermatitis/hyperkeratosis/acanthosis*	U	L	U	L	U	L
Dead embryo*	H	U	U	U	U	U
Cardiovascular (cardiomyopathy/avascular necrosis)*	L	L	U	U	U	U

The rationale for the rankings listed in Table 3 are summarised in Table 4 below. This provides transparency but a subsequent step of referencing the literature on each hazard should be completed to validate or revise these estimates and maximise the evidence-base for the rankings.

Table 4: Rationale for Rankings in Table 3.

Hazard	Captive RHE	Captive to wild RHE Transmission	Captive RHE to other wild bird transmission
Macroparasites			
Feather mites*	Known to be present in captive RH and bird to bird transmission is facilitated by close contact of birds in a confined space. However no associated clinical signs noted to date so impact on the captive population is considered low.	Mites are present in captive birds and low numbers may be missed on pre-release physical examination therefore it is highly likely captive birds could result in transmission to wild RH through close contact. Feather mites without clinical signs are commonly found in many wild bird species, therefor the impacts on the wild RH population is likely to be low.	Feather mites are generally species-specific so the likelihood of transmission from captive RH to other species is considered low and the impact on these species is also likely to be low or negligible.
Nasal mites	Has been isolated from honeyeaters (not Regent) but is rare and of little clinical significance to the captive population.	As the mites have not been found in nearly 100 Regent Honeyeater necropsies likelihood of transmission by them to free-living birds is considered low. As reports of nasal mites as disease causing agents in wild birds is also rare the likely impact on wild birds, if transmission did occur, is also considered low.	

Feather lice*	Known to be present in captive RH and bird to bird transmission is facilitated by close contact of birds in a confined space. However no associated clinical signs noted to date so impact on the captive population is considered low. See detailed risk assessment, this report.	Feather lice are present in captive birds and low numbers may be missed on pre-release physical examination therefore it is highly likely captive birds could result in transmission to wild RH through close contact but, as lice have not been observed in wild RH there is a high level of uncertainty to this assumptions. Low numbers of feather lice without clinical signs are commonly found in many wild bird species, therefore the impacts on the wild RH population is likely to be low.	Feather lice are generally species-specific so the likelihood of transmission from captive RH to other species is considered low and the impact on these species is also likely to be low or negligible.
Ticks	Rarely found in captive honeyeaters and of little clinical significance to the captive population.	Readily identified and removed during pre-release quarantine so negligible likelihood of transfer from captive to wild birds.	
Proventricular nematodes[9] (Microtetrameres sp. ?)	Rarely found in captive honeyeaters and of little clinical significance to the captive population.	Requires invertebrate intermediate host so no direct transfer from captive Regent Honeyeaters possible.	

[9] Since the workshop, one of these has been identified in a Regent Honeyeater, suspect ID is Microtetrameres sp. (C.Sangster. *pers.comm.*).

Cestodes*	Rarely found in captive honeyeaters and of little clinical significance to the captive population	Require invertebrate intermediate host so no direct transfer from captive Regent Honeyeaters possible.	
Infectious hazards			
F. Microparasites			
Trypanosoma spp.*	Trypanosomes have been identified in a small number of captive RH. However the diagnostic method used (blood smear examination) has low sensitivity so the current knowledge of prevalence is likely to be an underestimate. Vectors of trypanosomes are present so the likelihood of exposure is considered high but until more sensitive diagnostic techniques are used to establish a more accurate estimate of prevalence there is a high level of uncertainty in this estimate. No clinical disease or pathology has been found in the captive RH examined to date so the impact on the captive populations is considered low. See detailed Risk Assessment	No systematic health surveillance of free living RH has been conducted to date. However trypanosomes have been observed in a small number of wild RH but it is not known if this is the same species found in captive RH. The likelihood of exposure of wild RH as a result of the release of captive bred RH is therefore unknown. On the basis of knowledge of trypanosome parasitism in other species which is frequently subclinical, the impact on with RH populations is considered low. See detailed Risk Assessment, this report	The expert panel had insufficient knowledge on which to assess the likelihood of exposure of wild birds as a result of the release of captive bred RH. On the basis of knowledge of trypanosome parasitism in other species which is frequently subclinical, the impact on other wild bird populations is considered low. See detailed Risk Assessment, this report

Isospora spp. – intestinal*	Very commonly found in Regent Honeyeaters so likelihood of exposure high. But rarely associated with pathology so impact on captive population low. See detailed Risk Assessment this report	*Isospora* spp. are widespread in free-living passerines including honeyeater species. Consequently likelihood of introduction of this parasite from captive to wild populations of Regent Honeyeaters is low. Captive Regent Honeyeaters are rarely clinically effected by coccidia so impact is also low. See detailed Risk Assessment this report	*Isospora* spp. are widespread in free-living passerines. Consequently likelihood of introduction of this parasite from captive to wild populations of Regent Honeyeaters is low. There is no field data to assess the impact of coccidia on other species at the destination site. See detailed Risk Assessment this report:
Coccidia – systemic*	Systemic form of coccidia only recently detected in captive Regent Honeyeaters so true prevalence and impact unknown. See detailed Risk Assessment this report	Assessment of likelihood of transmission and potential impacts on free-living populations of this parasite is pending further research to identify the parasite and its life cycle. See detailed Risk Assessment this report	
Other protozoa - systemic	Other systemic protozoa, apart from haemoprotozoa have not been identified in captive RHE	Since not identified in captive RHE , likelihood of transmission to wild RHE or other bird species considered low	
Microsporidia	No previous reports in RHEs so likelihood suspected to be low, but impact is unknown	No previous reports in RHEs so likelihood suspected to be low, but impact is unknown	No previous reports in RHEs so likelihood suspected to be low, but impact is unknown
Encephalitozoon hellum	No previous reports in RHEs so likelihood suspected to be low, but impact is unknown	No previous reports in RHEs so likelihood suspected to be low, but impact is unknown	No previous reports in RHEs so likelihood suspected to be low, but impact is unknown

Plasmodium spp.	*Plasmodium* spp. have not previously been recorded in RHEs	As vector species are continuous throughout the range of RHEs and the captive environment, captive RHEs are not thought to represent an increased risk of this disease.	As vector species are continuous throughout the range of RHEs and the captive environment, captive RHEs are not thought to represent an increased risk of this disease.
Other haemoprotozoa	Have not been previously recorded in RHEs despite high numbers of birds screened by blood smear examination. Risk is unknown but likely to be low.	No previous reports in RHEs so likelihood suspected to be low, but impact is unknown	No previous reports in RHEs so likelihood suspected to be low, but impact is unknown
G. Viruses			
Avipox virus	Avipox virus has not been identified in captive RHE. Avipox virus is transmitted by mosquito vectors and by contact with material from pox lesions on unfeathered parts of the body. Avipox is usually family or order specific. Moderate chance of exposure from vectors. Impact unknown, likely low to moderate.	Pox lesions are identified by close examination and the exclusion of birds with pox-like lesions should make the likelihood of transmission by vectors or close contact with wild birds low.	Pox lesions are identified by close examination and the exclusion of birds with pox-like lesions should make the likelihood of transmission by vectors or close contact with wild birds low.
H. Bacteria			

Salmonella spp.	Outbreaks of disease due to this pathogen have occurred in species sharing aviaries with captive RHEs, thus representing a medium likelihood of exposure. During the stresses of translocation a captive RHE could break with this infection, compromising its release, thus the medium impact. See detailed risk assessment in this report	Shedding by a clinically or subclinically affected RHE is unlikely to result in sufficient contamination of the wild environment to represent a risk to wild birds	Shedding by a clinically or subclinically affected RHE is unlikely to result in sufficient contamination of the wild environment to represent a risk to wild birds
Mycobacterium avium	Environmental levels of *M. avium* in captive situations are high, leading to a high likelihood of exposure. However, despite this high likelihood, no cases of mycobacteriosis have been recorded in captive RHEs, making the impact low.	Environmental contamination from an infected released bird likely to be low. Mycobacteriosis in not transmitted directly bird-to bird, so impact is low	Environmental contamination from an infected released bird likely to be low. Mycobacteriosis in not transmitted directly bird-to bird, so impact is low
Klebsiella pneumoniae *Klebsiella oxytoca* *Aeromonas hydrophila* *Proteus mirabilis* *Streptococcus* sp. *Enterococcus* sp.	Various enteric and integumentary infections due to these bacteria have been documented in Meliphagidae species on an incidental basis. However, the likelihood and impact of any one of these pathogens on captive RHEs is thought to be low.	Captive RHE undergo clinical examination and close observation for a period prior to release to ensure birds are in good health and hence are unlikely to be shedding large numbers of these pathogens. Subclinical shedding is possible, but in open environments, direct or indirect transmission is unlikely.	Captive RHE undergo clinical examination and close observation for a period prior to release to ensure birds are in good health and hence are unlikely to be shedding large numbers of these pathogens. Subclinical shedding is possible, but in open environments, direct or indirect transmission is unlikely.
I. Fungi			

Aspergillus spp.*	Aspergillus is ubiquitous in the environment, making likelihood of exposure high. Disease often develops in response to stress, so risk during the translocation process can be elevated. See detailed risk assessment in this report.	Aspergillus is ubiquitous in the environment and is not transmitted directly from bird to bird, making the likelihood and impact of exposure to an infected captive bird low.	Aspergillus is ubiquitous in the environment and is not transmitted directly from bird to bird, making the likelihood and impact of exposure to an infected captive bird low.
Candida spp. *Candida albicans* *Mucor* sp. *Penicillium* spp. *Pantoea aglommerans*	Various fungal infections have been recorded in Meliphagidae, but these tend to be isolated, opportunistic infections. No infections with these fungi have been recorded in RHE and hence likelihood of infection and impact are thought to be low.	These organisms are typically obtained opportunistically from the environment and thus there is little risk of transmission from infected captive birds.	These organisms are typically obtained opportunistically from the environment and thus there is little risk of transmission from infected captive birds.
Non-Infectious Hazards			
Metabolic bone disease*	MBD has been seen in fledglings at a low incidence in the captive population. . If there is a genetic component to the disease, future generations could be impacted	Birds with a history of MBD will not be selected for release. If there is a genetic component to MBD there is the possibility of future generations of wild RHE developing MBD but the likelihood and impact is unknown.	N/A
Malnutrition (Includes vitamin D toxicity and thiamine deficiency)	Diet of captive RHEs is closely monitored, making the likelihood low. However, if a calculation error was made in the production of the food, the impacts could be high	N/A	N/A

Neoplasia*	Neoplasia has occurred in captive populations, but there has been no evidence of an infectious agent. Neoplasia is more likely to affect post-reproductive birds and thus have little impact on birds at the time of release	N/A	N/A
Harness injury	Has occurred in the past and had a severe impact (death) on a few individuals. Harness has since been modified with no further issues.	N/A	N/A
Trauma/Predation/ Misadventure	There is opportunity for trauma to occur during the translocation process, which should be minimized if at all possible	N/A	N/A
Foreign body/obstruction	The is no component of the translocation process which should increase the chance of this occurring	N/A	N/A
Toxins (including snake bite)	Low likelihood in captive enclosures, chemicals use in enclosures carefully monitored.	N/A	N/A
Developmental	None identified, likelihood and impact unknown	N/A	N/A
Hazards of Unknown Cause			
Dermatitis/hyperkeratosis/acanthosis*	Cause is unknown, but infectious aetiologies have not been found and impact on the population is assumed to be low	Cause is unknown, but infectious aetiologies have not been found hence likelihood of transmission to wild RHE low	Cause is unknown, but infectious aetiologies have not been found hence likelihood of transmission to wild birds low

Dead embryo*	Reduced breeding success could impact on the captive breeding program overall. Further research into the cause of embryonic deaths recommended.	Insufficient information	Insufficient information
Cardiovascular (cardiomyopathy/a vascular necrosis)*	Has occurred rarely and is unlikely to be a result of infection. Impact on captive population has been low.	Insufficient information	Insufficient information

Appendix III: Risk Management Option Evaluations.

Two methods to identify and select risk management options were trialled during the DRA workshop: An Option Evaluation Matrix and the process of Structured Decision Making.

1. The Option Evaluation Matrix

Described by Jakob-Hoff *et al*. (2014) this method can provide a valuable starting place for decision making before specific measures are developed and evaluated further. As shown in the following matrices, based on expertise available at the workshop, each option was considered according to the group's assessment of feasibility and effectiveness. Ideally options chosen should be both feasible and highly effective. These evaluations were initiated at the DRA workshop and completed subsequently by the expert panel.

Risk management evaluations – apply to general management of Regent Honeyeaters in captivity as well as the pre-release period

Hazard: *Aspergillus fumigatus*

Options	Feasibility	Efficacy	Explanation	Decision
Pre-release isolation (PRI) period (21-30d)	H	L	Feasible but stress may predispose to aspergillosis. PRI may therefore increase susceptibility	No
Screening – haematology/ biochemistry	H	M-L	Useful as an indirect diagnostic aid to identify potentially diseased individuals. Birds with high WCC and monocytosis excluded	Yes
Stress mitigation (overcrowding, catch-ups, moves)	H	H	Readily implemented and a major method of reducing susceptibility	Yes
Avoid decomposing vegetation in aviaries	M	H-M	Minimising exposure to fungal spores is a key preventative measure. However more difficult to do in naturally planted aviaries	Yes
Dry, clean, well ventilated transport boxes	H	H	Readily achieved and effective in avoiding exposure to spores	Yes
Fumigate enclosures	M	L	Only effective in the short term. Difficult on open aviaries	No
Prophylactic treatment during PRI	M	L-M	Difficult to monitor dose ingested when treatment provided in food. Potential subclinical effect on fitness/health of birds. Disease is sporadic and no cases previously seen in release cohorts both pre- and post-release. Given these and other risk management option to reduce exposure, treatment cannot be justified. However in the unlikely event a case is diagnosed in one or more birds during the pre-release period prophylactic treatment may be implemented for the remaining release cohort.	No
Release site	H	H	Unlikely to be high at release sites therefore	Yes

Options	Feasibility	Efficacy	Explanation	Decision
with low spore counts			minimal exposure risk	

Hazard Feather Lice (*Brueelia* spp. *Menacanthus* spp.)

Options	Feasibility	Efficacy	Explanation	Decision
Isolation 21-30 days	H	M	Isolation from other species that may be potential hosts (particularly exotics) may be effective but still exposed to wild birds. Efficacy conditional on treatment. Overcrowding may facilitate transmission and be stressful. A *Brueelia* spp. has egg incubation of 5-7 d, and *Menacanthus stramineus* 4-5 d, suggesting a 7 d isolation period and 2 treatments 7 d apart may be more effective. Logistics and stress associated with isolating batches of birds for 7 d may outweigh benefit and efficacy	Isolation only from exotic species or those not found within RHE range.
Reduce stocking density	H	M	Limited available aviary space, husbandry requirements and direct and potentially indirect parasite transmission via phoresis	Yes
Treatment for control	H	M	Treatment easy but may not be 100% effective. Given egg incubation periods 2 treatments 7 d apart may be more effective. Due to reasons stated above, very low to no lice burdens and low risk, treatment at pre-release health check and just prior to transport to release site is preferable	Yes
Screening	H	L	Difficult to identify low burdens. Low sensitivity. Heavily infested birds will be excluded. Lice will be collected for further identification of species	Yes
Treatment for eradication	L	H	Treatment may not be 100% effective, low burdens difficult to detect, potential ongoing source of infestations from other species	No
Treat environment	L	U	Needs further research re life cycle and transmission	No
Permanent isolation of captive population	L	H-M	Aviary and other resource limitations. Birds would need to be excluded from exposure to wild birds and insects. Adverse effects of permanent isolation on behaviour, predator avoidance, fitness, adaptation to changes, outweigh benefits of isolation.	No
Do not release infected birds	H	H	Highly effective to prevent transfer to free-living populations but feasibility may be impacted by resource limitations. Heavily infested individuals will be removed from the release cohort	

Hazard: Intestinal and systemic coccidia

Options	Feasibility	Efficacy	Explanation	Decision
Pre-release isolation period (21-30d)	H	L	All birds infected anyway and no effort is made to eradicate. Species specific coccidia. Stress of PRI may exacerbate loads and stress may induce coccidiosis	No
Screening (faeces and blood)	H	L	All birds infected anyway and faecal oocyte counts are variable and do not correlate with risk of coccidiosis. Blood smears will be examined for systemic coccidia and samples will be collected for the purposes of further identification of the parasite. Birds with heavy systemic burdens may be retained for further investigation of this parasite	Yes*
Limit intake of sporulated oocysts by chicks	L	L	Chicks are almost certainly infected in the nest before fledging by ingesting oocysts shed by the parents. Preventing this would be impossible and exposure of chicks is desirable. It would also be impossible to prevent	No
Husbandry and disinfection to reduce environmental load in aviaries	M	L	RHEs are very arboreal and are unlikely to be exposed to faeces even in situations where stocking density is high	No
Hygiene to minimise faecal contamination of food/water	H	H	Nectar bottles would not be contaminated and food bowls are not usually placed under perches	Yes
Minimise stress (handling, transport, overcrowding)	H	H	Low stocking densities and good husbandry practices	Yes
Manage concurrent disease e.g. MBD	L	L	Occasional chicks with MBD and others with concurrent disease have been diagnosed with coccidiosis. Incidence is low	No
Use of coccidiostats prior to translocation	H	M	Given all birds infected, no correlation between oocyst shedding and disease and incidence of coccidiosis is low it is difficult to justify treatment. Potential subclinical effect on fitness/health of birds	No

* Faecal screening (wet prep and floatation) will be conducted but not for the specific purpose of detecting coccidia, but for other potential enteric parasites such as cestodes. Systemic coccidia will be screened for on blood smears.

Hazard: Trypanosome

Options	Feasibility	Efficacy	Explanation	Decision
Pre-release isolation period (21-30d)	H	L	Easy to do but stress and overcrowding may facilitate transmission. Vectors would have access to birds regardless and would be impossible to control without insect proofing	No
Screening	H	L	Blood smear examination has low sensitivity, culture is higher. Primarily indicated for collection of specimens for research and identification. Birds with high burdens may be removed from the release cohort for further investigation on identify and clinical effect on birds	Yes
Vector control	L	M-L	The vector is unknown. This would require use of insecticides and insect exclusion barriers	No
Do not release positive birds	H	L	Given the limited evidence of pathogenicity and worldwide distribution there is insufficient evidence to support this. Cannot guarantee negative birds are free. 2 positive birds released in 2013 had good survivorship. All positive birds retained in 2013 remained healthy.	No

Hazard: Salmonella

Options	Feasibility	Efficacy	Explanation	Decision
Pre-release isolation period (21-30d)	H	L	Easy to do but stress may predispose to disease. Salmonellosis has not been reported in RHEs. Isolation may reduce possibility of infection from other captive birds but not wild birds. Does nothing with regard to RHEs that may be carriers	No
Screening (faecal/cloacal swab culture)	M	L	Organism shed intermittently. What would a positive result mean? Very resource hungry (lab work). Previous screenings have all been negative and Salmonellosis has not been seen in RHEs.	No
Minimise stress (handling, transport, overcrowding)	H	H	Low stocking densities and good husbandry practices	Yes
Good hygiene and husbandry to minimise stress and faecal contamination of food and water	H	H	Nectar bottles would not be contaminated and food bowls not usually placed under perches. RHEs are very arboreal and are unlikely to be exposed to faeces even in situations where stocking density is high	Yes
Treatment	H	L	Not recommended	No
Positive birds excluded from release	H	L	Salmonellosis not documented in RHEs, risk to wild birds low	No

- All actions are subject to change based on new information that may become available prior to or during the pre-release period. Any decision is conditional.
- Recommendations:
    - After consideration of the above hazards and any other potential hazards a specific pre-release isolation/quarantine period for the purposes of managing hazard risks is not necessary. Mustering and holding Regent Honeyeaters in aviaries separated from other species has other benefits such as socialisation, observation for reduced fitness, and facilitates management prior to release. Standard biosecurity and hygiene measures will be applied to minimise introduction of potential pathogens to the release cohort. Strict barrier keeping principals will not apply.
    - In most cases standard husbandry practices that are already in place will reduce risks associated with identified hazards. Overcrowding will be avoided and if any adverse effects (e.g. aggression) of having birds in large flock is identified, birds may be separated into smaller groups.
    - Apart from 2 treatments for lice, no specific treatments will be used during the pre-release period unless indicated.

2. Structured Decision Making

For each of the five fundamental objectives of the DRA, a measurable attribute was identified to allow comparisons between possible risk management actions in terms of their expected outcomes. The impact on other species should be measured both in terms of the number of species that decline and the magnitude of such declines. Eventually, declines by more than 30% were agreed on, since they represent the threshold for status up- or down-grading. Finally, welfare is recognised as an extremely difficult parameter to quantify rigorously. Although the survival of individuals that go through the translocation process is the attribute presented here, this is also recognised as an imperfect indicator of welfare.

Table 5: Fundamental objectives, desired directions and measurable attributes for Regent Honeyeater DRA

Objective	Direction	Attribute
1. Impact of disease on wild Regent Honeyeaters	Minimise	Proportional change in population growth rate as a result of disease
2. Impact of disease on captive Regent Honeyeaters	Minimise	Proportional change in population growth rate as a result of disease
3. Impact of disease on other species in the wild	Minimise	N of species that decline by more than 30%
4. Cost	Minimise	A$ / bird
5. Impacts of disease on welfare of individual birds that go through the process for release	Minimise	Proportion of released birds that die as a result of disease

During the workshop, the set of fundamental objectives presented in Table 5 was discussed and each participant was asked to rank objectives in terms of their proportional importance. These weightings reflect the importance of objectives to the different stakeholders when needing to make a decision. For example, if impact on wild honeyeaters was considered the most important objective, and cost was considered only half as important, Objectives 1 and 4 would be given scores of 100 and 50 respectively. Objectives of equal importance would receive equal weights. Objective weightings were then normalised to sum to 1, and averaged across participants.

Table 6: Subjective weightings of fundamental objectives, reflecting their relative importance, averaged across the group

Objective	Impact of disease on wild Regent Honeyeaters	Impact of disease on captive Regent Honeyeaters	Impact of disease on other species in the wild	Cost	Impacts of disease on welfare of individual birds that go through the process for release
Weighting	0.28	0.19	0.22	0.14	0.17

Note that the average weightings in Table 4 are not meant to represent a consensus position of the stakeholders; rather, they provide an overview of the general preferences within the group. The diversity of preferences among individual workshop attendees is DELWPcted in Figure 4.

Figure 4: Subjective scores reflecting relative preferences for objectives. A greater value indicates higher importance: for example, an objective with a score of 0.4 is considered twice as important as one with a score of 0.2.

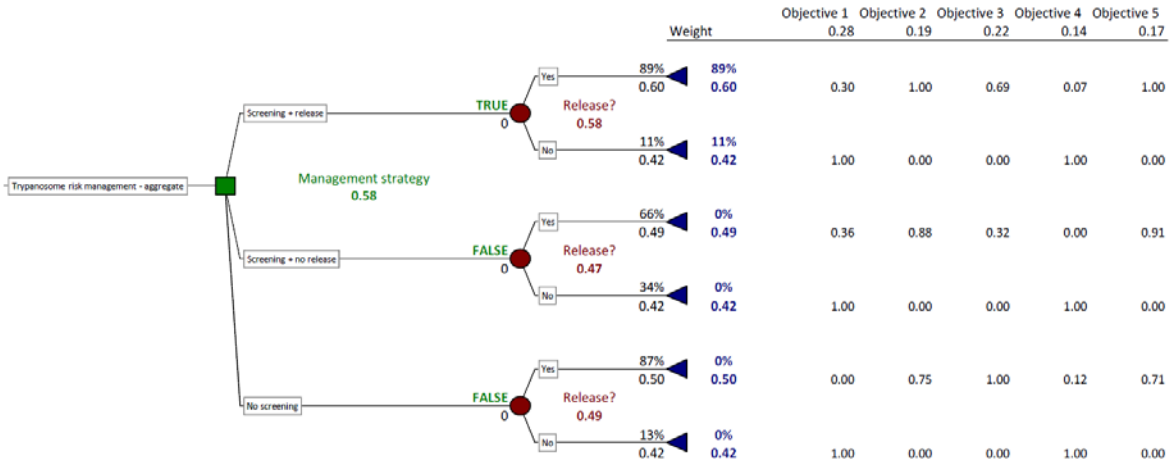
Alternative management actions

Three general strategies were identified as available for management of risks from Trypanosome: (1) screening for Trypanosome and releasing all animals; (2) screening and releasing only animals that test negative; (3) not screening any animal. Once these strategies were identified, we developed a decision tree to visualise how they would influence the level of risk, by modifying the probability of disease outbreaks and their expected outcomes. A decision tree is a graphical representation of a decision process, which calculates the expected value of alternative choices, accounting for uncertainty (Behn & Vaupel 1982). In a decision tree, the problem is represented as a flow chart, where paths traced by connecting decision nodes (choices amongst decision alternatives, conventionally represented as squares) and chance nodes (possible outcomes of stochastic processes, represented as circles) lead to a series of discrete outcomes (represented as hexagons). When a specific decision (i.e., a branch of a decision node) leads to a stochastic node with a number of possible discrete outcomes, the expected value for that decision is the average value of the outcomes weighted by their probability of occurrence.

We built the decision tree for the Trypanosome hazard using the PrecisionTree® add-in for MS Excel (Figure 5). After the chosen management action is carried out, there is a certain probability that the pathogen is released into the wild. When all animals are released, regardless of test results, and when no screening is done, this probability of release depends on the prevalence of the pathogen in the release cohort (based on available evidence, 6 out of 45 RH in 2013 only; mean prevalence 0.133, 95% binomial confidence interval 0.059, 0.266; note that this value is still subject to additional uncertainty due to the imperfect sensitivity of the methods used). When positive animals are retained after the screening, there is still a possibility that infected individuals are released, since the screening does not have perfect sensitivity: in this case, the probability of release of the pathogen depends both on its prevalence in the released bird cohort and on the sensitivity of the test.

Applying different management actions can thus change the probability of release of the pathogen (p). If the release occurs (with probability p_A for action A), a given range of outcomes can be expected; conversely, if it does not occur, these outcomes may differ. For example, the number of other species that decline *as a result of disease* will be 0 if the release does not occur and the estimated number n of species affected if the release occurs. On the other hand, other outcomes such as cost may occur regardless of whether the pathogen is released, since they are related to the management action itself (for example, the cost of screening all individuals is incurred before releasing them into the wild and is the same regardless of whether the release of the pathogen then occurs or not).

Figure 5: Decision tree for Trypanosome, built using the PrecisionTree® add-in for MS Excel



Consequences of management actions

The third step of the analysis focuses on estimating the expected consequences of each action on the risk of disease. This is necessary to be able to produce quantitative comparisons of the effectiveness of each action, which can then serve as a guide for making decisions. Each workshop participant was asked to provide estimates of how each proposed management strategy would affect (1) the expected probability that the pathogen is released into the wild and (2) the expected outcomes of an outbreak, expressed in terms of the metrics for the fundamental objectives (Figure 6).

Inevitably, uncertainty will affect estimates of risks. This is recognised and indeed explicitly quantifying such uncertainty is the only way of addressing it rigorously, accounting for its influence on decision-making and formally identifying research priorities. Therefore, in addition to their best estimate, workshop participants also provided their estimated worst- and best-case scenarios. These would represent confidence intervals which would be used to assess the effect of uncertainty on decision-making. Only one round of elicitation was achieved due to time constraint and the recognition that qualitative discussion would be adequate in the context of this DRA. The results of round one elicitation are presented here to highlight the method of DRA and show the result.

Figure 6: Sample elicitation form for estimated consequences of Trypanosome infestation

	TRYPANOSOME		Name:							
	Strategy	**p(release)**			**Impact on wild honeyeaters**			**Impact on captive honeyeaters**		
		Minimum	Most likely	Maximum	Minimum	Most likely	Maximum	Minimum	Most likely	Maximum
1	Screening+release									
2	Screening+no release									
3	No screening									
	Reference (no release)									
		Probability (0-1)			Metric: growth rate of the captive population (0-1: decline, 1=stable, >1: increase)			Metric: growth rate of the captive population (0-1: decline, 1=stable, >1: increase)		

	Strategy	**Impact on other species**			**Cost**			**Impact on welfare of individuals**		
		Minimum	Most likely	Maximum	Minimum	Most likely	Maximum	Minimum	Most likely	Maximum
1	Screening+release									
2	Screening+no release									
3	No screening									
	Reference (no release)									
		Measure: number of species which will decline by more than 30% as a result of disease			A$ spent for bird released			Proportion of released individuals that die as a result of disease		

Analysis and results

From the discussion of objectives and management strategies for Trypanosome, the decision tree described in Figure 5 was built in MS Excel using the PrecisionTree ® add-in. The average outcome of actions was then calculated. To account for uncertainty, the elicited estimates (worst-case, best-case and most likely) were used to define a beta-PERT distribution for each elicited parameter (the probability of release and the outcomes for each of the five fundamental objectives). For each of 5,000 simulation runs, the decision tree was populated by drawing values from these distributions. The final output was a distribution of expected outcomes for each action which reflect expectations and uncertainty. In addition to outcomes for each objective, a measure of aggregate impact was also calculated using a multi-attribute technique:

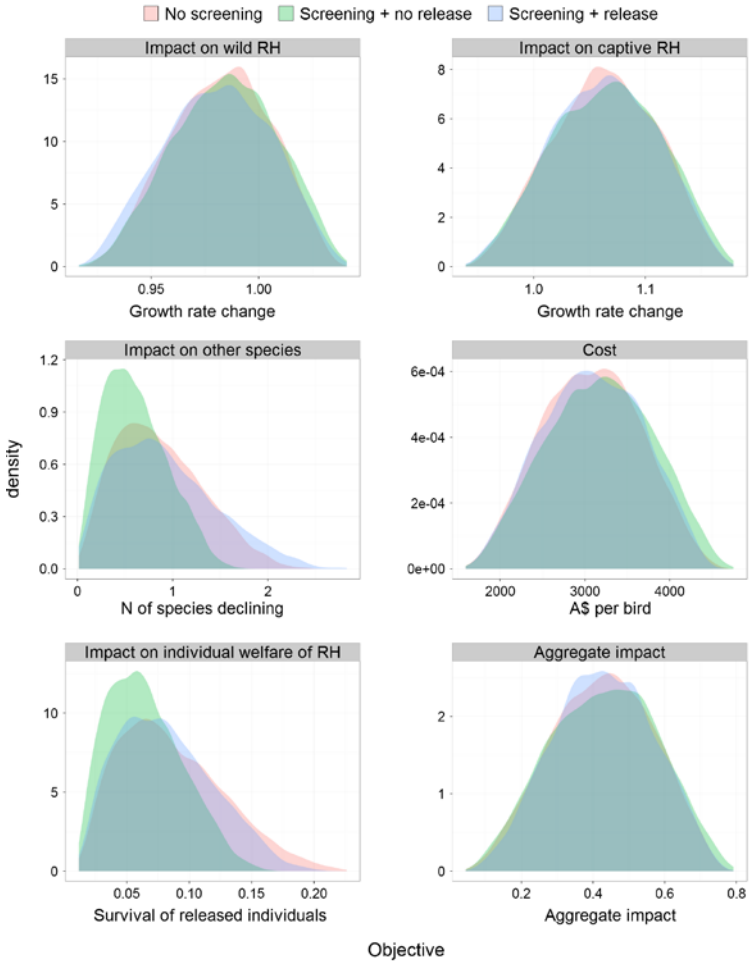
$$EV_{Atot} = EV_{A1} * w_1 + EV_{A2} * w_2 + EV_{A3} * w_3 + EV_{A4} * w_4 + EV_{A5} * w_5$$

where EV_{Atot} is the aggregate outcome of action *A* (for example, screening and release), EV_{Ai} is the expected outcome of action A relative to objective *i* (for example, the expected impact of disease on wild Regent Honeyeaters when

applying screening and release) and w_i is the weight of objective I reflecting its perceived importance (as elicited on workshop day 1; see Table 3).

The three available actions for *Trypanosoma* did not differ markedly in their expected outcome for individual objectives or aggregate impacts (Figure 7). The only marginal difference was in the impact to other species and in the survival of release individuals. The analysis suggests that choosing one management strategy over the others has only marginal consequences on the fundamental DRA objectives. Moreover, the uncertainty for all actions mostly overlaps, suggesting that learning about the effectiveness of different management actions would not be expected to lead to significantly improved management outcomes. It could be hypothesised that developing an improved method for trypanosome screening could lead to more effective management of this risk. However, the analysis does not support this hypothesis: increasing the sensitivity of the test in the simulations (i.e., the probability of releasing animals with *Trypanosoma* even when retaining all individuals that tested positive) to 0.75 and even 0.9 did not greatly alter the relative outcomes of management strategies.

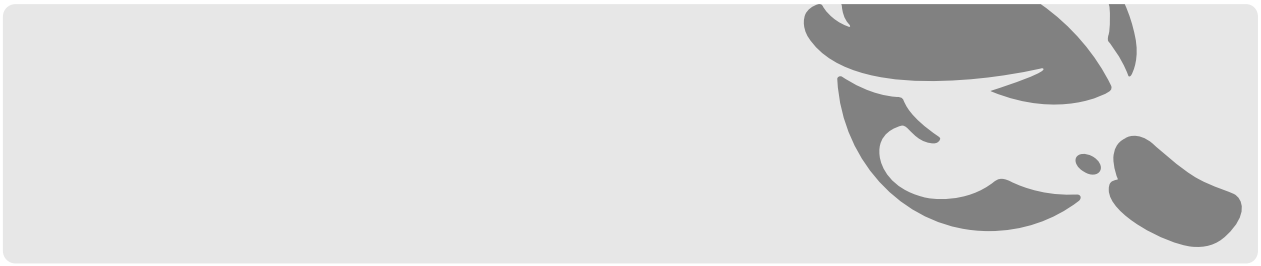
Figure 7: Estimated outcomes of management actions for Trypanosome, expressed in terms of the metrics for the fundamental objectives described in Table 1.



Reference

Behn, R. D., and J. W. Vaupel 1982. Quick analysis for busy decision makers. Basic Books, New York.

Appendix IV: Taronga Zoo Pre-Release Health Management Protocol



Purpose

The purpose of this protocol is to ensure that zoo bred Regent Honeyeaters selected for translocation to the wild as part of the Recovery Program for this species are physically healthy, fit and do not harbour any pathogens that may pose a risk to the individual birds being released or other birds of the same or different species in the wild. This protocol is based on the outcomes of a formal Disease Risk Assessment.

Any bird that fails pre-release health assessment and fitness will be removed from the release cohort.

This protocol applies only to the April 2015 release.

Pre-release protocol

All birds in the 2015 release cohort have been bred and held at Taronga Zoo.

Pre-release period (PRP)

After consideration of hazards identified through the DRA and any other potential hazards, a specific pre-release isolation/quarantine period for the purposes of managing hazard risks is not necessary. However, marshalling and holding Regent Honeyeaters in aviaries separated from other species has other benefits such as socialisation, observation for fitness, and facilitates management prior to release. Standard biosecurity and hygiene measures will be applied to minimise introduction and spread of potential pathogens to the release cohort. Strict barrier keeping principals will not apply.

In most cases standard husbandry practices that are already in place will reduce risks associated with identified hazards. Divisional husbandry procedures, biosecurity, hygiene and basic barrier keeping should be followed at all times. Overcrowding will be avoided and if any adverse effects (e.g. aggression) of having birds in large flock is identified, birds may be

separated into smaller groups. Aviaries will be maintained to avoid build-up of faecal material, food scraps, rotting vegetation and perches and browse will be maintained and replaced as needed. Food and water containers will be positioned to avoid faecal contamination and changed and/or cleaned daily.

Pre-release aviaries in which the release cohort will be marshalled should be separate from other collection bird aviaries. Exposure to wild birds is unavoidable.

- To accommodate the large number of birds (approximately 80) in the 2015 release cohort, health screening and entry into pre-release aviaries will be staggered over a period of 3-4 weeks.
- The release will occur the week beginning 13th April.
- Marshalling and screening of birds will commence the week beginning 16th February.
- Up to 5 birds a day will be screened Monday to Thursday (see schedule). After screening, the birds in each screening cohort will be held in an aviary until results are received such that birds that fail screening can be readily captured if necessary. Once cleared screening they can be released into aviaries with other birds that have completed screening and lice treatment.
- All birds are to be observed daily for signs of disease and fitness assessment.
- Any sick birds identified during PRP will be investigated and either removed from the release cohort or returned if deemed fit and healthy for release.
- Any birds that die will undergo necropsy investigation.
- The results of investigations on sick or dead birds may dictate measures and treatments that may be applied to the release cohort or stop the release from proceeding.
- All birds must be surgically or morphologically sexed and banded.
- Within 30 days prior to release, group faecal samples (one sample per 5 birds) from marshalled birds are to be checked for parasites (wet preparation and flotation) once a week for 3 weeks. If positive for helminths treatment may be indicated.

Health assessment

Each Regent Honeyeater is to undergo a physical examination and health screening under general anaesthesia prior to the PRP. After screening, the birds in each screening cohort will be held in an aviary and if the entire batch is deemed healthy and free of pathogens of concern once results are received, they will enter PRP aviaries. If a disease or pathogen of concern is identified, the affected bird/s will be further investigated. Results will determine if the individual/s will be released or if there will be any impact on the release of other birds.

The following examination procedures will be performed:

1. Thorough physical examination and weight.
2. Blood collection (up to 0.35ml from the right jugular vein). Note: the order below is in order of priority. The actual samples provided will depend on volume of blood collected.
    a. 1ml lithium heparin tube (green top) for routine haematology and biochemistry.
    b. Smear for examination for blood parasites.
    c. Plasma storage at -70^{o}C (if available).
    d. Blood sample for systemic coccidia identification
        i. Place 1-2 drops into Eppendorf tube with DNA stabilising agent (shake vigorously for 10-15 seconds to mix well, label the tube (date, time, animal ID), store at room temperature)
    e. Blood sample for trypanosome culture (see separate document for more details)
        i. Place (cleanly) 1-2 drops of blood into the culture flask with biphasic media
        ii. Close tube for transport and keep horizontally (keep away from direct sunlight, label the tube (date, time, animal ID), keep at room temperature.
    f. Blood sample for genetic analysis
        i. One drop into Eppendorf tube with 99% ethanol
        ii. One drop on Whatman FTA card

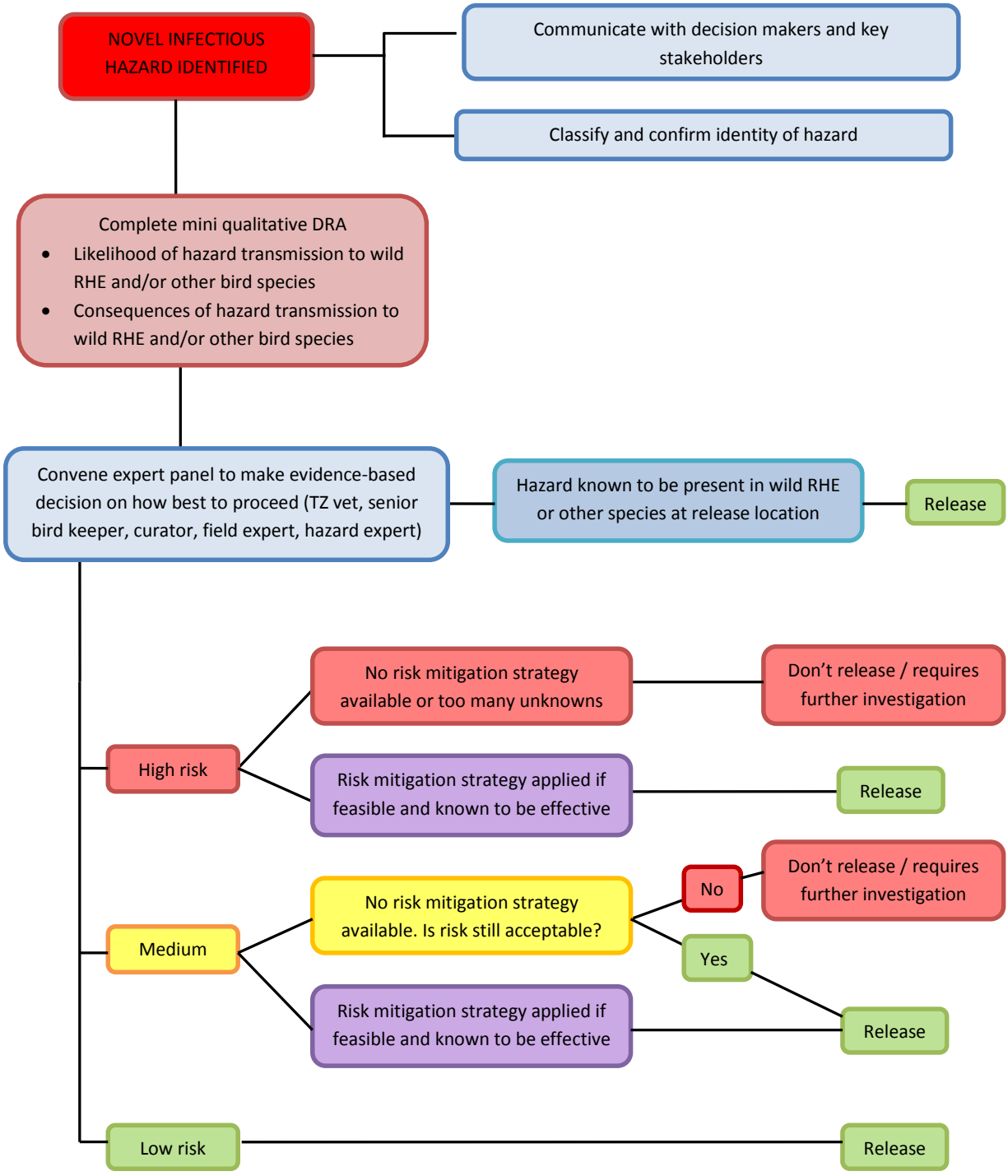
3. Careful examination of plumage for presence of feather mites and lice.
    a. Lice collection (once several lice have been collected there may be no need for more however all birds to be sprayed)
        i. Hold the bird up and puff Avitrol bird mite and lice spray onto the bird avoiding the face
        ii. Place bird in white paper bag and wait a few minutes.
        iii. Remove bird and tear bag open. Examine the paper carefully for lice (should be dead or dying). Using fine tweezers pick them up and place them in an Eppendorf tube with 70% ethanol.
        iv. Write, in pencil the date, time and bird ID on a small strip of paper and place in the tube.

Pre-release treatments:

1. Lice – each bird will be sprayed with Avitrol bird mite and lice spray (pyrethrins 0.5g/L, piperonyl butoxide 5g/L) at the time of health assessment and again when being boxed for transport to the release site.
2. During the PRP birds will be treated for cestodes using moxidectin plus in nectar.

Appendix V: Action Flow Chart for Novel Infectious Hazards

Decision tree for response to identification of a novel infectious hazard during pre-release screening of Regent honeyeaters



See communication list associated with this action flowchart below.

Communication list for decision makers and key stakeholders to be informed in response to identification of a novel infectious hazard during pre-release screening of Regent Honeyeaters

Taronga		
Position	**Current holder**	**Contact details**
General Manager, Life Sciences, Research and Conservation	Simon Duffy	0438 471 343, sduffy@zoo.nsw.gov.au
Bird curator	Paul Andrew	0409 036 063 pandrew@zoo.nsw.gov.au
Species Coordinator	Judith Gillespie	0419 410 772 jgillespie@zoo.nsw.gov.au
Australian Fauna Precinct Manager	Nick Boyle (acting)	0459 81 1024 nboyle@zoo.nsw.gov.au
Birds Unit Supervisor	Michael Shiels	0412 226 637 mshiels@zoo.nsw.gov.au
Media Relations Manager	Mark Williams	0417 293 507 mwilliams@zoo.nsw.gov.au
National Recovery Team		
Position	**Current holder**	**Contact details**
Co-Chairperson	Peter Menkhorst	0488 463 018 Peter.Menkhorst@DELWP.vic.gov.au
Member (Recovery coordinator, BirdLife Australia)	Dean Ingwersen	0409 348 553 dean.ingwersen@birdlife.org.au
Member (Victorian Government)	Glen Johnson	0418 501 936 Glen.Johnson@DELWP.vic.gov.au
Co-chairperson (NSW Government)	Peter Christie	0427835331 Peter.Christie@environment.nsw.gov.au

Appendix VI: Regent Honeyeater Diagnostic Sampling Protocols

REGENT HONEYEATER 2014 (WILD SAMPLING):

Contact / shipping address:

Jan Slapeta
McMaster Bld B14, Parasitology
Faculty of Veterinary Science
University of Sydney, NSW 2006

JAN will provide all RED items (~30 of each)

The aim of this protocol is to evidence presence/absence/prevalence of parasites in wild Regent Honeyeaters. We focus on two main parasites: coccidia and Trypanosoma that were detected in the captive population. Samples will be "banked" to form a resource for additional investigations such as prospective parasitology survey.

PARASITES: Coccidia (genus *Isospora*) and *Trypanosoma*

Coccidia can be detected in faecal samples (only afternoon samples) [Part A] using microscopy or, under some circumstances, in blood using molecular tools such as PCR [Part B].

Trypanosomes are best detected using blood culture [Part C] or, under some circumstances, in blood using molecular tools such as PCR [Part B].

PART 1) FECAL SAMPLES COLLECTION:

- bird material needed:
  - FECAL MATERIAL
- material from the lab:
  - **Eppendorf (epp.) tubes each with 1mL of 4% potassium dichromate**
  - **Small wooden spatula**
- PROCEDURE:
  - Use small wooden spatula to scoop the faecal sample (no urine)
  - Deposit the faeces into the epp. tube
  - Dispose spatula
  - Store epp. tube with faeces
  - Label epp. tube (date, time, animal ID)
- NOTES:
  - must be collected after 2 pm (ideally after 3 pm); from ZOO we know that they don't shed until 2pm and we know that at 5pm 100% birds shed oocysts in faeces
  - faecal samples collected (no urine) into epp. tube with 4% potassium dichromate (orange solution)

- all epp. tube to be labelled with date, time, bird ID.
- keep at room temperature, don't expose to >30C heat and <10C
- deliver to lab (USYD) within 2-3 days

PART 2) BLOOD SAMPLING FOR DNA ISOLATION:

- bird material needed:
  - BLOOD
- material from the lab:
  - **Eppendorf (epp.) tubes each with 100uL of DNA stabilising agent**
- PROCEDURE:
  - 1-2 drops (50uL) into a tube with stabilizing agent (for DNA)
  - Shake vigorously for 10-15 seconds to mix well
  - Store at room temperature
  - all epp. tube to be labelled with date, time, bird ID.
  - deliver to lab (USYD)
  -

PART 3) BLOOD FOR TRYPANOSOMA CULTURE:

- bird material needed: BLOOD
- material from the lab:
  - **Blood agar slopes in small culture flasks (keep in cool place, out of sunlight)**
  - **Overlay medium (keep in cool place, out of sunlight)**
- PROCEDURE
  - Add ~1mL of the overlay medium into blood agar slope to make biphasic medium
  - collect (cleanly) 1-2 drops of blood into the culture flask with biphasic media
    - try not to contaminate the culture flask with any other material
    - open drop the blood in and close immediately; culture needs oxygen
  - CLOSE TUBE FOR TRANSPORT AND KEEP HORIZONTALLY (keep away from direct sunlight)
  - keep at room temperature, don't expose to >30C heat and <10C
  - deliver to lab (USYD) within 2-3 days; if they cannot be delivered immediately open and close the tube once a day to allow oxygen in
  - all cultures to be labelled with date, time, bird ID

Regent Honeyeater field sample collection protocol for identification and collection of parasites

The aim of this protocol is to provide evidence for the presence or absence and prevalence of parasites in wild Regent Honeyeaters.

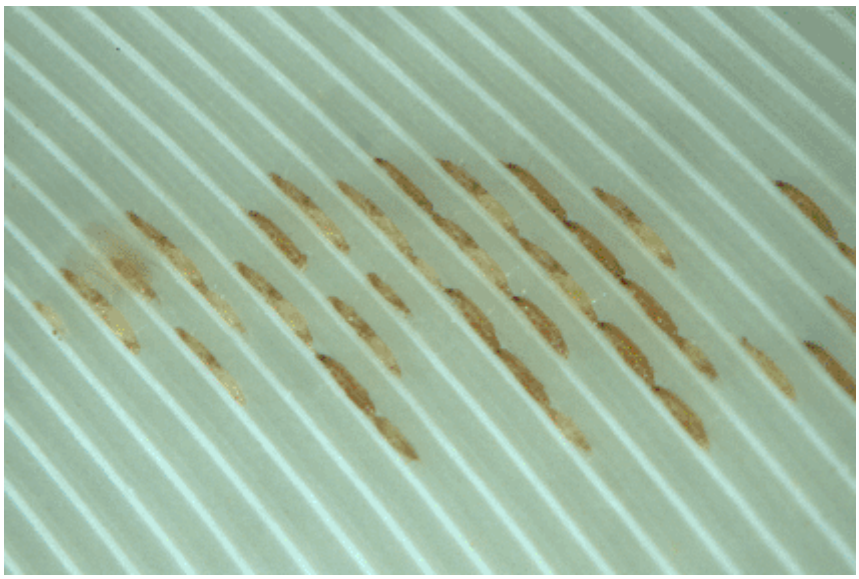
We will focus on the following parasites: coccidia (both systemic and enteric), trypanosomes, lice and mites. These have been found in the captive population.

Samples to be collected include: blood, faeces, lice and mites on feathers.

Methods:

2. Faecal sample collection (if available in bag or box from known individual bird)
    a. Use a small wooden spatula to scoop the faecal sample without urates (if there is an option to collect faeces after 3pm that is preferable as oocyte numbers are higher in the afternoon)
    b. Deposit the faeces into an Eppendorf tube with 1mL of 4% potassium dichromate
    c. Label the tube (date, time, animal ID)
    d. Sore at room temperature, don't expose to >30^{O}C and <10^{O}C
    •
3. Blood collection
    a. One person physically restrains the bird
    b. A second person gently swabs the underside of the elbow using a cotton ball soaked in 70% methanol and chlorhexidine. This will expose the wing vein
    c. Prick the vein using a 25G hypodermic needle
    d. Collect the dripping blood into 2 heparinised capillary tubes
    e. Attached capillary tube to end of plastic pipette to deliver blood
    f. Place 1-2 drops into Eppendorf tube with DNA stabilising agent
        i. Shake vigorously for 10-15 seconds to mix well
        ii. Label the tube (date, time, animal ID)
        iii. Store at room temperature
    g. Blood sample for trypanosome culture (see separate document for more details)
        i. Place (cleanly) 1-2 drops of blood into the culture flask with biphasic media
        ii. Close tube for transport and keep horizontally (keep away from direct sunlight)
        iii. Label the tube (date, time, animal ID)
        iv. Keep at room temperature, don't expose to >30^{O}C and <10^{O}C
        •
4. Mite collection
    a. Examine the vein of the wing and tail coverts carefully (magnification may be required). If present, mites can be seen in rows along the feather barbs.

b. Using scissors, snip the end of a feather with mites and place directly into an Eppendorf tube with 70% methanol.
c. Write, in pencil the date, time and bird ID on a small strip of paper and place in the tube.



5. Lice collection
    a. Examine the bird closely for the presence of lice. If present they may be seen scurrying in the feathers. They are approximately 0.5mm long and dark in colour.
    b. Even if lice are not found, hold the bird up and puff Avitrol bird mite and lice spray onto the bird avoiding the face. Note: this product is specifically for use on birds and is safe even if fumes are inhaled.
    c. Place the whole bird in the white paper bag, hold it closed and wait a few minutes. If the bird is not moving around in the bag, lightly shake the bag to encourage the bird to flutter its wings so dead lice are more likely to fall off. Note: the bags are not airtight so there is no risk of asphyxiation and the bird is only in it for a few minutes.
    d. Remove bird and tear bag open. Examine the paper carefully for lice (should be dead or dying). Using fine tweezers pick them up and place them in an Eppendorf tube with 70% methanol.
    e. Write, in pencil the date, time and bird ID on a small strip of paper and place in the tube.

Supplies provided by Taronga and University of Sydney

1. Small wooden spatulas
2. Eppendorf tubes with 1mL of 4% potassium dichromate
3. Heparinised capillary tubes
4. Plastic pipettes
5. Eppendorf tubes with 100uL of DNA stabilising agent

6. Blood agar slopes in small culture flasks and overlay medium (keep in cool place, out of sunlight)
7. Eppendorf tubes with 70% methanol
8. Avitrol bird mite and lice spray (pyrethrins 0.5g/L, piperonyl butoxide 5g/L) and puffer spray bottle
9. White paper bags
10. Fine tweezers