A tactics-based approach for improving the outcomes of eastern bettong (*Bettongia gaimardi*) reintroductions



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Declaration

This thesis is my own work, except where otherwise acknowledged and no part of this compilation has been submitted for any other academic qualification (see Preface and Acknowledgements).

William Batson

October 2015

Preface

This thesis complies with The Australian National University, Collage of Medicine, Biology and Environment guidelines for a 'Thesis by Compilation'. As such, this thesis is structured as a series of connected *papers* (journal articles and book chapters) which have been published, or submitted for publication at the time of thesis submission. Each paper is intended as an independent publication; therefore, there are areas of overlap and repetition between them (e.g. background information). The papers are listed at the end of this preface and are referred to in-text by their Roman numeral. The Extended Context Statement provided at the beginning of the thesis is not intended to be a complete literature review as included in many 'traditional theses', but rather to provide a framework for understanding the overall project, and the relationships between different aspects of the research.

I undertook the vast majority of the work for each paper included in this thesis. My efforts included concept and question development, experimental design, data collection, analysis and manuscript authorship. My role was supported by my supervisors (Adrian Manning, Iain Gordon and Don Fletcher), and other respective co-authors (Tim Portas, Racheal Abbott and Kate Richardson). The key areas of input for each collaborator are listed below. The author contribution statements below have been agreed to in writing by all authors in the respective author lists. Other assistance is acknowledged in the acknowledgments section at the end of each paper.

Papers included in thesis and author contributions:

I. Batson, WG; Gordon, IJ; Fletcher, DB & Manning, AD. (2015) Translocation tactics: A framework to support the IUCN Guidelines for wildlife translocations and improve the quality of applied methods. *Journal of Applied Ecology*, 52, 1598-1607.

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Appendix

I. Batson, WG; Fletcher, DB; Portas, T; Crisp, H; Ryan, S; Wimpenny, C; Gordon, IJ & Manning, AD. (Accepted). Reintroduction of eastern bettong to a critically endangered woodland habitat in the Australian Capital Territory, Australia. In: *IUCN Global Reintroduction Perspectives Series: 2016*. Editor: Sooare, PS. IUCN, Species Survival Commission, Reintroduction Specialist Group.

Conceptualisation: WGB & ADM; Manuscript drafting: WGB; Manuscript editing: WGB, DBF, TP, HC, SR, CW, IJG, & ADM.

II. A2. Portas, T; Cunningham, R; Spratt, D; Devlin, J; Holz, P; Batson, WG and Manning, AD. (in review) Changes in eastern bettong (*Bettongia gaimardi*) health parameters following reintroduction to the Australian mainland. *Oryx*

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Outside of the research outcomes, one of the most valuable things that I have learnt is that the key to applied conservation is the passion, dedication and expertise of the people involved. The sheer volume of people involved in the bettong reintroduction makes it impossible to name them all here. Instead, I would like to acknowledge the 'teams' involved, and highlight specific individuals who have directly assisted with my research. First and foremost, I sincerely thank the ANU team, especially the Research Officers Helen Crisp and Jenny Newport. They are both a pleasure to work with, and have generously picked up many of the jobs that fall between our roles allowing me to concentrate on my thesis. I also thank Nicky Munro, Karen Ikin, Steve Holliday, and Chris Davey for their help with endless hours of radio-tracking. Over the course of the project, there have also been dozens of volunteers who have generously given up their nights to assist with trapping, and I thank all of them for their help.

I sincerely thank the Mulligans Flat Woodland Sanctuary team across its many layers. I need to give a special mention to the Rangers Grant Woodbridge, John Lawler and Dave Whitfield for their help, hospitality and numerous cups of coffee in the field. Moving out a layer, I would like to

acknowledge other members of the ACT Government and the Woodlands & Wetlands Trust who co-manage Mulligans Flat. I am excited by their collective vision for the reserve. The involvement of Tidbinbilla has been essential to my research and the broader project. I especially thank Tim Portas, Elyce Fraser, David Dobroszczyk, Scott Ryan and Leith Collard for taking time out of their busy schedules to respond to my seemingly random requests. I firmly believe that the collaboration between Mulligans and Tidbinbilla highlights the benefit of establishing strong relationships between *in-situ* and *ex-situ* conservation initiatives. I have shared my time at Fenner with fellow students and colleagues who have helped keep me sane. I especially thank the people previously mentioned, and Laurence Berry, Darren Le Roux, Ben Scheele, Claire Foster, Nélida Villaseñor, John Evans, Claudia Benham, Philip Barton, Laura Rayner, Nick Engerer and Alessio Mortelliti for their friendship and support.

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despite the extreme sleep deprivation he inspires me to succeed and make the world a better place for him to live.

Thesis Abstract

Reintroductions aim to re-establish self-sustaining populations of the focal species within its indigenous range, but their outcomes are variable. An issue commonly perceived as limiting reintroduction success is the tendency for decisions to be based on personal opinion and general assumption. Reintroduction outcomes are ultimately determined by the relative forces of mortality, dispersal and recruitment; but these are influenced by a myriad of proximate factors that may need to be managed. This has led to a diverse array of management techniques being developed; however, comprehensive records of these are rarely available. As certain techniques can induce unpredictable effects they need to be tested to ensure that they are used appropriately. In the initial part of this thesis, I develop the concept of *Translocation Tactics* which I define as "techniques capable of influencing post-release individual performance or population persistence" (Paper I). This concept is founded on a review of 195 peer-reviewed scientific articles, the IUCN/SSC Guidelines for reintroductions and other conservation translocations ('*the Guidelines'*), and 73 case-studies from the IUCN/SSC Global Reintroduction Perspectives Series. Through this review, I identified 30 tactics used during bird and mammal translocations which I organised into The *Translocation Tactics Classification System (TTCS)* providing a structural framework to help practitioners anticipate threats, and identify appropriate tactics. I use the TTCS to assess the coverage of tactics in the Guidelines, and conclude that they offer an extensive, but not exhaustive coverage. The absence of six tactics reinforces the benefit of developing context-specific resources to support their broadly applicable approach. I expand upon this concept by outlining the theoretical basis of common *release tactics* (e.g. delayed- and immediate-release, the number of founders, behavioral training) and provide examples of their application and evidence of their effectiveness during Australasian reintroductions (Paper II).

The second part of this thesis empirically investigates the biological, behavioral and physiological effects of pre-release captivity on reintroduced eastern bettong (*Bettongia gaimardi*), to evaluate the potential use of captivity as a tactic. This research focuses on founders at Mulligans Flat

Woodland Sanctuary (MFWS) a *mainland-island* and *outdoor laboratory* in southeast Australia. Founders were released using three tactics (1) *wild-wild* or *immediate-release*, (N=16) incorporating wild founders without captive experience, (2) *wild-captive-wild* or *delayed-release*, (N=16) incorporating wild founders released after 3-18 months in captivity, (3) *captive-wild*, (N=6) incorporating captive-bred founders. Founders were monitored for up to 18 months post-release, and the data were used to compare a range of variables including performance (survival and reproduction), physiology (stress and body-mass), and behaviors (movement and nesting). My results suggest that exposing wild founders to captivity did not alter their performance or body-mass post-release, despite being heavier and having fewer pouch young when released. However, the lack of a population-level effect may reflect the high-quality and low-risk (e.g. predator-free) release-site which resulted in optimum performance irrespective of release tactic (Paper III). Pre-release captivity did induce a range of sub-lethal responses including influencing the stress physiology of wild founders which may be associated with chronic stress (Paper IV). In addition, wild and captive-bred founders tended to display wider exploratory movements, and higher rates of activity when released with captive experience; while the wild-captive-wild group also tended to build poor quality nests (Paper V). I interpreted these results based on their expected effect on establishment during subsequent reintroductions *beyond-the-fence* which carry a greater risk of failure. Overall, I recommend captivity is avoided when possible to reduce the risk to founders, except during reintroductions requiring pre-release quarantine.

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Extended context statement

General Introduction

Reintroductions are conservation translocations that aim "*to re-establish a viable population of the focal species within its indigenous range*" (IUCN/SSC, 2013, pp 3). Reintroduction is a common conservation management tool that has been applied to a plethora of threatened species across many taxonomic groups (Soorae, 2008, 2010, 2011, 2013). Despite the volume of reintroductions being attempted the probability of success is far from guaranteed. For example, Fischer & Lindenmayer (2000) estimated that only 26% of fauna reintroductions were successful; whereas, Beck, Rapaport, Price *et al.* (1994) estimated that the rate of success reduces to 11% when captive-bred founders are released. However, non-success does not necessarily equate to failure and the outcomes of a majority of reintroductions cannot be reliably assessed due to insufficient monitoring, or evidence not being presented in a publicly accessible manner (Fischer & Lindenmayer, 2000, Sutherland, Armstrong, Butchart *et al.*, 2010). It is also possible to gain significant conservation benefits from failed reintroductions by learning from them, and using the information to improve future projects (Seddon, Armstrong & Maloney, 2007). The inability to confidently evaluate outcomes is indicative of a general tendency for reintroductions to be conducted and monitored in an *ad-hoc* fashion, with "*let's put some animals out there and see if they survive*" a common mantra (Seddon *et al.*, 2007, pp 305).

The International Union for Conservation of Nature (IUCN), Species Survival Commission (SSC) established the Reintroduction Specialist Group (RSG) in 1988. The primary object of the RSG is to "*combat the ongoing and massive loss of biodiversity by using reintroductions as a responsible tool for the management and restoration of biodiversity through actively developing and promoting sound inter-disciplinary scientific information, policy, and practice*" (www.iucnsscrsg.org). To improve standards the RSG published the 'Guidelines for Re-Introductions' (1998), which were superseded by the 'Guidelines for Reintroductions and Other Conservation Translocations' (2013) (referred to as the *Guidelines* in this thesis). The Guidelines

"*provide guidance on the justification, design and implementation of any conservation translocation*" with the content '*based on principle rather than example*' (IUCN/SSC 2013 p. 1). The Guidelines are widely acknowledged as setting universal standards for reintroduction practice, and many projects appear to adhere to their recommendations (Soorae, 2008, 2010, 2011, 2013).

The outcomes of reintroductions are ultimately determined by the relative forces of mortality, dispersal and recruitment, but the strength of the effects is often amplified by the small size of reintroduced populations (Caughley, 1994). To have any chance of success, founders (i.e. the individuals released) must transition through the *establishment phase* and overcome a myriad of challenges associated with the reintroduction process, and exposure to the novel conditions at the release-site (Letty, Marchandeau & Aubineau, 2007, Armstrong & Seddon, 2008). Failure often occurs during the establishment phase because the behavioural and physiological responses to translocation increase the vulnerability to threats including starvation, predation and dispersal (e.g. Bennett, Doerr, Doerr *et al.*, 2012). If the founder population successfully transitions through the establishment phase it enters the *persistence phase* which is influenced by factors including genetic viability, and habitat suitability that act more slowly. Therefore, management may need to be designed to account for challenges that change across time (Armstrong & Seddon, 2008).

Reintroduction biology refers to research undertaken to improve the outcomes of conservation translocations (Armstrong & Seddon, 2008). Over the past few decades, reintroduction biology has developed into an accepted discipline of conservation science, and accumulated a significant body of literature including 100s of peer-reviewed articles published in respected conservation journals (Fischer & Lindenmayer, 2000, Seddon *et al.*, 2007). However, many studies are limited in their scientific rigor, and often lack fundamental components of robust experiments including clearly defined hypotheses, repetition and control (Sheean, Manning & Lindenmayer, 2012, Kemp, Norbury, Groenewegen *et al.*, 2015). Instead, a majority of studies offer opportunistic and

retrospective evaluations of aspects of the translocation process, or general summaries of reintroduction outcomes limiting the opportunity for scientific learning (Seddon *et al.*, 2007). Expanding the scientific understanding of factors that influence success relies heavily on reintroductions being conducted within experimental frameworks, but the opportunities for experimentation are often restricted by inherent issues including small sample sizes, and financial cost (Kemp *et al.*, 2015).

Reintroductions can be superficially viewed as relatively simple processes involving capture/breeding, transportation and release; but in reality, well-designed reintroductions are very complex. Much of the complexity is derived from the diversity of factors that can influence outcomes, and the broad range of techniques used to manage their effects. However, comprehensive records of the diversity of techniques are rarely available. A key function of reintroduction biology is to experimentally evaluate the effectiveness of various techniques to ensure that they are used appropriately, and avoid undesirable effects (Kemp *et al.*, 2015). Despite evaluations of techniques being common, they tend to focus on only a few of the common techniques, for example, delayed- vs immediate-release, and predator avoidance training (Griffin, Blumstein & Evans, 2000, Seddon *et al.*, 2007, Parker, Dickens, Clarke *et al.*, 2012). The absence of comprehensive records of techniques, and limited availability of evidence regarding their effectiveness, increases the risk that valuable techniques will be overlooked, and inhibits evidence-based decision-making.

Faunal reintroductions appear to be more commonly applied on isolated islands including Australia (strictly a continent), compared to other regions (Fischer & Lindenmayer, 2000), but mainland reintroductions also appear to increasing. This geographic bias largely reflects the significant impact that introduced predators have on island biodiversity, and reintroductions being attempted into areas subject to predator control or eradication programmes (Craig, Anderson,

Clout *et al.*, 2000, Short, 2009). In Australia, predation by introduced red foxes (*Vulpes vulpes*) and feral cats (*Felis catus*) has caused the extinction or decline of many native species, and remains the most significant threat to native biodiversity (Woinarski, Burbidge & Harrison, 2015). The impact has been especially profound on ground-dwelling mammals within the 35 g - 5.5 kg critical-weight-range, which have suffered many extinctions, and extant species tend to occupy a fraction of their indigenous range (Burbidge & Mckenzie, 1989, Burbidge, Mckenzie, Brennan *et al.*, 2009).

There have been numerous reintroductions involving native Australian mammals, but many have failed, primarily through unsustainable predation (Short, 2009, Clayton, Pavey, Vernes *et al.*, 2014). In response, reintroductions are increasingly being conducted into areas without foxes and cats including *mainland islands*, used here to describe predator-free fenced reserves (Dickman, 2012). The fence surrounding a mainland island is primarily designed to inhibit pest recolonisation, but can also provide additional benefits including restricting dispersal by the reintroduced population. However, fences can also be problematic by increasing population density to unnatural levels, and limiting population grown and colonisation. A common motivation behind the establishment of mainland islands is to provide a low-risk environment to re-establish populations of threatened species to provide a viable source for subsequent reintroductions. Secondary reintroductions are often targeted *beyond-the-fence* (i.e. unfenced environments with predator control), which carry a substantial risk of failure (Moseby, Read, Paton *et al.*, 2011). The increased level of risk beyond-the-fence can deter secondary reintroductions being attempted, effectively marooning populations within mainland islands which can bring their conservation value into question (Scofield, Cullen & Wang, 2011).

Mainland islands can also be considered *outdoor laboratories* if they are used to conduct ecological experiments with a level of environmental control that is unattainable beyond-the-fence

(Manning, Wood, Cunningham *et al.*, 2011, Shorthouse, Iglesias, Jeffress *et al.*, 2012, Manning, Eldridge & Jones, 2015). Therefore, reintroductions into mainland islands present the ideal opportunity to experimentally evaluate the effectiveness of different techniques without exposing genetically, demographically and economically valuable founders to unnecessary risk (Manning *et al.*, 2011, Kemp *et al.*, 2015, Manning *et al.*, 2015). The results of these experiments can then be used to optimise reintroduction protocols, and develop new hypotheses to guide future experiments. However, in the absence of the primary threats to establishment (e.g. predation and dispersal), conclusions may need to be drawn from proximate responses (e.g. behavioural and physiological), and pre-cautionary recommendations based on their anticipated effect on establishment beyond-the-fence (e.g. vulnerability to predation).

There are a number of features that can influence a founder's ability to overcome the challenges associated with reintroduction, including its life-history (Letty *et al.*, 2007). A key aspect of life-history is experience of captivity. Captivity is usually considered undesirable during reintroductions due to the high rates of failure when captive-bred founders are released (Griffith, Scott, Carpenter *et al.*, 1989, Beck *et al.*, 1994, Fischer & Lindenmayer, 2000). Many of the issues associated with captivity stem from behavioural, physiological and genetic adaptations that can occur across multiple generations. The effects of those factors on reintroduction success have received substantial scientific attention (Mathews, Orros, Mclaren *et al.*, 2005, Robert, 2009). In some situations wild founders are temporarily held in captivity prior to release for purposes including quarantine. Temporary captivity can induce adaptations within the individual, but the impact this has on post-release establishment has rarely been studied, compared to multi-generational captivity (Calvete, Angulo, Estrada *et al.*, 2005, Degregorio, Weatherhead, Tuberville *et al.*, 2013). Contrary to the expected detrimental effects of captivity, the inclusion of temporary captivity within a reintroduction process can actually benefit establishment in certain situations. For example, establishment is higher in wild Canadian lynx (*Lynx canadensis*) released after captivity compared to those released directly into the recipient environment (Devineau, Shenk,

Doherty *et al.*, 2011). As temporary captivity can influence establishment probabilities, it could potentially be used (or excluded) as a tactic.

Introduction to thesis and summary of methods and results

This thesis is structured in two parts encompassing a central theme *Translocation Tactics*. The first part (Papers I & II) is used to develop this novel concept and outline its theoretical basis. In the second part of this thesis (Papers III-V & Appendix I-II), I apply this concept to a series of experiments conducted to evaluate the potential use of pre-release captivity as a viable tactic during eastern bettong (*Bettongia gaimardi*) reintroductions.

Part One

In Paper I, I present the results of a literature review conducted to develop the Translocations Tactics concept. In this paper, I define 'translocation tactics' as "*the techniques capable of influencing post-release individual performance or population persistence*". I argue that the importance of individual components of a translocation process are often underappreciated, and the absence of comprehensive records of their diversity inhibits the ability to anticipate threats, and assign appropriate applied responses. To address these issues, I undertook a systematic review of 195 peer-reviewed articles, the IUCN/SSC Guidelines for reintroductions and other conservation translocations (IUCN/SSC 2013), and 73 case-studies from the IUCN/SSC Global Reintroduction Perspectives Series (Soorae, 2008, 2010, 2011). From this body of literature, I recorded descriptions of techniques which fulfilled my definition for translocation tactics, especially those applied during bird and mammal translocations. Using the descriptions, I identified and defined 30 tactics which I organised into the *Translocation Tactics Classification System (TTCS)*.

The TTCS is an ecologically relevant framework designed to communicate the theoretical and operational basis of translocation tactics. The TTCS serves a number of functions including

providing an easy-to-follow checklist to ensure that all available tactics are considered when developing reintroduction protocols. By defining specific tactics, I was also able to detect their presence, or absence in published resources. From the results, I conclude that the Guidelines comprehensively, but not exhaustively cover tactics detected in the wider literature. This outcome provides empirical support for the breadth and relevance of the Guidelines, but also highlights the benefit of supporting their broad content with context-specific resources. I also conclude full methodological accounts of translocations are rare in the peer-reviewed literature, which is further confounded by the absence of information outlining the tactical basis of selected methods. Based on the empirical results and my experience reviewing the literature, I make recommendations aimed at improving the communication of translocation tactics, and the quality of translocation methods.

In Paper II, I build upon the translocation tactics concept by outlining the theoretical basis of common *release tactics*. I also provide specific examples of Australasian fauna reintroductions to highlight their application and effects. The release tactics selected include the deliberate manipulation of the size and demographic and genetic composition of founder populations; and the spatial and temporal distribution of release events including delayed- and immediate-release. These tactics essentially represent tactics within the *animal* and *environment release design tactical groups* presented in the TTCS. I adapt two of Armstrong & Seddon's (2008) "key questions in reintroduction biology" to provide an appropriate structure to this essay-style paper, to make it easy to relate the content to broader theory:

1. How is establishment probability affected by the size and composition of the release group?
2. How are establishment probabilities affected by the design of release events?

Addressing these questions will help the broad spectrum of the conservation community, especially those with non-science backgrounds, understand the theoretical basis of release tactics and improve their ability to integrate theory into the design of reintroduction protocols.

Part Two

Papers III-V focus on experiments conducted to assess the biological, physiological and behavioural effects of pre-release captivity, and its potential use as tactic for reintroducing eastern bettong. The experiments were conducted during the reintroduction of eastern bettong to the Australian Capital Territory (ACT). This project represents the first attempt to reintroduce this species to mainland Australia after a 100 year absence (Short, 1998). Two populations were established in the ACT, one in captivity at Tidbinbilla Nature Reserve (TNR), and one at Mulligans Flat Woodland Sanctuary (MFWS), a mainland island and outdoor laboratory. My research primarily focused on the founder population at MFWS, and the results were used to optimise reintroduction protocols for secondary reintroductions beyond-the-fence. Text-box 1.1. presents background information regarding the study-species, study-sites and the reintroduction and experimental design. A detailed account of the overall project is also provided in Appendix I, and study-specific information is presented in Papers III-V and Appendix II.

Text-box 1.

Study-species

Eastern bettong (also known as Tasmanian bettong) is the most abundant species within the *Bettongia* genus, colloquially known as rat-kangaroos. Prior to European colonisation, the distribution of the eastern bettong extended from southeast Queensland to southwest Victoria, and Tasmania (Rose & Rose, 1998). Extinction occurred in the ACT by ca.1905, and on the mainland by the 1920s (Short, 1998, Menkhorst, 2008). Despite being relatively common in Tasmania, the eastern bettong is considered to be of conservation concern due to the likelihood of foxes becoming established in Tasmania (Menkhorst, 2008).

Young eastern bettong (*Bettongia gaimardi*). Image by Stephen Corey, © Woodlands and Wetlands Trust.

Both sexes reach maturity at ~40 weeks, and have a life-expectancy in the wild of five to six years (Rose, 1987, 1989). There is little sexual dimorphism with adults weighing between 1200-2240 g (Rose & Rose, 1998, Claridge, Seebeck & Rose, 2007). Eastern bettongs are nocturnal, usually rest within densely woven nests during daylight, and have large overlapping home ranges (males 47-85 ha, females 38-63 ha) (Taylor, 1993, Rose & Rose, 1998, Claridge *et al.*, 2007). Eastern bettongs tend to use a number of nests at irregular intervals (Taylor, 1993). This species is predominantly mycophagous, although vegetation and invertebrates are also consumed (Taylor, 1992, Rose & Rose, 1998). Females can reproduce constantly (e.g. always carrying pouch young), producing up to three independent young per year (Rose, 1987). Both sexes display little social interaction (Rose, 1987, Rose & Rose, 1998). Eastern bettongs are considered an ecosystem engineer because they dig extensively while foraging which alters soil dynamics, hydrology and nutrient cycling; and in turn, germination and ecological succession (Fleming, Anderson, Prendergast *et al.*, 2014, Manning *et al.*, 2015).

Study-sites

Tidbinbilla Nature Reserve

Tidbinbilla Nature Reserve played an integral role in this research as the captive setting for the wild-captive-wild release tactic, and the source of captive-bred founders. TNR is an accredited member of the Zoo Aquarium Association and operates captive breeding programmes for native species (www.tidbinbilla.act.gov.au). A population of eastern bettongs was established at TNR during this reintroduction to provide insurance against catastrophic failure at MFWS, enable breeding management, facilitate equipment trials (e.g. radio-collars), and conduct quarantine. The conditions at TNR can be considered 'low-intensity captivity' due to the large enclosures, low level of human interaction, and daily provision of food and water.

Mulligans Flat Woodland Sanctuary

Mulligans Flat Woodland Sanctuary is a mainland island adjacent to the suburbs of Canberra (www.mulligansflat.org.au). It is situated within one of the largest, and least degraded expanses of box-gum grassy woodland in public ownership. MFWS is part of a large-scale, long-term ecological restoration experiment involving several experimental treatments including the eradication of introduced predators, the addition of coarse woody debris, manipulated grazing and fire regimes, and species reintroductions (www.mfgowoodlandexperiment.org.au, Manning *et al.*, 2011, Shorthouse *et al.*, 2012). Previous reintroductions have included New Holland mice (*Pseudomys novaehollandiae*), bush-stone curlew (*Burhinus grallarius*) and brown tree-creeper (*Climacteris picumnus*) (www.bettongs.org, Bennett *et al.*, 2012). Eastern chestnut mice (*Pseudomys gracilicaudatus*), yellow footed antechinus (*Antechinus flavipes*), and eastern quoll (*Dasyurus viverrinus*) are due to be reintroduced in the near future.

Reintroduction and experimental design

Sixty adults and 28 pouch young were translocated from wild Tasmanian populations to the ACT during 2011-12. The translocated population was deliberately female-biased (2:1 sex ratio). Females carrying large pouch young, or with young-at-foot were excluded in Tasmania. No other selection criteria were applied. The selected bettongs were translocated to the ACT by air and road, with the translocation process designed to minimise stress (e.g. short transit times, mild sedation of adults). Translocation processes were initially trialled on small groups, before being optimised and applied for large translocation events. Upon arrival each bettong received an extensive health assessment which was repeated periodically thereafter.

This reintroduction project had three major ecological/conservation objectives:

1. To re-establish a locally extinct ecosystem engineer as part of an ecological restoration experiment.
2. To establish viable populations on the mainland to provide insurance against future declines in Tasmania.
3. To establish a viable source population for secondary mainland reintroductions.

The MFWS founder population was released using three experimental tactics:

1. *Wild-wild* or *immediate-release* incorporated wild founders translocated from Tasmania and released at MFWS within 24 hours.
2. *Wild-captive-wild* or *delayed-release* incorporated wild founders translocated from Tasmania and released at MFWS after a period of 3-18 months at TNR.
3. *Captive-wild* incorporated captive founders born at TNR and translocated to MFWS.

The wild-wild and wild-captive-wild were included in Papers III-V; whereas, the captive-wild group was only included in Paper V. Founders within the wild-wild and wild-captive-wild groups were monitored intensively for 1 year using VHF- and GPS-collars and targeted trapping; whereas, the captive-wild group was monitored

using GPS-collars for 1 month, and via opportunistic trapping thereafter. Data collected via VHF-telemetry, GPS-telemetry and trapping were used to statistically compare the biological, physiological and behavioural responses among the experimental treatment groups.

It was vital to conduct this reintroduction within an experimental framework due to the novelty of reintroduction for the focal species. The focus on the effects of pre-release captivity was selected because quarantine was required to manage ecological risk, and captive-breeding to establish multiple populations in the ACT, but the impacts on establishment probabilities were unknown.

In Paper III, I compare the performance ((1) survival and (2) female reproduction) and (3) physiological condition (body mass) of the wild-wild and wild-captive-wild groups up until 18 months post-release. To enable meaningful temporal comparison, I divided the data into periods:

Acquisition - capture in Tasmania

Release - release at MFWS (same as acquisition for the wild-wild group)

1-60 days post-release - data collected between 0-2 months at MFWS

61-180 days post-release - data collected between 2-6 months at MFWS

181-360 days post-release - data collected between 6-12 months at MFWS

361-540 days post-release - data collected after 12 months at MFWS

The results suggest that the tactic used did not have a significant effect on the three variables post-release, despite the wild-captive-wild group being heavier and carrying fewer pouch young when released. This suggests that, in this species, post-release performance and body-condition rapidly

adapted to the environmental conditions at the release-site, and physiological differences at release held little legacy post-release. However, the favourable conditions at MFWS (e.g. absence of predators and limited competition) may have induced near optimal performance and body-condition, potentially masking effects that could become apparent in more challenging environments. I base the assumption of 'near-optimality' on the indication that all of the indices met, or exceeded our pre-release expectations. I therefore conclude that founder population at MFWS successfully transitioned through the establishment phase and management should now primarily focus on persistence. Based on the results, I recommend pre-release captivity should be avoided when possible to reduce cost as it is unlikely to improve establishment in suitable habitats, but temporary captivity appears justifiable for quarantine. These recommendations are based on my interpretation of the results that captivity does not induce other physiological and behavioural responses that could influence later establishment beyond-the-fence (e.g. predation and dispersal).

In Paper IV, I compared stress indices between the wild-wild and wild-captive-wild groups up until 18 months post-release. I drew statistical comparisons using faecal glucocorticoid metabolites (FGM), and behavioural assessment data collected during trapping events. To enable meaningful temporal comparison, I divided the data into the periods described in Paper III, but without 'acquisition' due to a lack of faecal samples from Tasmania. The results suggest that pre-release captivity affected the stress physiology of the wild-captive-wild group prior to release, and over the medium-term, post-release. The observed physiological response may indicate that this group was experiencing some form of chronic stress, which may be inhibiting reproductive recruitment at TNR. The elevated FGM in the wild-captive-wild group at release also indicated that they did not acclimatise to captivity despite the prolonged extent of the captive period. FGM fluctuated seasonally and were lowest in spring, which suggests that this is an appropriate time to schedule subsequent releases to reduce the accumulated effects of multiple stress responses. I hypothesised that *in-trap behaviour* (subjective 1-3 score based on observation) would be influenced by pre-release captivity, and that behavioural variation would be related to FGM, but the results did not

support either hypothesis. Based on the results, I recommend pre-release captivity should be avoid when possible to avoid the impact on the stress physiology of founders, which could detrimentally affect establishment.

In Paper V, I compared movement behaviours between all three experimental groups, and nesting behaviour between the wild-wild and wild-captive-wild groups across the first month post-release. I drew statistical comparisons using location and observation data collected via VHF- and GPS-telemetry. The movement behaviours included *activity*, *exploration* and *dispersal*; while the nesting behaviours included *nest occupancy*, *nest reuse* and *nest construction*. These behaviours were selected on the basis that they could influence susceptibility to predation and the likelihood of dispersal beyond-the-fence, or provide indices for settlement. The results suggest that founders with captive experience tended to make longer exploratory movements, and spend a greater proportion of the night active than founders without captive experience. Captive founders tended to settle closer to their release-site than wild founders. In addition, the wild-captive-wild group tended to build poorer quality nests than the wild-wild group. Based on the results, I predicted that the risk of predation could be higher for founders with pre-release captive experience because their exploration, activity and nest construction behaviours during the establishment phase of reintroduction could increase the probability of encountering, and be detected by predators. At the same time, the risk of dispersal may be lower in captive-bred founders. Therefore, I recommend captivity is avoided when possible as a precaution, except when dispersal is considered as the primary threat; in which case, captive founders may be preferable. Overall, the results may suggest that exposure to captivity may alter how founders perceive the risk of predation post-release.

In addition to the main Paper with this thesis, I have included two chapters as appendixes. These both support the main body of research. Appendix I presents the ACT bettong reintroduction project as a case-study to be included in the next edition of IUCN Global Reintroduction

Perspectives Series. I led the authorship of this case-study because I firmly believe in the value of publishing both experimental and experiential learning outcomes. This case-study provides a detailed and extensive account of the reintroduction design, methodology, outcomes and experience based learnings. Appendix II presents a study led by the project veterinarian Dr Tim Portas which evaluates changes to blood chemistry and health parameters pre- and post-translocation, and between the two ACT populations. This paper provided valuable insight into the use of health parameters to evaluate reintroduction outcomes. This study is included as an appendix because I was not the primary author, but I was heavily involved in its development and implementation.

Synthesis of Conclusions

The value of a tactics based approach for designing translocations

The design of the actions within a translocation process are important as an applied conservation tool. A translocation process effectively extends from conceptualisation, through to post-release monitoring and communicating results (IUCN/SSC 2013). One way to view this process is as a collection of individual components. The design of specific components can influence translocation outcomes and requires careful consideration; hence, my deliberate use of the term *tactics*. Ideally, the design of every tactic would be founded on robust, reliable and relevant evidence, but the complexity of translocations means that this will never be possible. Therefore, it is vital for reintroduction biologists to not only conduct experiments to provide empirical evidence, but also develop theories which facilitate better decision-making when faced with uncertainty. The research included within this thesis aims to serve both functions.

Through the first part of this thesis, I developed the Translocation Tactics concept to improve translocation outcomes by increasing the ability to anticipate and respond to threats. In essence, this concept provides a mechanism for developing applied responses to the *key questions in*

reintroduction biology relevant at the population-level (Figure 1.1, Armstrong & Seddon, 2008). Surveying the diversity, and developing definitions for tactics encourages them to be viewed as independent units. This increases the ability to evaluate, communicate and interpret their application, design and effectiveness. Inconsistent language is a common feature of reintroduction biology, and establishing standardised terminology would increase the efficacy of communication by making it easier to find and detect relevant information. Using the definitions of tactics, I was able to systematically assess their communication across the three forms of literature (IUCN Reintroduction Guidelines, case-studies, and scientific articles). The Guidelines are the most widely accepted resource governing translocation practice. They are specifically designed to improve translocation standards by avoiding ecologically irresponsible translocations being attempted, and increasing the standard of translocations attempted. My results provide quantitative support for coverage of tactics within the Guidelines, and their value in serving the second function. Given the broad scope of the Guidelines it is not surprising that several tactics were 'missing', but these 'gaps' could be filled effectively by using the TTCS as a complementary resource.

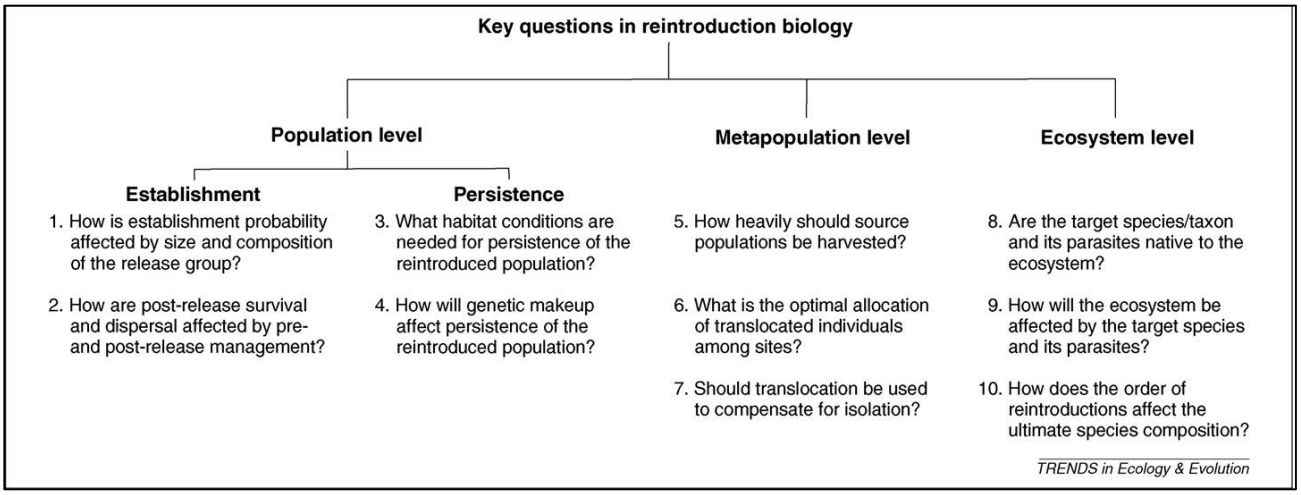

Figure 1. The key questions in reintroduction biology as presented by Armstrong & Seddon (2008). The translocation tactics concepts provides a mechanism for developing applied responses to the four questions relevant to 'establishment' and 'persistence'

The primary (scientific) literature is important for communicating translocation-related information, but my results highlight its shortcomings for communicating methodological information. The low detection rates of tactics in scientific articles suggests that most translocations apply few tactics, or that full accounts of tactics are rarely described. To be successful, a translocation only requires the sub-set of tactics which can ensure establishment and persistence. Therefore, the number of tactics applied does not necessarily reflect the 'quality' of the process. However, the accumulated effects of many tactics may be required to successfully translocate challenging species, or establish populations in challenging environments. Viewing actions as tactics encourages translocations to be conducted within the adaptive management framework, which is commonly advocated, but seldom applied (Mccarthy, Armstrong & Runge, 2012). For example, tactics are applied to induce an expected positive response, which encourages the effects to be monitored, and facilitates the process being evaluated and adapted. Adopting an adaptive process will improve the tactical strength of projects, and increase the probability of success, especially during difficult projects.

From reviewing the primary literature, it is clear that the level of methodological detail recommended by Sutherland *et al.* (2010) is rarely achieved in scientific articles. Therefore, using the primary literature as the sole method for communicating methodological information would restrict the ability to model future translocations on previous projects. The IUCN Global Reintroduction Perspectives Series (Soorae, 2008, 2010, 2011, 2013) provides a more suitable platform for communicating translocation methods, and this is reflected in my results. However, specific methodological details only represents one aspect of the information required to fully interpret methodological design. An important piece of information that is often neglected, is the tactical basis of methodological decisions. Without this information the audience can only understand '*what was done'*, and not '*why it was done'*. The absence of information regarding the theoretical basis of decisions inhibits the ability to adapt those theories to guide decision-making across different translocation contexts. Encouraging the recording of this type of information may

require new resource to be developed specifically designed to serve that purpose. The accumulation of *'tactical'* information would improve the ability to model projects based on past experiences, and avoid mistakes being repeated. It would also enable broad-scale meta-analyses to better predict the effectiveness of specific tactics within specific contexts.

The potential use of pre-release captivity as a release tactic for eastern bettong reintroductions

A tactic can only be applied effectively if its effects can be confidently predicted based on reliable evidence. Therefore, it is vital to undertake empirical research to assess the effectiveness of tactics by conducting translocations within experimental frameworks (Kemp *et al.*, 2015). The second part of this thesis was designed to investigate the biological, physiological and behavioural effects of pre-release captivity to evaluate its potential use and effectiveness as a tactic for reintroducing eastern bettongs.

The results from Paper III suggest that temporary captivity did not alter two fundamental determinates of reintroduction outcomes; post-release survival and reproduction. In addition, this study indicated that increasing the body-mass of founders in captivity prior to release did not influence their body mass over the medium-term post-release. When interpreting these results, it is important to consider the context of the reintroduction. For example, without context the results of Paper III could simply be interpreted as indicating pre-release captivity does not affect the probability of establishment. However, this conclusion may only be relevant for eastern bettong reintroductions into low-risk high-quality recipient environments similar to MFWS. Therefore, it may also be valuable to investigate sub-lethal responses, and use the results to develop new hypotheses regarding the potential effects of focal tactics when applied during projects with different contexts (e.g. a high-risk recipient environment).

The experiments presented in Papers IV and V were conducted to investigate two sub-lethal responses that may influence establishment beyond-the-fence, physiology and behaviour. The results suggest that temporarily housing wild eastern bettongs in captivity influenced their stress physiology over the short-to-medium term, which could influence establishment through a number of mechanisms including disease, starvation or predation (Teixeira, De Azevedo, Mendl *et al.*, 2007, Dickens, Delehanty & Romero, 2010). Although, these responses did not induce a population-level effect at MFWS, the favourable conditions may have buffered the effects. Similarly, both forms of pre-release captivity (captive-breeding and temporary captivity) influenced post-release movement, and temporary captivity influenced nest construction. In general, founders with captive experience tended to display behaviours that can be expected to increase risk beyond-the-fence. The only potentially beneficial behavioural response which appeared to be associated with captivity was a potential reduction in dispersal in captive-bred founders which could be desirable in small unfenced areas of predator control.

Based on the accumulated results of Papers III-V, I generally recommend that captivity is avoided whenever possible. This is based on the assumption that the observed responses are more likely to have a detrimental effect on establishment, than a positive effect, which would not justify the extra economic cost. Therefore, I conclude that pre-release captivity does not represent a viable tactic for this species (but it could be tactically avoided), and potentially other macropods. However, the absence of a positive response to captivity does not necessarily mean it could not be justifiably used to obtain another benefit (e.g. quarantine). Therefore, I advocate that the potential ecological benefits of pre-release captivity (e.g. avoiding detrimental co-introductions of pathogens, parasites, or disease), is balanced against the potential biological costs (e.g. increase predation), and economic cost when planning reintroductions.

Conducting research focused on a few key hypotheses can often produce valuable secondary learning outcomes. By focusing on the physiological and behavioural responses to a potential release tactic, I gathered evidence that can be used to improve reintroduction practice including scheduling releases in spring, and avoiding using behavioural proxies to assess physiological stress. It is important to communicate these learning outcomes, and integrate them in to the design of reintroductions because reintroduction success, and maintaining feasibility may require the accumulated effects of many tactics, rather than a single 'silver-bullet' solution.

It is equally important to consider the context when evaluating experimental design, as it is when interpreting results. Releasing founders into a low-risk mainland island may be seen as an experimental weakness (because the absence of predators and the barrier to dispersal), meaning that recommendations for reintroductions beyond-the-fence can only be founded on educated assumptions. However, it must also be appreciated that the loss of founders may have inhibited the ability to detect responses and develop such recommendations. For example, any predation during the monitoring period would have inevitably reduced the data available for analysis, and exaggerated statistical issues including sample size, and insufficient statistical power.

Future Directions

In light of the expected continuation of biodiversity declines in Australia and around the world, translocation is likely to increase in application and conservation value (Thomas, 2011, Seddon, Griffiths, Soorae *et al.*, 2014, Woinarski *et al.*, 2015). Therefore, it is essential that the field of reintroduction biology also continues to develop. It is vital that in the pursuit of scientific advancement (e.g. novelty), the value of testing '*what we actually do'* does not diminish. In response to threats to biodiversity, environmental refuges including mainland islands are also likely to increase in number and conservation value (Hayward & Kerley, 2009). To maximise the

conservation benefits achieved by establishing these expensive resources, it is important that they are viewed as stepping-stones back to the wild, rather than reservoirs of threatened biota.

Moving into the future, I believe substantial ecological benefits could be gained by following the procedural model of this reintroduction project, in addition to the research outcomes presented in this thesis. The ACT eastern bettong reintroduction has the long-term objective of re-establishing this species beyond-the-fence, using the mainland island population as the initial source of founders. Adding the intermediary step within the translocation model (wild → mainland island/outdoor laboratory → wild) has allowed the initial pool of tactics (identified from limited information), to be tested and refined prior to secondary reintroductions being attempted. The ability to identify and improve specific practices could be pivotal to future success in the broader landscape. Without the knowledge gained through the outdoor laboratory, founders released beyond-the-fence may have been deliberately exposed to pre-release captivity without considering the potential impact on establishment. Based on my results, this would have been likely to have increased the vulnerability of founders, and ultimately increased the risk of failure.

There are obvious costs associated with failed reintroductions (e.g. the loss of individuals, financial expense), but the costs are likely be greater during initial reintroduction attempts, than secondary reintroductions. Much of the extra cost is associated with the barriers created by reintroduction failure which can deter future attempts. If these barriers cannot be overcome it effectively stops the adaptive reintroduction model ever coming to fruition. We must not be afraid to fail, but must learn from failures if they occur, rather than cease trying. In our project, the risk of failure was managed in a number of ways, including releasing founders into low-risk environments, involving veterinarians throughout the process, and conducting trials. The benefits gained from the reduction of risk were built upon with knowledge gained by conducting the reintroduction within an experimental framework. This research has provided key information that

can improve the probability of future success. Adopting this model has influenced risk in two important ways. Initially it reduced the risk of failure, and in the future it will encourage risks to be taken (e.g. releases beyond-the-fence). It is ultimately, reintroductions that carry risk that will achieve the greatest ecological benefits. Facilitating these projects, and improving the probability of success needs to be a central focus of reintroduction biology as it develops.

References

Armstrong, D.P. & Seddon, P.J. (2008). Directions in reintroduction biology. Trends in Ecology & Evolution 23, 20-25.

Beck, B.B., Rapaport, L.G., Price, M.R.S. & Wilson, A.C. (1994). Reintroduction of captive-born animals. In Creative Conservation: 265-286. Olney, P. J. S., Mace, G. M. & Feistner, A. T. C. (Eds.). Springer Netherlands.

Bennett, V.A., Doerr, V.a.J., Doerr, E.D., Manning, A.D. & Lindenmayer, D.B. (2012). The anatomy of a failed reintroduction: a case study with the Brown Treecreeper. Emu 112, 298-312.

Burbidge, A.A. & Mckenzie, N.L. (1989). Patterns in the modern decline of western Australia's vertebrate fauna: Causes and conservation implications. Biological Conservation 50, 143-198.

Burbidge, A.A., Mckenzie, N.L., Brennan, K.E.C., Woinarski, J.C.Z., Dickman, C.R., Baynes, A., Gordon, G., Menkhorst, P.W. & Robinson, A.C. (2009). Conservation status and biogeography of Australia's terrestrial mammals. Australian Journal of Zoology 56, 411-422.

Calvete, C., Angulo, E., Estrada, R., Moreno, S. & Villafuerte, R. (2005). Quarantine length and survival of translocated European wild rabbits. Journal of Wildlife Management 69, 1063-1072.

Caughley, G. (1994). Directions in Conservation Biology. Journal of Animal Ecology 63, 215-244.

Claridge, A.W., Seebeck, J. & Rose, R.W. (2007). Bettongs, potoroos and the musky rat-kangaroo. Collingwood, VIC: CSIRO Publishing.

Clayton, J.A., Pavey, C.R., Vernes, K. & Tighe, M. (2014). Review and analysis of Australian macropod translocations 1969-2006. Mammal Review 44, 109-123.

Craig, J., Anderson, S., Clout, M., Creese, B., Mitchell, N., Ogden, J., Roberts, M. & Ussher, G. (2000). Conservation Issues in New Zealand. Annual Review of Ecology and Systematics 31, 61-78.

Degregorio, B.A., Weatherhead, P.J., Tuberville, T.D. & Sperry, J.H. (2013). Time in captivity affects foraging behavior of ratsnakes: implications for translocation. Herpetological Conservation and Biology 8, 581-590.

Devineau, O., Shenk, T.M., Doherty, P.F., White, G.C. & Kahn, R.H. (2011). Assessing release protocols for Canada lynx reintroduction in Colorado. The Journal of Wildlife Management 75, 623-630.

Dickens, M.J., Delehanty, D.J. & Romero, L.M. (2010). Stress: An inevitable component of animal translocation. Biological Conservation 143, 1329-1341.

Dickman, C. (2012). Fences or Ferals? Benefits and Costs of Conservation Fencing in Australia. In Fencing for Conservation: 43-63. Somers, M. J. & Hayward, M. (Eds.). Springer, New York.

Fischer, J. & Lindenmayer, D.B. (2000). An assessment of the published results of animal relocations. Biological Conservation 96, 1-11.

Fleming, P.A., Anderson, H., Prendergast, A.S., Bretz, M.R., Valentine, L.E. & Hardy, G.E.S. (2014). Is the loss of Australian digging mammals contributing to a deterioration in ecosystem function? Mammal Review 44, 94-108.

Griffin, A.S., Blumstein, D.T. & Evans, C. (2000). Training captive-bred or translocated animals to avoid predators. Conservation Biology 14, 1317-1326.

Griffith, B., Scott, J.M., Carpenter, J.W. & Reed, C. (1989). Translocation as a Species Conservation Tool - Status and Strategy. Science 245, 477-480.

Hayward, M.W. & Kerley, G.I.H. (2009). Fencing for conservation: Restriction of evolutionary potential or a riposte to threatening processes? Biological Conservation 142, 1-13.

IUCN/SSC (2013). Guidelines for reintroductions and other conservation translocations. Gland, Switzerland: IUCN Species Survivial Commission.

Kemp, L., Norbury, G., Groenewegen, R. & Comer, S. (2015). The roles of trials and experiments in fauna reintroduction programs In Advanves in reintroduction biology of Australian and New Zealand fauna: 73-90. Armstrong, D. P., Hayward, M. W., Moro, D. & Seddon, P. J. (Eds.). Clayton South, VIC, Australia: CSIRO Publishing.

Letty, J., Marchandeau, S. & Aubineau, J. (2007). Problems encountered by individuals in animal translocations: Lessons from field studies. Ecoscience 14, 420-431.

Manning, A., Wood, J., Cunningham, R., Mcintyre, S., Shorthouse, D., Gordon, I. & Lindenmayer, D. (2011). Integrating research and restoration: the establishment of a long-term woodland experiment in south-eastern Australia. Australian Zoologist 35, 633-648.

Manning, A.D., Eldridge, D.J. & Jones, C.G. (2015). Policy implications of ecosystem engineering for multiple ecosystem benefits. In Advances in reintroduction biology of Australian and New Zealand fauna: 167-184. Armstrong, D. P., Hayward, M. W., Moro, D. & Seddon, P. J. (Eds.). Clayton South, Australia: CSIRO Publishing.

Mathews, F., Orros, M., Mclaren, G., Gelling, M. & Foster, R. (2005). Keeping fit on the ark: assessing the suitability of captive-bred animals for release. Biological Conservation 121, 569-577.

Mccarthy, M.A., Armstrong, D.P. & Runge, M.C. (2012). Adaptive Management of Reintroduction. In Reintroduction Biology: Integrating Science and Management.: 256-289. Ewen, J. G., Armstrong, D. P., Parker, K. A. & Seddon, P. J. (Eds.). Oxford, U.K.: Blackwell Publishing Ltd.

Menkhorst, P. (2008). *Bettongia gaimardi*. In IUCN 2014. IUCN Red List of Threatened Species. Version 2014.1).

Moseby, K.E., Read, J.L., Paton, D.C., Copley, P., Hill, B.M. & Crisp, H.A. (2011). Predation determines the outcome of 10 reintroduction attempts in arid South Australia. Biological Conservation 144, 2863-2872.

Parker, K.A., Dickens, M.J., Clarke, R.H. & Lovegrove, T.G. (2012). The theory and practice of catching, holding, moving and releasing animals. In Reintroduction biology: integrating science and management: 105-137. Ewen, J. G., Armstrong, D. P., Parker, K. A. & Seddon, P. J. (Eds.). Oxford, U. K.: Wiley-Blackwell Publishing Ltd.

Robert, A. (2009). Captive breeding genetics and reintroduction success. Biological Conservation 142, 2915-2922.

Rose, R.W. (1987). Reproductive biology of the Tasmanian Bettong (*Bettongia gaimardi*: Macropodidae). Journal of Zoology 212, 59-67.

Rose, R.W. (1989). Age Estimation of the Tasmanian Bettong (*Bettongia gaimardi*) (Marsupialia, Potoroidae). Wildlife Research 16, 251-261.

Rose, R.W. & Rose, R.K. (1998). *Bettongia gaimardi*. Mammalian Species 584, 1-6.

Scofield, R.P., Cullen, R. & Wang, M. (2011). Are predator-proof fences the answer to New Zealand's terrestrial faunal biodiversity crisis? New Zealand Journal of Ecology 35, 312-317.

Seddon, P.J., Armstrong, D.P. & Maloney, R.F. (2007). Developing the science of reintroduction biology. Conservation Biology 21, 303-312.

Seddon, P.J., Griffiths, C.J., Soorae, P.S. & Armstrong, D.P. (2014). Reversing defaunation: Restoring species in a changing world. Science 345, 406-412.

Sheean, V.A., Manning, A.D. & Lindenmayer, D.B. (2012). An assessment of scientific approaches towards species relocations in Australia. Austral Ecol. 37, 204-215.

Short, J. (1998). The extinction of rat-kangaroos (Marsupialia:Potoroidae) in New South Wales, Australia. Biological Conservation 86, 365-377.

Short, J. (2009). The characteristics and success of vertebrate translocations within Australia: a progress report. Department of Agriculture, Australia.

Short, J., Bradshaw, S.D., Giles, J., Prince, R.I.T. & Wilson, G.R. (1992). Reintroduction of macropods (marsupialia, macropodoidea) in Australia - a review. Biological Conservation 62, 189-204.

Shorthouse, D.J., Iglesias, D., Jeffress, S., Lane, S., Mills, P., Woodbridge, G., Mcintyre, S. & Manning, A.D. (2012). The 'making of' the Mulligans Flat – Goorooyarroo experimental restoration project. Ecological Management & Restoration 13, 112-125.

Soorae, P.S. (Ed.) (2008) Global re-introduction perspectives: re-introduction case-studies from around the globe, Abu Dhabi, UAE, IUCN/SSC Re-introduction Specialist Group.

Soorae, P.S. (Ed.) (2010) Global re-introduction perspectives: Additional case-studies from around the globe, Abu Dhabi, IUCN/SSC Re-introduction Specialist Group.

Soorae, P.S. (Ed.) (2011) Global Re-introduction Perspectives: 2011. More case studies from around the globe., Gland, Switzerland: IUCN/SSC Re-introduction Specialist Group and Abu Dhabi, UAE: Environment Agency-Abu Dhabi.

Soorae, P.S. (Ed.) (2013) Global Re-introduction Perspectives: 2013 Further case-studies from around the globe. Gland, Switzerland, SSC Re-introduction Specialist Group and Abu Dhabi, UAE: Environment Agency-Abu Dhabi.

Sutherland, W.J., Armstrong, D., Butchart, S.H.M., Earnhardt, J.M., Ewen, J., Jamieson, I., Jones, C.G., Lee, R., Newbery, P., Nichols, J.D., Parker, K.A., Sarrazin, F., Seddon, P.J., Shah, N. & Tatayah, V. (2010). Standards for documenting and monitoring bird reintroduction projects. Conservation Letters 3, 229-235.

Taylor, R.J. (1992). Seasonal-changes in the diet of the tasmanian bettong (*Bettongia gaimardi*), a mycophagous marsupial. Journal of Mammalogy. 73, 408-414.

Taylor, R.J. (1993). Home range, nest use and activity of the Tasmanian bettong, *Bettongia gaimardi*. Wildlife Research 20, 87-95.

Teixeira, C.P., De Azevedo, C.S., Mendl, M., Cipreste, C.F. & Young, R.J. (2007). Revisiting translocation and reintroduction programmes: the importance of considering stress. Animal Behaviour 73, 1-13.

Thomas, C.D. (2011). Translocation of species, climate change, and the end of trying to recreate past ecological communities. Trends in Ecology & Evolution 26, 216-221.

Woinarski, J.C.Z., Burbidge, A.A. & Harrison, P.L. (2015). Ongoing unraveling of a continental fauna: Decline and extinction of Australian mammals since European settlement. Proceedings of the National Academy of Sciences 112, 4531-4540.

Paper I: Translocation tactics: A framework to support the IUCN Guidelines for wildlife translocations and improve the quality of applied methods.

The translocation process is comprised of multiple components. Many components can be specifically used or designed to improve the probability of success, and therefore, represent *tactics*. Despite the effect tactics can have on translocation outcomes, they are rarely defined, and comprehensive records of their diversity are rarely available. This increases the risk that potentially valuable tactics will be overlooked. This paper presents the results of a broad review of three prominent forms of translocation-related literature. From the results, I developed definitions for tactics used during bird and mammal translocations, and created the Translocation Tactics Classification System (TTCS). This system organises the diversity of tactics into an ecologically relevant framework. I also assess the communication of tactics among different forms of translocation literature, and provide recommendations that could improve the use of tactics in future translocations.

Batson, W.G., Gordon, I.J., Fletcher, D.B. & Manning, A.D. (2015) Translocation tactics: A framework to support the IUCN Guidelines for wildlife translocations and improve the quality of applied methods. Journal of Applied Ecology. 52, 1598-1607.

Abstract

1. Translocation is a popular conservation tool, but the outcomes are variable. Many tactics can be used to improve the probability of success, but a comprehensive summary of these does not exist. This increases the risk that valuable tactics will be overlooked, and inhibits effective communication.
2. We assess the diversity of 'translocation tactics' used in mammal and bird translocations, by reviewing the IUCN/SSC Guidelines for Re-introduction and other Conservation Translocations, 195 peer-reviewed articles, and 73 case-studies from the IUCN/SSC Global Re-introduction Perspectives Series.
3. We recorded descriptions of every technique used to influence the post-release performance of translocated wildlife. We developed the Translocation Tactics Classification System (TTCS) which defines a collection of 30 tactics and organize them into an ecologically-relevant framework. We also assess the occurrence of tactics within the Guidelines, the primary literature, and the case-studies to evaluate how tactics are communicated within these media.
4. Our results indicate that the Guidelines are a valuable resource, but do not exhaustively cover tactics, and that detailed methodological accounts are rarely made publicly accessible. This highlights the need to develop context-specific resources to support the Guidelines, and to develop and exploit mediums that facilitate recording of methodological detail, the tactical rationale behind the design, and evaluations of effectiveness. Although some forms of grey-literature address this issue, the general lack of information limits the ability to investigate the relationship between tactics and translocation success.
5. *Synthesis and applications.* The Translocation Tactics Classification System (TTCS) provides a checklist which ensures that the full diversity of tactics are considered when developing translocation processes. Standardizing the communication of tactics, and encouraging detailed accounts of applied methodologies to be recorded, along with the tactical reasoning behind the design, will provide operational models and the data required to conduct broad meta-analyses.

Introduction

"*Strategy without tactics is the slowest route to victory. Tactics without strategy is the noise before defeat*." ― Sun Tzu, The Art of War (c.500 BC).

'Conservation translocations' (hereafter referred to as 'translocations') describe the deliberate movement of wildlife for the purposes of conservation (Seddon 2010; IUCN/SSC 2013). Despite the growing popularity of translocations as a conservation tool, the outcomes remain variable due to the myriad of factors that can affect translocated populations (Griffith et al. 1989; Wolf et al. 1996; Fischer & Lindenmayer 2000). Translocations inherently fail from a population perspective when the effects of mortality, dispersal and disrupted reproduction cannot be mitigated (Soorae 2008, 2010, 2011, 2013). The probability of success can be improved by using 'tactics' which are techniques capable of influencing post-release individual performance or population persistence. The selection and design of tactics should be founded on 'strategy' which are clearly defined objectives (e.g. minimizing dispersal) that guide the selection of tactics which maximize the probability of success, whilst maintaining the efficiency and feasibility of the overall project. Many tactics are commonly applied during translocations, including controlling the number of individuals released, selecting suitable areas of habitat and incorporating a confinement period prior to release (Miller et al. 1999; Armstrong & Seddon 2008; Parker et al. 2012; Batson, Abbott & Richardson 2015). However, a comprehensive summary of the diversity of tactics is not currently available, which reduces the standardization of terminology, and increases the risk that potentially valuable tactics could be overlooked in translocation design, especially given the specific needs of different translocations.

Although tactics can improve the probability of success, they are unlikely to be effective unless they are integrated into an appropriately designed process. We use the terms strategy and tactics deliberately to highlight the need to consider the design of translocation processes within the

context of these concepts. Ideally, translocations are designed by interdisciplinary groups making evidence-based decisions (IUCN/SSC 2013), but many decisions appear to be founded upon personal knowledge, opinions and experience (Parker et al. 2012). This reliance on human cognisance may affect the quality of translocation processes due to variability in the knowledge of tactics. The potential for variability in the implementation of translocations is compounded by the complexity of translocations and the unpredictability of biological and behavioural responses to different methods (Miller et al. 1999; Seddon, Strauss & Innes 2012; Moseby, Hill & Lavery 2014). Therefore, increasing the conceptual and theoretical understanding of tactics could help to improve conservation outcomes by increasing the general quality of translocation processes.

The IUCN Reintroduction Specialist Group (RSG) was established in 1988 in response to the proliferation of poorly-managed translocations (Seddon, Armstrong & Maloney 2007). Since inception the RSG has advocated universal standards for translocation by publishing the Guidelines for Reintroduction (1998) and the Guidelines for Reintroduction and other Conservation Translocations (2013). The purpose of these Guidelines is to "provide guidance on the justification, design and implementation of any conservation translocation" and the content is "based on principle rather than example" (IUCN/SSC 2013 p. 1). The Guidelines are widely accepted by the conservation community, who generally adhere to the recommended standards (Soorae 2008, 2010, 2011, 2013). However, the necessary broad scope and non-taxon specific nature of the Guidelines restricts the depth of information regarding specific aspects of translocation practice. This suggests that there is a need to support the Guidelines with resources with a specific focus. In recognition of the benefits associated with recording practical information, the IUCN/SSC produced the Global Re-introduction Perspectives Series (Soorae 2008, 2010, 2011, 2013). This series presents reintroduction case-studies with a focus on application, key learnings and ultimate outcomes.

Here we present a tactics-focused resource to complement the Guidelines. Our objective is to identify, define and organize the diversity of tactics used to improve the outcomes of bird and mammal translocations. These taxonomic groups were selected because they are involved in a significant proportion of translocation projects and are over-represented in the translocation-related literature (Fischer & Lindenmayer, 2000, Seddon et al., 2005, Bajomi et al., 2010). To achieve this we reviewed the content of the 2013 edition of the Guidelines, a collection of 195 articles from the primary literature, and 73 case-studies from the Global Re-introduction Perspectives Series (Soorae 2008, 2010, 2011). From this review, we developed the Translocation Tactics Classification System (TTCS) which supports the Guidelines by placing the diversity of tactics into a logical and ecologically-relevant framework. The purpose of the TTCS is to ensure that conservation groups are fully aware of the range of tactics available when designing translocations. We also evaluate the communication of tactics within all three media, and provide recommendations to improve the standard of communication in the future.

Methods

We reviewed the content of the Guidelines and recorded descriptions of every technique that fulfilled our criteria for a tactic. To maintain a specific focus we excluded techniques associated with species-selection, monitoring, capture, handling and transportation because these are often taxon-specific and have also been reviewed elsewhere (Kleiman 1989; Letty, Marchandeau & Aubineau 2007; Parker *et al.* 2012). We also excluded techniques associated with non-ecological or biological aspects of translocation (e.g. economic, social and political), and those stated as being associated with translocations not involving birds or mammals. We then repeated this process on a collection of 195 articles accessed using the ISI Web of Science data base in January 2013. We identified articles using the search terms Translocat* OR Reestabl* OR Re-establ* OR Reintroduc* OR Re-introduc*, Introduc* OR Relocat* OR Re-locat* entered in 'Topic', with the 'Research Area' restricted to 'Biological Conservation' which produced 1499 hits. This was reduced to 195 articles using the following criteria: (i) a full pdf. version of the article was

accessible using the Find Full Text function in Endnote X6, (ii) the article focused on mammal or bird translocation(s) (including simulation models and reviews, and (iii) the article included a description of at least one tactic. We used the same criteria to select 73 case-studies published in the mammal and bird sections of Global Reintroduction Perspectives Series (Soorae 2008, 2010, 2011).

We recorded a description of each technique that was indicated as being implemented or excluded to improve post-release performance or persistence (e.g. survival or reproduction), induce a desirable behavioural response (e.g. settlement) or to avoid/mitigate a potential threat (e.g. mortality or genetic viability). We also recorded descriptions of techniques that were recommended to be used in subsequent projects, and those suggested as being beneficial in reviews and modelling articles. We excluded techniques associated with capture, handling and transportation on the assumption that these are predominantly taxon-specific. We developed definitions of tactics using the descriptions, ensuring that every technique described was accounted for within a definition. We grouped tactics according to operative similarities into a hierarchical framework to produce the TTCS. We also recorded presence or absence of each tactic within the Guidelines, articles and case-studies to allow the communication of tactics to be compared between mediums.

Results

We identified and defined a total of 30 tactics during the review process (Table 1). Each tactics was identified in the collection of case-studies, 29 were detected within the collection of articles, and 24 were detected in the Guidelines (Table 1). The tactics were organized into the TTCS based on operational similarities. The uppermost tier of the framework is the *tactical pool* which represents the entire collection of tactics. The tactical pool is divided to form the *tactical focus groups* which differentiate between *animal-focused tactics* and *environment-focused tactics* based

on aspect of the translocation process in which they operate. The tactical focus groups are further subdivided into *tactical groups* by differentiating between tactics that operate according to the principles of *selection, preconditioning, release design* or *post-release management*. Each tactical group is subdivided into specific *tactics* (Figure 1). The detection rates of the tactical groups and tactics in the three media are presented in Table 1.

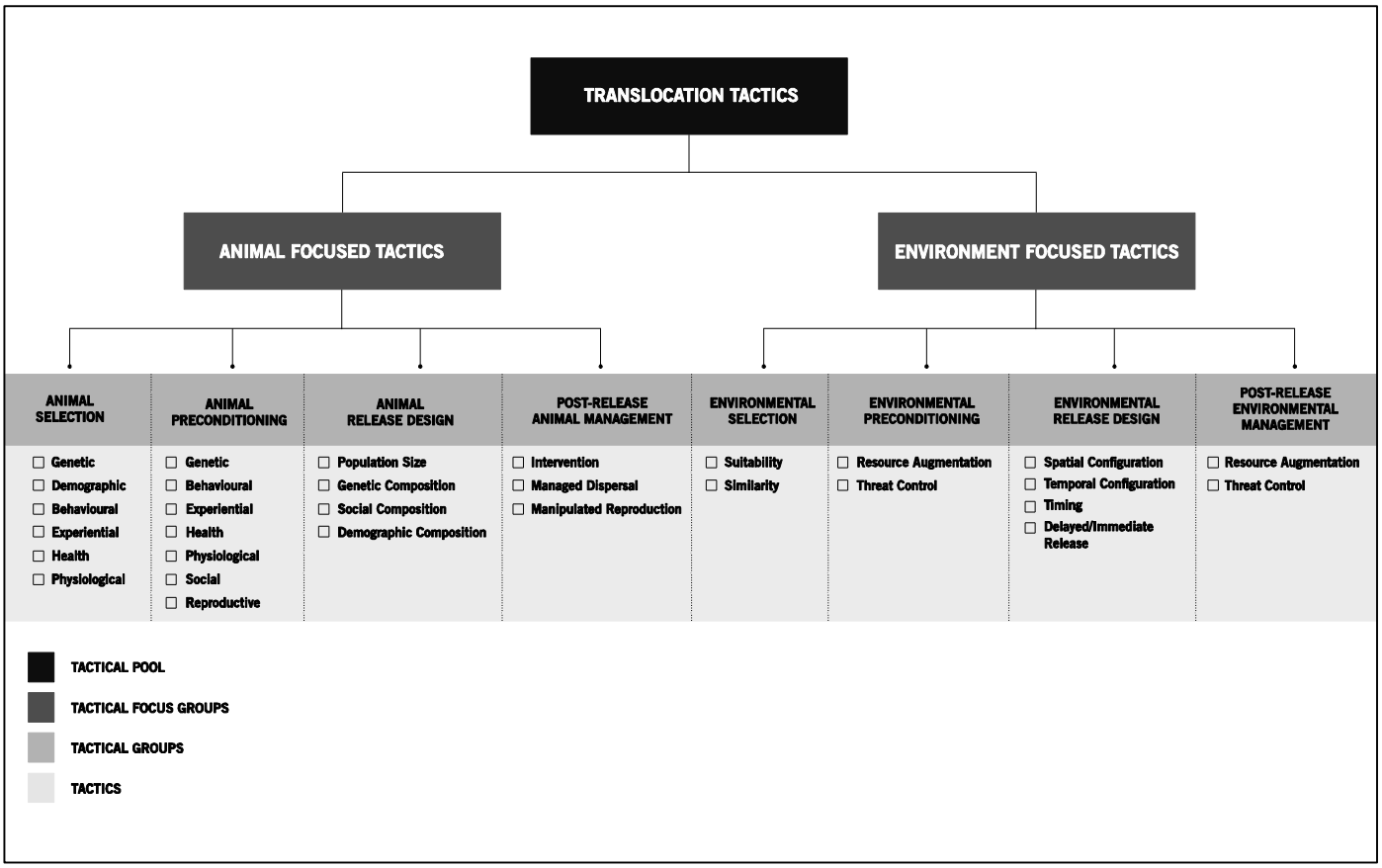

Figure 1. The Translocation Tactics Classification System. This framework represents a hierarchical organization of the tactical options identified from the literature review. The groupings are created according to operational similarities.

Table 1. The definition of the tactical options presented in the Translocation Tactics Classification System. Each definition is supported with examples of how a tactical option can be implemented in a translocation, and relevant references (max. 3). The two right-hand columns indicate whether a tactical option was detected in the IUCN/SSC Guidelines for Reintroductions and Other Conservation Translocations (2013), and the detection rates in the collection of 195 articles, and 73 case-studies assessed during this study. Where possible the references provided present both a theoretical, and an applied account of the respective tactic.

Tactical option	Definition	Example	References	Detected in Guidelines	Detection rate in articles (%)	Detection rate in case-studies (%)
Animal Selection	The deliberate selection of an individual or source population based on the relative prevalence for a discernible trait				34	40
Behavioural Selection	The deliberate selection of individuals or groups from multiple candidates based on a behavioural trait	Selection for or against behavioural boldness, shyness or wildness	(Miller *et al.* 1999; Bremner-Harrison, Prodohl & Elwood 2004; Le Gouar, Mihoub & Sarrazin 2012)	Y	3	7
Demographic Selection	The deliberate selection of individuals or groups from multiple candidates based on a demographic trait	Selection for or against sex, age, reproductive or social status	(Miller *et al.* 1999; Sarrazin & Legendre 2000; Aaltonen *et al.* 2009)	Y	16	29
Genetic Selection	The deliberate selection of individuals or groups from multiple candidates based the prevalence for a genetic trait	Selection for or against heterozygosity or level of genetic differentiation	(Elliott, Merton & Jansen 2001; Letty, Marchandeau & Aubineau 2007; Jamieson & Lacy 2012)	Y	7	1
Physiological Selection	The deliberate selection of individuals or groups from multiple candidates based the prevalence for a	Selection for or against body-mass, or body-condition	(Calvete *et al.* 2005; Letty, Marchandeau & Aubineau 2007)	Y	3	4

	physiological trait					
Health Selection	The deliberate selection of individuals or groups from multiple candidates based the prevalence for a health trait	Selection for or against immunology, pathogen or parasite load or injury (often involves health screening)	(Mathews *et al.* 2006; Faria, van Oosterhout & Cable 2010; Ewen *et al.* 2012)	Y	8	15
Experiential Selection	The deliberate selection of individuals or groups from multiple candidates based on pre-release experiences	Selection for or against source type (wild vs. captive), raising conditions (hand-reared vs. cross-fostered) or predator experience	(Jule, Leaver & Lea 2008; Zidon *et al.* 2009; Parlato & Armstrong 2013)	Y	10	4
Animal Preconditioning	The deliberate alteration of a trait within an individual or group prior to release				29	41
Behavioural Preconditioning	The deliberate alteration of a behavioural trait within individuals or group prior to release	Preconditioning through predator avoidance or resource acquisition training	(Shier & Owings 2006; Alonso *et al.* 2011; White *et al.* 2012)	Y	6	3
Genetic Preconditioning	The deliberate alteration of genetic traits within an individual or group prior to release	Preconditioning through controlled breeding in captivity	(Frankham 1995; Christie 2009)	N	3	1
Physiological Preconditioning	The deliberate alteration of physiological traits within individuals prior to release	Preconditioning through wing-clipping or improved body-condition	(Combreau & Smith 1998; Calvete *et al.* 2005; Letty, Marchandeau & Aubineau 2007)	N	4	1
Social Preconditioning	The deliberate alteration of social relationships within	Preconditioning through communally housing of individuals to	(Tear & Ables 1999; Gusset, Slotow & Somers 2006)	Y	4	10

	individuals prior to release	establish social networks				
Experiential Preconditioning	The deliberate alteration of environmental characteristics of the source environment prior to release	Preconditioning through the provision of wild environmental features whilst in captivity	(Shepherdson 1994; Biggins *et al.* 1999; Letty, Marchandeau & Aubineau 2007)	Y	12	26
Health Preconditioning	The deliberate alteration of health characteristics of individuals prior to release	Preconditioning through immunization or the treatment of pre-existing conditions (often incorporating quarantine)	(Mathews *et al.* 2006; Faria, van Oosterhout & Cable 2010; Ewen *et al.* 2012)	Y	6	12
Reproductive Preconditioning	The deliberate alteration or control of the reproductive status of individuals prior to release	Preconditioning through the removal of pouch-young from marsupials	(Andrews, Bigwood. & Barlow, 2010)	N	0	1
Animal Release Design	The deliberate control of the size or composition of a founder population				38	40
Population Size	The deliberate selection of the number of individuals included in a translocated cohort	Deliberately maximizing the size of a cohort, or releasing a predetermined number of individuals	(Komers & Curman 2000; Tracy *et al.* 2011, Batson *et al*. 2015)	Y	26	21
Genetic Composition	The deliberate control of the genetic makeup of a translocated cohort	Deliberately maximizing genetic diversity within cohort, or mimicking the genetic makeup of a wild population	(Robert *et al.* 2004; Biebach & Keller 2012; Batson *et al*. 2015)	Y	5	11
Demographic Composition	The deliberate control of the demographic makeup of a translocated population or cohort	Deliberately designed sex-bias, age-bias or wild-like demographic structure in a translocated population	(Komers & Curman 2000; Jamieson & Lacy 2012; Batson *et al*. 2015)	Y	12	16
Social Composition	The deliberate control of the social makeup of a translocated	Deliberately designed social composition established by translocating multiple	(Bennett *et al.* 2012; Shier & Swaisgood 2012, Batson *et al*. 2015)	Y	9	16

	population or cohort	members of an established social group				
Post-release Animal Management	Management actions undertaken on translocated individuals post-release				10	16
Intervention	Actions undertaken in order to mitigate issues based on post-release observations	The treatment of an injury or the removal of problem of individuals based on post-release observations	(Elliott, Merton & Jansen 2001; Mathews *et al.* 2006; Ewen *et al.* 2012)	Y	6	8
Manipulated reproduction	Actions undertaken to influence the reproductive cycles or offspring of translocated individuals	The removal of offspring from translocated adults to hand-raise or cross-foster	(Elliott, Merton & Jansen 2001)	N	3	3
Managed Dispersal	Action undertaken to establish and maintain meta-population dynamics	The translocation of individuals among translocated sub-populations	(Davies-Mostert, Mills & Macdonald 2009; Gusset *et al.* 2009; Jamieson & Lacy 2012)	N	3	5
Environmental selection	The selection of a source or recipient environment based on the relative prevalence for a discernible trait				42	62
Suitability Selection	The deliberate selection of an environment from multiple candidates based on the level of suitability to the translocated wildlife	Selection based on resource availability, threat abundance suitability, or climatic suitability	(Miller *et al.* 1999; Osborne & Seddon 2012; White *et al.* 2012)	Y	38	62
Similarity Selection	The deliberate selection of an environment from multiple candidates based on the level of similarity between the source and	Selection based on resource availability, threat abundance suitability, or climatic similarity	(Letty, Marchandeau & Aubineau 2007; Osborne & Seddon 2012; Parlato & Armstrong 2013)	Y	8	3

	recipient environments					
Environmental Preconditioning	The deliberate alteration of a trait within a recipient environment				17	25
Pre-release Resource Augmentation	The deliberate augmentation of resources within the recipient environment pre-release	Environmental preconditioning through habitat restoration, artificial resources or biological markers (e.g. broadcasting con-specific scat)	(Veitch 1995; Manning, Lindenmayer & Fischer 2006; Osborne & Seddon 2012)	Y	8	16
Pre-release Threat Control	The deliberate control of threats within the recipient environment pre-release	Environmental preconditioning through fencing and predator control	(Moseby *et al.* 2011; Burns, Innes & Day 2012; Osborne & Seddon 2012)	Y	9	10
Environmental Release Design	The control of the spatial or temporal dynamics of releases				44	67
Spatial Configuration	The deliberate control of the number and configuration of release-sites	Deliberately designing the number of release-sites, distance between release-sites or distance between source and recipient sites	(Saltz 1998; Rout, Hauser & Possingham 2009; Berger-Tal, Bar-David & Saltz 2012)	Y	9	12
Temporal Configuration	The deliberate control of the number and configuration of release-events	Deliberately designing the number of release-events and period between release-events	(Gusset *et al.* 2009; Faria, van Oosterhout & Cable 2010; Batson *et al.* 2015)	Y	14	19
Release Timing	The deliberate control of the timing of a release event(s)	Deliberately designing the timing of a release event according to seasonal, behavioural or biological cycles	(Tavecchia *et al.* 2009; Bright & Morris, 1994; Batson *et al*. 2015))	Y	11	23
Delayed or Immediate Release	The deliberate inclusion, exclusion and design of a holding period immediately	Deliberately including or excluding a period temporary confinement immediately	(Letty, Marchandeau & Aubineau 2007; Richardson *et al.* 2013,	Y	25	45

	preceding release	preceding release	Batson *et al*. 2015)			
Post-Release Environmental Management	Management actions undertaken on recipient environment post-release				24	45
Post-release Resource Augmentation	The deliberate augmentation of resources within the recipient environment post-release	Post-release management through ecological restoration, artificial resources or biological markers	(Swaisgood 2010; Bradley *et al.* 2011; Chauvenet *et al.* 2012)	Y	17	30
Post-release Threat Control	The deliberate control of threats within the recipient environment post-release	Post-release management through predator or pathogen control	(Short *et al.* 1992; Armstrong *et al.* 2006; Moseby *et al.* 2011)	N	10	21

Discussion

The abundance and diversity of the tactics indicates the compositional complexity of translocations. This complexity presumably increases the likelihood that the knowledge of tactics will vary among conservation groups involved in translocation projects. The TTCS provides a framework that can improve the ability to identify, select and design tactics which help to achieve defined strategies. The TTCS also outlines the fundamental theory behind the operation of each tactic. The TTCS complements the Guidelines by placing key recommendations within a logical framework, and will ultimately improve the ability to identify and counter potential threats to translocation success. Using the TTCS as a checklist will also encourage and facilitate standardized and systematic design processes to be adopted. The TTCS will also improve the ability to interpret and communicate tactics among people from various disciplines by providing a standardized set of definitions.

The 2013 version of the Guidelines presents a broader range of recommendations compared to the previous version. As a majority of tactics identified in the primary literature also featured in the Guidelines, we conclude that the 2013 version of the Guidelines presents a comprehensive, but not exhaustive record of the tactics used in bird and mammal translocations. It appears that the absence of the missing tactics can attributed to their rare use, as indicated by the relatively low detection rates in articles and case-studies; whereas, the absence of post-release threat control can be attributed to the Guidelines not differentiating between threat control measures that occur pre- and post-release. Despite the substantial coverage of tactics in the Guidelines, we believe that the distribution of these references amongst different sections of the document reduces the ease with which they can be accessed and interpreted by the reader highlighting the benefit of the TTCS as a supportive framework. The extensive coverage of tactics within the Guidelines validates the breadth of information presented, and the process used to develop the resource. The extent of coverage of tactics in the Guidelines is encouraging given the prominent role the Guidelines have in advocating responsible translocation standards. Conversely, the absence of some tactics highlights the need to develop concept-specific resources to complement the Guidelines.

As the TTCS is based on subjective interpretations of applied techniques, its structure and definitions may appear arbitrary in places. However, any debate surrounding its validity is unlikely to reduce its effectiveness as a resource for practitioners as long as the operational basis of each tactic is understood. It is also inevitable that there will be additional tactics that do not appear within the TTCS due to the method used to develop this framework. It is also important to recognize that despite the focus on bird and mammal translocations in this study, many of the tactics presented will also be relevant to other taxa. In the future we would encourage the expansion of the TTCS to encompass additional tactics, those that related to non-biological and non-ecological elements, and those associated with other taxa. Expanding the framework will ultimately improve the conceptual understanding of the translocation process in its entirety.

The broad-scope of the Guidelines and the TTCS make general recommendations regarding the design or implementation of tactics for translocations within specific characteristics inappropriate. However, these types of recommendations have been presented elsewhere. For example, Jones and Merton (2012) advocate, predominantly supported by experimental evidence, the use of immediate-releases for translocating wild-birds, and delayed-releases for those involving captive-birds (Mitchell *et al.* 2011; Richardson *et al.* 2013). However, sweeping recommendations are relatively uncommon in the translocation literature due to the complexity of an animal's response to different methods (Parker *et al.* 2012). Therefore, the design of translocation processes needs to be considered on a project-by-project basis (IUCN/SSC 2013), and be conducted within adaptive and experimental frameworks to constantly improve the quality of translocation practices (Seddon, Armstrong & Maloney 2007; McCarthy, Armstrong & Runge 2012). One of the primary functions of the TTCS is to encourage each tactic to be carefully evaluated, and decisions founded on empirical evidence and previous experience where possible. Adopting this systematic design process will ultimately improve the general quality of translocation methods (meaning the probability that the process will achieve the ultimate objectives of the project) and avoid the implementation of poorly planned projects.

The primary literature is important for communicating translocation-related information (IUCN/SSC 2013). However, it is apparent that scientific articles rarely present detailed accounts of translocation methods. This was recognized by Sutherland *et al.* (2010) who outline how the lack of detail description of methodology impacts on the ability to interpret methods, draw comparisons among projects and conduct broad-scale systematic meta-analyses. There are many factors responsible for the lack of methodological detail in the primary literature including publication constraints (e.g. word-limits), and the concise focus of scientific articles (Armstrong & McCarthy 2007). The level of detail is further restricted by other factors including the lack of involvement by scientists in many projects, the limited resource available to produce scientific articles, personal motivations and the required scientific rigor (e.g. sample size) needed to publish

in many scientific journals. These factors provide substantial barriers to the reporting in the primary literature, and can often shift the focus of articles that are produced away from the methodological concepts (Seddon, Armstrong and Maloney 2007, Armstrong & McCarthy 2007, Sutherland *et al.* 2010).

The need for increased reporting and access to practical information motivated the IUCN/SSC to produce the Global Re-introduction Perspectives Series. This series is specifically designed to record practical information without many of the barriers associated with the primary literature. The central focus of this series encompasses the design, application, key learnings, and the ultimate outcomes of re-introductions. The higher detection rates of tactics in the case-studies compared to the articles assessed in this study suggests a greater level of methodological detail is being provided in the case-studies. However, it can be assumed that only a small number of re-introduction projects are reported in this series which presents a potential loss of valuable information.

It was apparent, in both the articles and case-studies, that the description of many techniques that could be considered tactics were overlooked because they were not supported by the tactical rationale behind their design. We argue that this information should be reported whenever possible because it allows the factors that influence methodological design to be interpreted and be used to guide the design of other projects. For example, it is beneficial to understand whether the number of individuals released was predominantly determined by restricted availability or as a tactic to ensure genetic viability. The limited volume of methodological information currently available also restricts the opportunity to investigate the effects of specific tactics on translocation success to only the most commonly described tactics (e.g. Fischer and Lindenmayer 2000) and the most commonly translocated taxa (e.g. Wolf *et al*. 1996). This restriction may lead to a perception that the most commonly described tactics are the most critical components of a translocation process,

but the validity of this assumption is remains to be tested. The ability to record full methodological accounts, and the tactical rationale of their design, is likely to require the creation of a new medium (e.g. a centralized data base) which is specifically designed to serve this purpose. Ultimately, the design and structure of any future resource would be founded on the TTCS framework. The value of developing such a resource would be the accumulation of information that could be used to conduct broad meta-analyses to assess the effectiveness various approaches, within specific translocation contexts. More detailed accounts would also provide conservation practitioners with operational models to help guide the design of translocation processes, and avoid mistakes being repeated.

The outcomes of translocations are strongly influenced by the ability to select and design appropriate tactics. As translocation methods are predominately shaped by the knowledge of the people involved in the project, those people need to be fully aware of the tactical options available. When faced with uncertainty, practitioners should make use of evidence-based recommendations accessed through various media including the primary literature, case-studies, and personal communication. Accessing this information will help to ensure that justifiable decisions are made and decrease the chances of making mistakes. The role of conservation biology is to develop the theoretical understanding of the factors that can influence conservation outcomes. However, striving for scientific novelty may cause fundamental components to be under-appreciated and under-represented in the scientific literature. To remedy this problem, conservationists should be encouraged to record and communicate their practical experiences, as well quantitative results to increase awareness across the community. Although, there is immense value provided by general Guidelines as produced by the IUCN/SSC, these need to be supported by context specific resources and practical case-studies which provide insight regarding application and design. Here, we provide a supporting resource that can be used by all members of the conservation community whatever their disciplinary background which will help to improve the tactical and strategic strength of translocation processes.

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Data accessibility

Data used to calculate the detection rates of tactics within each article, case-study and the Guidelines are accessible at doi:10.5061/dryad.gm6mc

References

Aaltonen, K., Bryant, A.A., Hostetler, J.A. & Oli, M.K. (2009) Reintroducing endangered Vancouver Island marmots: Survival and cause-specific mortality rates of captive-born versus wild-born individuals. Biological Conservation, 142, 2181-2190.

Alonso, R., Orejas, P., Lopes, F. & Sanz, C. (2011) Pre-release training of juvenile little owls *Athene noctua* to avoid predation. Animal Biodiversity and Conservation, 34, 389-393.

Andrews, L., Bigwood, S. & Barlow, S. (2010) The re-introduction of yellow-footed rock-wallaby to the northern Flinders Ranges, South Australia. *Global re-introduction perspectives: Additional case-studies from around the globe.* (ed. P.S. Soorae), pp. 215-219. IUCN/SSC Re-introduction Specialist Group & Environment Agency-Abu Dhabi.

Armstrong, D.P. & McCarthy, M.A. (2007) Big decisions and sparse data: Adapting scientific publishing to the needs of practical conservation. Avian Conservation and Ecology, 2.

Armstrong, D.P., Raeburn, E.H., Lewis, R.M. & Ravine, D. (2006) Estimating the viability of a reintroduced New Zealand robin population as a function of predator control. Journal of Wildlife Management, 70, 1020-1027.

Armstrong, D.P. & Seddon, P.J. (2008) Directions in reintroduction biology. Trends in Ecology & *Evolution*, 23, 20-25.

Bajomi, B., Pullin, A.S., Stewart, G.B. & Takacs-Santa, A. (2010) Bias and dispersal in the animal reintroduction literature. Oryx 44, 358-365.

Batson, W., Abbott, R. & Richardson, K.M. (2015) Release strategies for fauna reintroductions: theory and tests. In *Advances in Reintroduction Biology of Australian and New Zealand Fauna*: 7-16. Armstrong, D. P., Hayward, M. W., Moro, D. & Seddon, P. J. (Eds.). Collingwood, Australia: CSIRO Publishing.

Bennett, V.A., Doerr, V.A.J., Doerr, E.D., Manning, A.D. & Lindenmayer, D.B. (2012) The anatomy of a failed reintroduction: a case study with the brown treecreeper. Emu, 112, 298-312.

Berger-Tal, O., Bar-David, S. & Saltz, D. (2012) Effectiveness of multiple release sites in reintroduction of Persian fallow deer. Conservation Biology, 26, 107-115.

Biebach, I. & Keller, L.F. (2012) Genetic variation depends more on admixture than number of founders in reintroduced alpine ibex populations. Biological Conservation, 147, 197-203.

Biggins, D.E., Vargas, A., Godbey, J.L. & Anderson, S.H. (1999) Influence of prerelease experience on reintroduced black-footed ferrets (*Mustela nigripes*). Biological Conservation, 89, 121-129.

Bradley, D.W., Ninnes, C.E., Valderrama, S.V. & Waas, J.R. (2011) Does ‘acoustic anchoring’ reduce post-translocation dispersal of North Island robins? Wildlife Research, 38, 69-76.

Bremner-Harrison, S., Prodohl, P.A. & Elwood, R.W. (2004) Behavioural trait assessment as a release criterion: boldness predicts early death in a reintroduction programme of captive-bred swift fox (*Vulpes velox*). Animal Conservation, 7, 313-320.

Bright, P.W. & Morris, P.A. (1994) Animal translocation for conservation: performance of dormice in relation to release methods, origin and season. Journal of Applied Ecology, 31, 699-708.

Burns, B., Innes, J. & Day, T. (2012) The use and potential of pest-proof fencing for ecosystem restoration and fauna conservation in New Zealand. *Fencing for conservation* (eds M.J. Somers & M.W. Hayward), pp. 65-90. Springer-US, NY, USA.

Calvete, C., Angulo, E., Estrada, R., Moreno, S. & Villafuerte, R. (2005) Quarantine length and survival of translocated European wild rabbits. Journal of Wildlife Management, 69, 1063-1072.

Chauvenet, A.L.M., Ewen, J.G., Armstrong, D.P., Coulson, T., Blackburn, T.M., Adams, L., Walker, L.K. & Pettorelli, N. (2012) Does supplemental feeding affect the viability of translocated populations? The example of the hihi. Animal Conservation, 15, 337-350.

Christie, S. (2009) Breeding far eastern leopards for reintroduction: the zoo programme perspective. *Reintroduction of Top-Order Predators* (eds M.W. Hayward & M. Somers), pp. 388-410. Wiley-Blackwell Hoboken, NJ, USA.

Combreau, O. & Smith, T.R. (1998) Release techniques and predation in the introduction of houbara bustards in Saudi Arabia. Biological Conservation, 84, 147-155.

Davies-Mostert, H.T., Mills, M.G.L. & Macdonald, D. (2009) A critical assessment of South Africa's managed metapopulation recovery strategy for African wild dogs. *Reintroduction of Top-Order Predators* (eds M.W. Hayward & M.J. Somers), pp. 10-42. Blackwell Publishing, Oxford, UK.

Elliott, G.P., Merton, D.V. & Jansen, P.W. (2001) Intensive management of a critically endangered species: the kakapo. Biological Conservation, 99, 121-133.

Ewen, J.G., Acevedo-Whitehouse, K., Alley, M.R., Carraro, C., Sainsbury, A.W., Swinnerton, K. & Woodroffe, R. (2012) Empirical consideration of parasites and health in reintroduction. *Reintroduction Biology: Integrating Science and Management* (eds J.G. Ewen, D.P. Armstrong, K.A. Parker & P.J. Seddon), pp. 290-335. Blackwell Publishing Ltd., Oxford UK.

Faria, P.J., van Oosterhout, C. & Cable, J. (2010) Optimal release strategies for captive-bred animals in reintroduction programs: Experimental infections using the guppy as a model organism. Biological Conservation, 143, 35-41.

Fischer, J. & Lindenmayer, D.B. (2000) An assessment of the published results of animal relocations. Biological Conservation, 96, 1-11.

Frankham, R. (1995) Genetic management of captive populations for reintroduction *Reintroduction Biology of Australian and New Zealand Fauna* (ed. M. Serena), pp. 31-34. Surrey Beatty and Sons, NSW, Australia.

Griffith, B., Scott, J.M., Carpenter, J.W. & Reed, C. (1989) Translocation as a species conservation tool - status and strategy. Science, 245, 477-480.

Gusset, M., Jakoby, O., Muller, M.S., Somers, M.J., Slotow, R. & Grimm, V. (2009) Dogs on the catwalk: Modelling re-introduction and translocation of endangered wild dogs in South Africa. Biological Conservation, 142, 2774-2781.

Gusset, M., Slotow, R. & Somers, M.J. (2006) Divided we fail: the importance of social integration for the re-introduction of endangered African wild dogs (*Lycaon pictus*). Journal of Zoology, 270, 502-511.

IUCN/SSC (1998) *Guidelines for re-introductions*. IUCN Species Survival Commission, Gland, Switzerland and Cambridge, UK.

IUCN/SSC (2013) *Guidelines for reintroductions and other conservation translocations*. IUCN Species Survival Commission, Gland, Switzerland.

Jamieson, I.G. & Lacy, R.C. (2012) Managing genetic issues in reintroduction biology *Reintroduction biology: Integrating science and management* (eds J.G. Ewen, D.P. Armstrong, K.A. Parker & P.J. Seddon), pp. 441-475. Blackwell Publishing Ltd., Oxford, UK.

Jones, C.G. & Merton, D.V. (2012) A tale of two islands: The rescue and recovery of endemic birds in New Zealand and Mauritius. *Reintroduction Biology: Integrating science and management* (eds J.G. Ewen, D.P. Armstrong, K.A. Parker & P.J. Seddon), pp. 33-72. Wiley-Blackwell, Oxford, UK.

Jule, K.R., Leaver, L.A. & Lea, S.E.G. (2008) The effects of captive experience on reintroduction survival in carnivores: A review and analysis. Biological Conservation, 141, 355-363.

Kleiman, D.G. (1989) Reintroduction of Captive Mammals for Conservation. Bioscience, 39, 152-161.

Komers, P.E. & Curman, G.P. (2000) The effect of demographic characteristics on the success of ungulate re-introductions. Biological Conservation, 93, 187-193.

Le Gouar, P., Mihoub, J. & Sarrazin, F. (2012) Dispersal and habitat selection: Behavioral and spatial constraints for animal translocations *Reintroduction Biology: Integrating Science and Management* (eds J. Ewen, D.P. Armstrong, K.A. Parker & P.J. Seddon), pp. 138-164. Wiley-Blackwell Publishing Ltd., Oxford, UK.

Letty, J., Marchandeau, S. & Aubineau, J. (2007) Problems encountered by individuals in animal translocations: Lessons from field studies. Ecoscience, 14, 420-431.

Manning, A.D., Lindenmayer, D.B. & Fischer, J. (2006) Stretch goals and backcasting: Approaches for overcoming barriers to large-scale ecological restoration. Restoration Ecology, 14, 487-492.

Mathews, F., Moro, D., Strachan, R., Gelling, M. & Buller, N. (2006) Health surveillance in wildlife reintroductions. Biological Conservation, 131, 338-347.

McCarthy, M.A., Armstrong, D.P. & Runge, M.C. (2012) Adaptive Management of Reintroduction. *Reintroduction Biology: Integrating Science and Management.* (eds J.G. Ewen, D.P. Armstrong, K.A. Parker & P.J. Seddon), pp. 256-289. Blackwell Publishing Ltd., Oxford, UK.

Miller, B., Ralls, K., Reading, R.P., Scott, J.M. & Estes, J. (1999) Biological and technical considerations of carnivore translocation: a review. Animal Conservation, 2, 59-68.

Mitchell, A.M., Wellicome, T.I., Brodie, D. & Cheng, K.M. (2011) Captive-reared burrowing owls show higher site-affinity, survival, and reproductive performance when reintroduced using a soft-release. Biological Conservation, 144, 1382-1391.

Moseby, K.E., Hill, B.M. & Lavery, T.H. (2014) Tailoring release protocols to individual species and sites: One size does not fit all. Plos One, 9, 1-12.

Moseby, K.E., Read, J.L., Paton, D.C., Copley, P., Hill, B.M. & Crisp, H.A. (2011) Predation determines the outcome of 10 reintroduction attempts in arid South Australia. Biological Conservation, 144, 2863-2872.

Osborne, P.E. & Seddon, P.J. (2012) Selecting suitable habitats of reintroductions: Variation, change and the role of species distribution modelling. *Reintroduction Biology: Integrating Science and Management* (eds J.G. Ewen, D.P. Armstrong, K.A. Parker & P.J. Seddon), pp. 73-104. Wiley-Blackwell Publishing Ltd., Oxford, UK.

Parker, K.A., Dickens, M.J., Clarke, R.H. & Lovegrove, T.G. (2012) The theory and practice of catching, holding, moving and releasing animals. *Reintroduction Biology: Integrating Science and Management* (eds J.G. Ewen, D.P. Armstrong, K.A. Parker & P.J. Seddon), pp. 105-137. Wiley-Blackwell Publishing Ltd., Oxford, UK.

Parlato, E.H. & Armstrong, D.P. (2013) Predicting post-release establishment using data from multiple reintroductions. Biological Conservation, 160, 97-104.

Richardson, K., Castro, I.C., Brunton, D.H. & Armstrong, D.P. (2015) Not so soft? Delayed release reduces long-term survival in a passerine reintroduction. Oryx, 49, 535-541.

Robert, A., Sarrazin, F., Couvet, D. & Legendre, S. (2004) Releasing adults versus young in reintroductions: Interactions between demography and genetics. Conservation Biology, 18, 1078-1087.

Rout, T.M., Hauser, C.E. & Possingham, H.P. (2009) Optimal adaptive management for the translocation of a threatened species. Ecological Applications, 19, 515-526.

Saltz, D. (1998) A long-term systematic approach to planning reintroductions: the Persian fallow deer and the Arabian oryx in Israel. Animal Conservation, 1, 245-252.

Sarrazin, F. & Legendre, S. (2000) Demographic approach to releasing adults versus young in reintroductions. Conservation Biology, 14, 488-500.

Seddon, P.J. (2010) From reintroduction to assisted colonization: Moving along the conservation translocation spectrum. Restoration Ecology, 18, 796-802.

Seddon, P.J., Armstrong, D.P. & Maloney, R.F. (2007) Developing the science of reintroduction biology. Conservation Biology, 21, 303-312.

Seddon, P.J., Soorae, P.S. & Launay, F. (2005) Taxonomic bias in reintroduction projects. Animal Conservation 8, 51-58.

Seddon, P.J., Strauss, W.M. & Innes, J. (2012) Animal Translocations: What are they and why do we do them? *Reintroduction Biology: Integrating Science and Management* (eds J.G. Ewen, D.P. Armstrong, K.A. Parker & P.J. Seddon), pp. 1-32. Wiley-Blackwell Publishing Ltd., Oxford, UK.

Shepherdson, D. (1994) The role of environmental enrichment in the captive breeding and reintroduction of endangered species. *Creative Conservation: interactive management of wild and captive animals* (eds P.J.S. Olney, G.M. Mace & A.T.C. Feistner). Chapman and Hall, London.

Shier, D.M. & Owings, D.H. (2006) Effects of predator training on behavior and post-release survival of captive prairie dogs (*Cynomys ludovicianus*). Biological Conservation, 132, 126-135.

Shier, D.M. & Swaisgood, R.R. (2012) Fitness costs of neighborhood disruption in translocations of a solitary mammal. Conservation Biology, 26, 116-123.

Short, J., Bradshaw, S.D., Giles, J., Prince, R.I.T. & Wilson, G.R. (1992) Reintroduction of macropods (marsupialia, macropodoidea) in Australia - a review. Biological Conservation, 62, 189-204.

Soorae, P.S. (2008) Global re-introduction perspectives: re-introduction case-studies from around the globe. IUCN/SSC Re-introduction Specialist Group, Abu Dhabi, UAE.

Soorae, P.S. (2010) Global re-introduction perspectives: Additional case-studies from around the globe. IUCN/SSC Re-introduction Specialist Group, Abu Dhabi, UAE.

Soorae, P.S. (2011) Global Re-introduction Perspectives: 2011. More case studies from around the globe. IUCN/SSC Re-introduction Specialist Group Gland, Switzerland and Environment Agency-Abu Dhabi, Abu Dhabi, UAE.

Soorae, P.S. (2013) Global Re-introduction Perspectives: 2013 Further case-studies from around the globe. SSC Re-introduction Specialist Group, Gland, Switzerland and Environment Agency-Abu Dhabi. Abu Dhabi, UAE.

Sutherland, W.J., Armstrong, D., Butchart, S.H.M., Earnhardt, J.M., Ewen, J., Jamieson, I., Jones, C.G., Lee, R., Newbery, P., Nichols, J.D., Parker, K.A., Sarrazin, F., Seddon, P.J., Shah,

N. & Tatayah, V. (2010) Standards for documenting and monitoring bird reintroduction projects. Conservation Letters, 3, 229-235.

Swaisgood, R.R. (2010) The conservation-welfare nexus in reintroduction programmes: a role for sensory ecology. Animal Welfare, 19, 125-137.

Tavecchia, G., Viedma, C., Martinez-Abrain, A., Bartolome, M.A., Gomez, J.A. & Oro, D. (2009) Maximizing re-introduction success: Assessing the immediate cost of release in a threatened waterfowl. Biological Conservation, 142, 3005-3012.

Tear, T.H. & Ables, E.D. (1999) Social system development and variability in a reintroduced Arabian oryx population. Biological Conservation, 89, 199-207.

Tracy, L.N., Wallis, G.P., Efford, M.G. & Jamieson, I.G. (2011) Preserving genetic diversity in threatened species reintroductions: how many individuals should be released? Animal Conservation, 14, 439-446.

Veitch, C.R. (1995) Habitat repair: A necessary prerequisite to translocation of threatened birds. *Reintroduction Biology of Australian and New Zealand Fauna*, (ed. M. Serena), 97-104. Surrey Beatty and Sons, NSW, Australia.

White, T.H., Collar, N.J., Moorhouse, R.J., Sanz, V., Stolen, E.D. & Brightsmith, D.J. (2012) Psittacine reintroductions: Common denominators of success. Biological Conservation, 148, 106-115.

Wolf, C.M., Griffith, B., Reed, C. & Temple, S.A. (1996) Avian and mammalian translocations: Update and reanalysis of 1987 survey data. Conservation Biology, 10, 1142-1154.

Zidon, R., Saltz, D., Shore, L.S. & Motro, U. (2009) Behavioral Changes, stress, and survival following reintroduction of Persian fallow deer from two breeding facilities. Conservation Biology, 23, 1026-1035.

Paper II: Release tactics for fauna reintroductions: theory and tests

The effective application of tactics requires a sound understanding of underlying theory. Developing a better understanding of theory can be facilitated by providing relevant examples. In this paper, I build upon the translocation tactics concept by describing common *release tactics*, their underlying theory, and providing examples to highlight their application and effectiveness for reintroducing Australasian fauna. The content of this paper differs slightly from the published version, especially in terminology. I have replaced the term *strategy* in the published version, with *tactics* in this thesis to maintain consistency with Paper I. The term tactic was considered inappropriate at the time of publication, because it preceded the publication of Paper I; and therefore, represented a novel term that required classification that was not possible in the printed version. The box number references also reflect the references given in the published version.

Batson, WG; Abbott, R. & Richardson, KM. (2015) Release strategies for fauna reintroductions: theory and tests. In: Advances in Reintroduction Biology of Australian and New Zealand Fauna. Editors: Armstrong, DP; Hayward, MW; Moro, D & Seddon, PJ. CSIRO Publishing: Collingwood, Australia, 7-16.

Abstract

Reintroductions have become an integral part of conservation management for a variety of threatened species in Australia and New Zealand. This popularity largely reflects the dramatic impact that exotic species have had on the indigenous fauna of these countries. With control and eradication of several of the most detrimental exotic species from defined areas, reintroductions can be initiated in the absence of the pressures that caused the original extinction. Despite the volume of reintroductions being undertaken, the probability that a project will achieve the re-establishment of a viable population is not guaranteed. Many of the difficulties associated with reintroductions relate to the inherent challenges animals are exposed to throughout the translocation process and following release. 'Release tactics' are components of the reintroduction process that can be deliberately designed to manage these problems. They can, therefore, improve post-release establishment probabilities. We review here several release tactics that are commonly implemented in Australasian fauna reintroductions, summarise the ecological theory underlying their design, and provide examples to highlight their influence on post-release establishment. The selected release tactics include the design of the composition and size of the release group, the timing and number of release events and the selection of release tactics (delayed versus immediate releases).

Introduction

Reintroduction is defined as the intentional movement and release of an organism inside its indigenous range from which it has disappeared (IUCN 2013). Over the course of the last century, reintroductions have become an integral tool for conserving hundreds of threatened species around the world (Seddon et al. 2005; Seddon et al. 2007). Despite their popularity, the probability that a reintroduction will achieve the ultimate aim of establishing viable and sustainable populations in the wild is not guaranteed (Fischer and Lindenmayer 2000; Soorae 2008, 2010, 2011). Reintroduction failure is usually attributed to extrinsic factors such as the suitability of the recipient environment and the impact of post-release predation (Wolf et al. 1996; Fischer and Lindenmayer 2000; White et al. 2012). However, there are also intrinsic factors that can influence reintroduction outcomes. These intrinsic factors include the characteristics of the founder group and the stress responses of reintroduced species to applied processes (Letty et al. 2007; Dickens et al. 2010). Although the intrinsic challenges primarily affect individuals, they induce population-level effects through dispersal, mortality and disrupted reproduction. Often the ability to manage the severity of these effects is dependent on the design of the reintroduction process (Dickens et al. 2010; Parker et al. 2012; Richardson et al. 2015, Parker et al.2015).

Reintroductions are a common conservation strategy in New Zealand and Australia (Soorae 2008, 2010, 2011). This regional popularity primarily reflects the dramatic impact that exotic mammals have had on the indigenous fauna of New Zealand (Craig et al. 2000) and Australia (Short and Smith 1994). Because many of the most detrimental pests can be controlled or eradicated from specific areas, including oceanic islands and mainland sanctuaries, which are abundant in Australia and New Zealand (Innes et al. 2015), reintroductions can be initiated in the absence of the pressures that caused the original extinction (Richards and Short 2003; Towns and Broome 2003; Innes et al. 2015). Reintroductions have played pivotal roles in the conservation of many species including the Campbell Island teal (*Anas nesiotis*) in New Zealand (McClelland and Gummer 2006) and the burrowing bettong (*Bettongia lesueur*) in Australia (Short and Turner 2000).

As the cost of failed reintroductions became apparent, the science of 'reintroduction biology' was developed to increase the understanding of the ecological processes that influence reintroduction outcomes (Armstrong et al. 1995a; Sarrazin and Barbault 1996). The number of reintroduction-related studies has increased dramatically, with the majority concentrating on the most accessible elements of reintroductions, including the post-release effects induced through methodological variations (Seddon et al. 2007). Despite this focus, the ability to make sweeping recommendations regarding the most appropriate methods to use is confounded by the complexity of interacting factors that influence post-release responses (Parker et al. 2012; Moseby et al. 2014). For a reintroduction to be successful, the population must survive the reintroduction process and transition through the phases of 'establishment' and 'persistence', both of which present unique sets of challenges that may require specific management actions embedded within the reintroduction process (Armstrong and Seddon 2008).

Here we focus on how various release tactics affect establishment probabilities during fauna reintroductions. We define a release tactic as an aspect of the reintroduction process that can be manipulated to influence the outcomes of a reintroduction. We have developed our terminology to be consistent with that used by the IUCN (2013), who associate release tactics with the spatial configuration of release-sites, the temporal configuration of release-events, the size and composition of a founder group, and the design of pre- and post-release management. To provide an appropriate structure for this paper, we consider two questions based on those described by Armstrong and Seddon (2008):

1. How is establishment probability affected by the size and composition of the release group?

2. How are establishment probabilities affected by the design of release events?

We answer these questions by summarising the theoretical basis of each release tactics to show how they can be used to improve reintroduction outcomes. To highlight the influence that different

release tactics have on establishment probabilities, we provide examples of research undertaken during fauna reintroductions in Australia and New Zealand. Given the complexity of the reintroduction process, we restrict our focus to release tactic that incorporate the demographic composition, social composition and size of release groups when considering Question 1, and the timing and number of release events, and the selection of release tactics (immediate versus delayed releases) when considering Question 2.

How is establishment probability affected by the size and composition of the release group?

One of the factors commonly associated with reintroduction failure is the small size of founder groups, because small populations are vulnerable to extinction (Pimm 1991). Therefore, increasing the size of a release group (release cohort or founder group) represents an intuitive tactic for improving the probability of success (Box 2.1). The effectiveness of this tactic is suggested by the positive relationship between the number of individuals released and reintroduction success (Griffith et al. 1989; Fischer and Lindenmayer 2000). However, the number of individuals available is often restricted by a range of factors, including the need to minimise the detrimental effects to a source population (Dimond and Armstrong 2007), and the substantial cost and logistical difficulty associated with acquiring many individuals from small population (Van Houtan et al. 2009).

Given that the size of a founder group is finite, establishment probabilities can be improved through careful design of the release group (Armstrong and Seddon 2008). When designing the optimal composition of a release group(s), the traits considered often encompass genetic, demographic and social characteristics (IUCN 2013). Although we acknowledge the importance of genetics in reintroductions, we do not include them here because they have been reviewed extensively elsewhere (e.g. Frankham 2009; Jamieson and Lacy 2012; Weeks et al. 2015).

Generally, there are two opposing tactics adopted when designing the composition of release groups: the first approach is to mimic the composition of a reference population; the alternative approach is to establish unnatural biases (IUCN 2013).

Translocating only a sub-set of a source population or mixing previously unfamiliar individuals often causes social disorganisation that acts as a stressor and influences post-release performances (Letty et al. 2007). One tactic that can be adopted to minimise the detrimental effects of social disruption is to reintroduce groups of familiar individuals. This could have a range of potential benefits including reducing post-release aggression, encouraging mating or facilitating anti-predator behaviour. Releasing established social groups has been shown to improve the probability of establishment in black-tailed prairie dogs (*Cynomys ludovicianus)* in the USA (Shier 2006). However, experiments undertaken with New Zealand forest birds and Australian tammar wallabies (*Macropus eugenii*) have not indicated any beneficial effects of familiarity, either because relationships were not maintained post-release, or because the hypothesised effects of familiarity did not occur (Armstrong 1995; Armstrong and Craig 1995; Armstrong et al. 1995b). Although these experiments did not show any benefits of familiarity, releasing intact colonies of black-eared miners (*Manorina melanoti*) appeared to facilitate post-release social cohesion in this socially complex species (Clarke et al. 2002), leading to a successful reintroduction (colonies still present in 2013; R.L. Boulton pers. comm.). A similar tactic appeared to facilitate post-release settlement of brown treecreepers (*Climacteris picumnus*) (Bennett et al. 2012), although this reintroduction was not successful in the longer term.

An alternative method to gain similar benefits is to house previously unacquainted individuals together before release to allow for relationships to be established. This approach did not influence post-release survival of translocated hihi (*Notiomystis cincta*) on Kapiti Island, New Zealand (Castro et al. 1995), but has proved beneficial for other species including African wild dogs

(*Lycaon pictus*) (Gusset et al. 2006). A variation on the theme of familiarity is ensuring the release of animals with similar vocal dialects to avoid reproductive discrimination. This has been identified as a potential issue in translocations of North Island kokako (*Callaeas wilsoni*) in New Zealand (Rowe and Bell 2007) and noisy scrub birds (*Atrichornis clamosus*) in Australia (Kemp et al. 2015).

The demographic structure of a release group in regard to age, sex and reproductive status can influence reintroduction outcomes (Letty et al. 2007; IUCN 2013). The optimal sex ratio for a release group will often be dictated by the mating system of the species. For example, for the polygynous bridled nailtail wallaby (*Onychogalea fraenata*), creating a female bias can increase the potential population growth rates without affecting genetic viability (Sigg et al. 2005). Conversely, an equal sex ratio will usually be appropriate for monogamous species such as the New Zealand robin because population growth is limited by the availability of both males and females (Jamieson 2011). Sex-biased dispersal can also shape the optimal composition of a founder group, as observed during a translocation of bridled nailtail wallabies where the release of male-only groups increased dispersal due to mate-finding behaviour (Hayward et al. 2012). The age structure of release groups also needs to be considered for species with age-dependent behaviours. This has been observed in South Island saddleback (*Philesturnus carunculatus*) where settlement and survivorship is greater in birds released as sub-adults compared to adults due to differences in territorial statuses when released (Masuda and Jamieson 2012). An alternative approach is to preferentially select adults with dependent young in order to reduce dispersal capabilities, as observed in translocated black stilts (*Himantopus novaezelandiae*) (van Heezik et al. 2009).

When planning a reintroduction project, the design of the founder group is paramount because its size and composition can influence reintroduction outcomes (IUCN 2013). Although a founder

group will ideally be designed to maximise the probability of success, other factors must also be taken into consideration, including the effect harvesting will have on the source population (Richardson et al., 2015). Currently, the conservation community has a good theoretical understanding of how the structure of a founder group can influence future population dynamics. However, more empirical studies are needed to distinguish between perceived and real effects (Kemp et al. 2015). Through the accumulation of empirical evidence, it may also be possible to improve population models which could be used to guide the design of future reintroductions (Chauvenet et al., 2015).

How are establishment probabilities affected by the design of the release?

The release event represents one of the most stressful elements of the reintroduction process and deserves careful consideration to reduce any detrimental effects (Dickens et al. 2010; Parker et al. 2012; Parker et al. 2015). The timing of a release event can have a strong influence on post-release performances (Box 2.1). Selecting the most appropriate timing for a release is often dictated by seasonal biological cycles of the species being translocated (Armstrong and McLean 1995; Letty et al. 2007). In New Zealand, release events for native birds are scheduled to avoid breeding periods, but also to avoid moulting periods due to potential stress associated with moult (Armstrong and McLean 1995).

The number of release events can affect establishment probabilities in a translocation (Bertolero et al. 2007); however, there is no consistent relationship between the number of release events and probability of reintroduction success (Griffith et al. 1989). Several potential benefits could be obtained by using multiple releases, including pre-established individuals facilitating the establishment of later releases (Brightsmith et al. 2005), controlling the population density at the release-site (Faria et al. 2010), allowing trial releases (Moseby et al. 2011; Kemp et al., 2015), and enabling release methods to be adjusted within adaptive management frameworks (McCarthy et al.

2012). However, the use of multiple events can have a detrimental effect on establishment. Survival rates of hihi on Kapiti Island were better in initial releases compared with later releases, and this was primarily attributed to the competitive exclusion of newcomers by pre-established birds (Castro et al. 1995).

One tactic that has received substantial attention is the use of immediate and delayed releases (sometimes referred to as 'hard' and 'soft' releases). A delayed release describes the practice of temporarily confining animals within a structure at the release site before release, whereas, immediate release describes releases directly into the recipient environment (Box 2.2). The delayed-release tactic potentially allows time for acclimatisation and recovery from the reintroduction process before release, and potentially reduces homing instincts and develop social relationships. However, confinement may also induce additional stress and increase the risk of injury (Hunter 1998, Parker et al. 2012; Parker et al, 2015; Box 2.2). The variable effects of these opposing release tactics cause debate about which is better (Wanless et al. 2002; Swaisgood 2010).

How a population responds to a release tactic is likely to be influenced by a range of factors, including the species' phylogenetic group and life history. Species-specific responses are suggested by different responses to release tactics in phylogenetically similar species. For example, implementing a delayed-release reduces the time taken to establish territories in reintroduced burrowing bettongs (*Bettongia lesueur*), but does not influence settlement in greater bilbies (*Macrotis lagotis*) (Moseby et al. 2014). Jones and Merton (2012) recognise the influence life history can have during bird reintroductions, and recommend delayed releases for captive birds because confinement can ease the transition into the wild, and immediate releases for wild birds due to the unfamiliarity to captivity. The results of several experiments are consistent with this rule. For example, delayed release improved survival, reproduction and site fidelity of captive-

sourced western burrowing owl (*Athene cunicularia hypugaea*) in Canada (Mitchell et al. 2011), but reduced post-release survival of wild-sourced hihi (Box 2.2).

Box 2.1 Effects of release season and release group size on short-term survival of reintroduced rowi

The rowi (*Apteryx rowi*) is the rarest species of kiwi, with the current population estimated to be ~400 individuals (DOC kiwi managers pers. comm. 2014) with a range of 11 000 ha of lowland podocarp forest on the west coast of the South Island of New Zealand (DOC 2006). The population has increased from 150 individuals in the 1990s through intensive management practices, including reintroductions (DOC 2006).

Data on 104 rowi released between 1996 and 2009 were analysed to investigate the effects of release season and release-group size on post-release survival during the 90-day critical period following release. Traditionally, many of the releases took place with individual or pairs of birds at each release site to mimic the adult rowi social system, where birds form highly territorial monogamous pairs (Taborsky and Taborsky 1999; Colbourne et al. 2005). Release groups are defined as birds released on the same date, within 1 km of one another. The release timing in relation to season was initially unspecified by the management plan, and early releases took place in all seasons. Estimates of cumulative survival probability were calculated using Kaplan–Meier analysis and covariates were compared with a log-rank test (White and Garrott 1990).

Survival probability following release varied significantly among seasons, with a clear difference between autumn and winter compared to summer and spring releases (Fig. 2.1). Survival probability at 90 days post release was 0.81 (n = 44) following release in spring, 0.92 (n = 51) following releases in summer, 0.33 (n = 3) following releases in autumn and 0.17 (n = 6) following releases in winter (Kaplan–Meier analysis $\chi 2 = 34.744$, df = 3, P = 0.000).

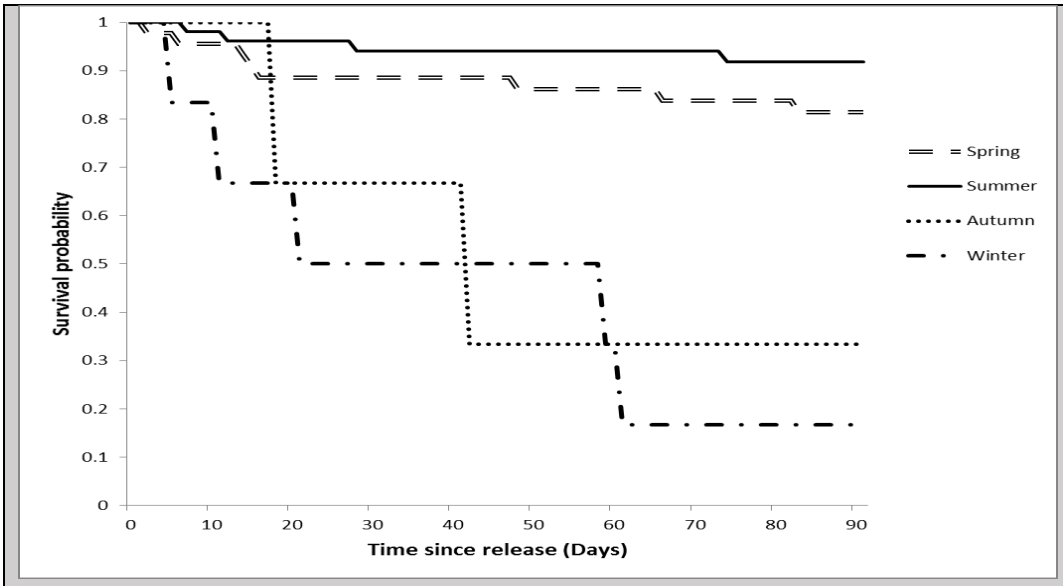

Figure 2.1. Estimated survival over time following release of rowi in different seasons: spring (n = 44), summer (n = 51), autumn (n = 3), and winter (n = 6). Spring and summer had a significantly higher survival rate than autumn and winter.

Release group size varied within and between years. Release groups were categorised and analysed as small (where group size was 1–3 birds), and large groups (with 4 or more birds). The probability of survival was 0.71 for small groups, and 0.89 for large groups, which is a statistically significant difference (Fig. 2.2, Kaplan–Meier analysis, $\chi 2 = 4.253$, df = 1, P = 0.039).

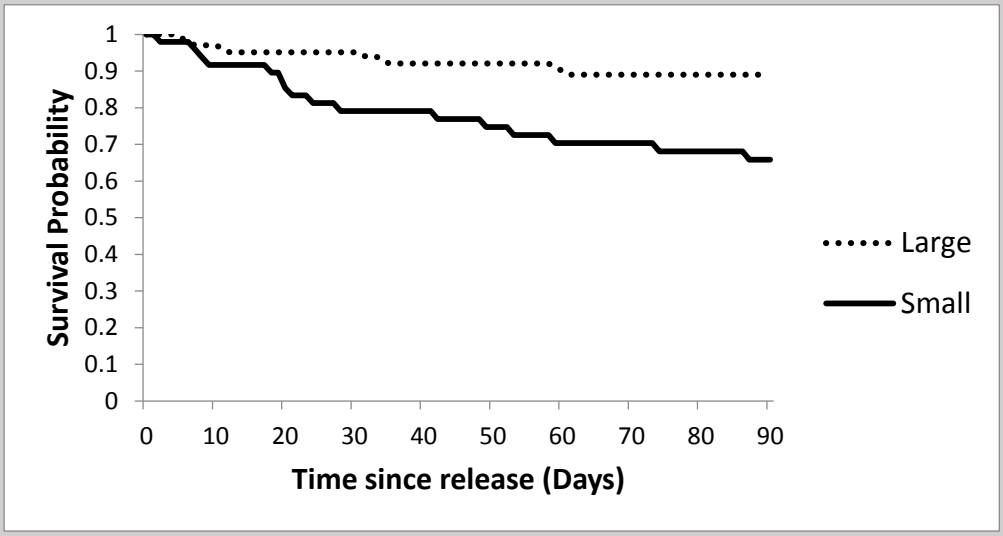

Figure 2.2. Estimated survival over time for small groups (1, 2 or 3 birds) (n = 48) and large groups (4, 5, 6, 8 or 14 birds) (n = 102) of reintroduced rowi. There is a significant difference in survival between large and small groups over the 90 days.

Box 2.2 Effects of release protocol on long-term survival of reintroduced hihi

Delayed release has been used in several reintroductions of the endangered New Zealand hihi (*Notiomystis cincta*, Figure. 2.3), with the post-release effects being assessed in two of these cases. Castro et al. (1995) examined the post-release survival of hihi by radio-tracking birds for the first 4 weeks after release on Kapiti Island, and found that immediate-release hihi had higher survival (75%) than those kept in an on-site aviary for 14 days before release (46%). The delayed release tactic was used again over a decade later for the translocations of hihi to Karori in 2005 and then Ark in the Park in 2007. In these translocations, half of the individuals were released immediately on arrival at the release site, whereas the other half were kept in an on-site aviary for 2–4 days before release.

Post-release survival was analysed following the 2007 Ark-in-the-Park translocations (Richardson et al. 2013), this time for up to 7 months after translocation. A multi-strata model was used to account for an effect of transmitters on detection probability. The results indicated that delayed release had a negative effect, this time on long-term survival, but with no effect apparent in the first 6 weeks. Based on the fortnightly survival probabilities estimated using the best model (0.98 for immediate release and 0.8 for delayed release), the overall probability of surviving the period from 6 weeks to 7 months post-release was estimated to be 0.77 ± s.e. 0.20 for immediate-release birds and 0.04 ± s.e. 0.06 for delayed-release birds (Fig. 2.3). In this case, the delayed release tactic alone could have been sufficient to cause translocation failure.

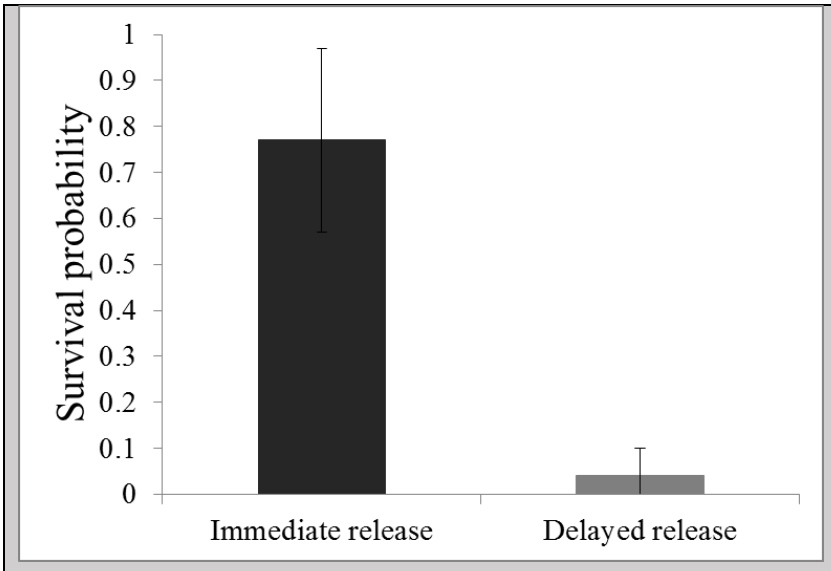

Figure. 2.3. Survival probabilities over the time period from 6 weeks to 7 months post-release for hihi reintroduced to Ark in the Park in 2007. Birds held for 2–4 days at Ark in the Park had significantly lower survival over this period than birds released immediately (error bars show SE). Inset shows an adult male hihi. Adapted from Richardson et al. (2013).

Consideration of biological context is essential in selection of an appropriate release tactic. Studies that have demonstrated a benefit of delayed release in other bird species have all involved captive-bred individuals, and it is probable that wild individuals perceive captivity differently. With wild-to-wild translocations, the priority should be to minimise stress and transfer individuals from the source to the release site as quickly as can be appropriately managed, unless there is a strong rationale to do otherwise.

Conclusion

The outcomes of reintroductions depend on many intrinsic and extrinsic factors. Once the catastrophic threats such as post-release predation have been accounted for, reintroduction practitioners must turn their attention to the finer details of reintroduction process including release tactics. Because reintroductions are typically expensive and labour intensive, each component within the process should be designed to maximise the probability of success. Multiple tactics will be required to achieve reintroduction success across a wide range of species and situations. Because reintroductions are often conducted in circumstances where the ideal tactics are unknown,

it is important that projects are undertaken within experimental or adaptive management frameworks to investigate the effectiveness of different protocols. Future research should aim to develop a holistic understanding of the interacting effects of causal mechanisms (e.g. release tactic), responses (e.g. stress) and consequences (e.g. survival) of different protocols across the variety of reintroduction contexts. The accumulated results of such studies can in turn be used to guide future practice, and therefore improve conservation outcomes.

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References

Armstrong DP (1995) Effects of familiarity on the outcome of translocations. 2. A test using New Zealand robins. Biological Conservation 71, 281–288.

Armstrong DP, Craig JL (1995) Effects of familiarity on the outcome of translocations. 1. A test using saddlebacks *Philesturnus carunculatus rufusater*. Biological Conservation 71, 133–141.

Armstrong DP, McLean IG (1995) New Zealand translocations: theory and practice. Pacific Conservation Biology 2, 39–54.

Armstrong DP, Seddon PJ (2008) Directions in reintroduction biology. Trends in Ecology & Evolution 23, 20–25.

Armstrong DP, Soderquist T, Southgate R (1995a) Designing experimental reintroductions as experiments. In Reintroduction Biology of Australian and New Zealand Fauna. (Ed. M Serena) pp. 27–29. Surrey Beatty and Sons, Sydney.

Armstrong DP, Lovegrove TG, Allen DG, Craig JL (1995b) Composition of founder groups for bird translocations: does familiarity matter? (Ed. M Serena) pp. 105–112. Surrey Beatty and Sons, Sydney.

Bennett VA, Doerr VAJ, Doerr ED, Manning AD, Lindenmayer DB (2012) The anatomy of a failed reintroduction: a case study with the brown treecreeper. Emu 112, 298–312.

Bertolero A, Oro D, Besnard A (2007) Assessing the efficacy of reintroduction programmes by modelling adult survival: the example of Hermann's tortoise. Animal Conservation 10, 360–368.

Brightsmith D, Hilburn J, del Campo A, Boyd J, Frisius M, Frisius R, et al. (2005) The use of hand-raised psittacines for reintroduction: a case study of scarlet macaws (*Ara macao*) in Peru and Costa Rica. Biological Conservation 121, 465–472.

Chauvenet, ALM, Parleto, EH., Gedir, JV & Armstrong, DP. (2015) Advances in modelling projections for reintroduced populations. In Advanves in reintroduction biology of Australian and New Zealand fauna: 91-104. Armstrong, D. P., Hayward, M. W., Moro, D. & Seddon, P. J. (Eds.). Clayton South, VIC, Australia: CSIRO Publishing.

Castro I, Alley JC, Empson RA O. ME (1995) Translocation of Hihi or stitchbird *Notiomystis cincta* to Kapiti Island New Zealand: transfer techniques and comparison of release strategies. In Reintroduction Biology of Australian and New Zealand fauna. (Ed M Serena) pp. 113–120. Surrey Beatty and Sons, Sydney

Clarke RH, Boulton RL, Clarke MF (2002) Translocation of the socially complex black-eared miner *Manorina melanotis*: a trial using hard and soft release techniques. Pacific Conservation Biology 8, 223–234.

Colbourne R, Bassett S, Billing T, McCormick H, McLennan J, Nelson A, et al. (2005) 'The development of Operation Nest Egg as a tool in the conservation management of kiwi. Science for Conservation 259. Department of Conservation, Wellington.

Craig J, Anderson S, Clout M, Creese B, Mitchell N, Ogden J, et al. (2000) Conservation issues in New Zealand. Annual Review of Ecology and Systematics 31, 61–78.

Dickens MJ, Delehanty DJ, Romero LM (2010) Stress: an inevitable component of animal translocation. Biological Conservation 143, 1329–1341.

Dimond WJ, Armstrong DP (2007) Adaptive harvesting of source populations for translocation: a case study with New Zealand robins. Conservation Biology 21, 114–124.

DOC (2006) Saving our kiwi. A stocktake of kiwi conservation in New Zealand: Progress with kiwi, who's doing the work and what the future holds for our national icon. Department of Conservation, Wellington, New Zealand, http://www.doc.govt.nz/publications/conservation/native-animals/birds/saving-our-kiwi.

Faria PJ, van Oosterhout C, Cable J (2010) Optimal release strategies for captive-bred animals in reintroduction programs: experimental infections using the guppy as a model organism. Biological Conservation 143, 35–41.

Fischer J, Lindenmayer DB (2000) An assessment of the published results of animal relocations. Biological Conservation 96, 1–11.

Frankham R (2009) Genetic considerations in reintroduction programs for large terrestrial predators. In The Reintroduction of Top-order Predators. (Eds MW Hayward and MJ Somers) pp. 371–387. Wiley-Blackwell, Oxford.

Griffith B, Scott JM, Carpenter JW, Reed C (1989) Translocation as a species conservation tool: status and strategy. Science 245, 477–480.

Gusset M, Slotow R, Somers MJ (2006) Divided we fail: the importance of social integration for the re-introduction of endangered African wild dogs (*Lycaon pictus*). Journal of Zoology 270, 502–511.

Hayward MW, L'Hotellier F, O'Connor T, Ward-Fear G, Cathcart J, Cathcart T, et al. (2012) Reintroduction of bridled nailtail wallabies beyond fences at Scotia Sanctuary – Phase 1. Proceedings of the Linnean Society of New South Wales 134, A27–A37.

Hunter LTB (1998) Early post-release movements and behaviour of reintroduced cheetahs and lions, and technical considerations in large carnivore restoration. In Cheetahs as Game Ranch Animals. (Ed. BL Penzhorn) pp. 72–82. Wildlife Group of the South African Veterinary Association, Onderstepoort, South Africa.

Innes, J., Burns, B., Sanders, A., & Haward, M.W. (2015). The impact of private santury networks on reintroduction programs. In Advanves in reintroduction biology of Australian and New Zealand fauna: 185-200. Armstrong, D. P., Hayward, M. W., Moro, D. & Seddon, P. J. (Eds.). Clayton South, VIC, Australia: CSIRO Publishing

IUCN (2013) Guidelines for Reintroductions and Other Conservation Translocations. Version 1.0. IUCN Species Survival Commission, Gland, Switzerland.

Jamieson IG (2011) Founder effects, inbreeding, and loss of genetic diversity in four avian reintroduction programs. Conservation Biology 25, 115–123.

Jamieson IG, Lacy RC (2012) Managing genetic issues in reintroduction biology. In Reintroduction Biology: Integrating Science and Management. (Eds JG Ewen, DP Armstrong, KA Parker and PJ Seddon) pp. 441–475. Wiley-Blackwell, Oxford.

Jones CG, Merton DV (2012) A tale of two islands: the rescue and recovery of endemic birds in New Zealand and Mauritius. In Reintroduction Biology: Integrating Science and Management. (Eds JG Ewen, DP Armstrong, KA Parker and PJ Seddon) pp. 33–72. Wiley-Blackwell, Oxford.

Kemp, L., Norbury, G., Groenewegen, R. & Comer, S. (2015). The roles of trials and experiments in fauna reintroduction programs In Advanves in reintroduction biology of Australian and New Zealand fauna: 73-90. Armstrong, D. P., Hayward, M. W., Moro, D. & Seddon, P. J. (Eds.). Clayton South, VIC, Australia: CSIRO Publishing.

Letty J, Marchandeau S, Aubineau J (2007) Problems encountered by individuals in animal translocations: Lessons from field studies. Ecoscience 14, 420–431.

Masuda BM, Jamieson IG (2012) Age-specific differences in settlement rates of saddlebacks (*Philesturnus carunculatus*) reintroduced to a fenced mainland sanctuary. New Zealand Journal of Ecology 36, 123–130.

McCarthy MA, Armstrong DP, Runge MC (2012) Adaptive management of reintroduction. In Reintroduction Biology: Integrating Science and Management. (Eds JG Ewen, DP Armstrong, KA Parker and PJ Seddon) pp. 256–289. Wiley-Blackwell, Oxford.

McClelland P, Gummer H (2006) Reintroduction of the critically endangered Campbell Island teal *Anas nesiotis* to Campbell Island, New Zealand. Conservation Evidence 3, 61–63.

Mitchell AM, Wellicome TI, Brodie D, Cheng KM (2011) Captive-reared burrowing owls show higher site-affinity, survival, and reproductive performance when reintroduced using a soft-release. Biological Conservation 144, 1382–1391.

Moseby KE, Read JL, Paton DC, Copley P, Hill BM, Crisp HA (2011) Predation determines the outcome of 10 reintroduction attempts in arid South Australia. Biological Conservation 144, 2863–2872.

Moseby KE, Hill BM, Lavery TH (2014) Tailoring release protocols to individual species and sites: One size does not fit all. PLoS ONE 9, e99753.

Parker, KA., Adams, L., Baling, M., et. al. (2015). Practical guidelines for planning and implimenting fauna translocations. In Advanves in reintroduction biology of Australian

and New Zealand fauna: 255-272. Armstrong, D. P., Hayward, M. W., Moro, D. & Seddon, P. J. (Eds.). Clayton South, VIC, Australia: CSIRO Publishing

Parker KA, Dickens MJ, Clarke RH, Lovegrove TG (2012) The theory and practice of catching, holding, moving and releasing animals. In Integrating Science and Management. (Eds JG Ewen, DP Armstrong, KA Parker and PJ Seddon) pp. 105–137. Wiley-Blackwell, Oxford.

Pimm SL (1991) The Balance of Nature? Ecological Issues in the Conservation of Species and Communities. The University of Chicago Press, Chicago, Illinois.

Richards JD, Short J (2003) Reintroduction and establishment of the western barred bandicoot *Perameles bougainville* (Marsupialia: Peramelidae) at Shark Bay, Western Australia. Biological Conservation 109, 181–195.

Richardson K, Castro IC, Brunton DH, Armstrong DP (2013) Not so soft? Delayed release reduces long-term survival in a passerine reintroduction. Oryx 49, 535-541.

Richardson, KM., Doerr, V. Ebrahami, M., et. al. (2015). Considering dispersal in reintroduction and restoration planning. In Advanves in reintroduction biology of Australian and New Zealand fauna: 59-72. Armstrong, D. P., Hayward, M. W., Moro, D. & Seddon, P. J. (Eds.). Clayton South, VIC, Australia: CSIRO Publishing.

Rowe SJ, Bell BD (2007) The influence of geographic variation in song dialect on post-translocation pair formation in North Island kokako (*Callaeas cinerea wilsoni*). Notornis 54, 28–37.

Sarrazin F, Barbault R (1996) Reintroduction: challenges and lessons for basic ecology. Trends in Ecology & Evolution 11, 474–478.

Seddon PJ, Soorae PS, Launay F (2005) Taxonomic bias in reintroduction projects. Animal Conservation 8, 51–58.

Seddon PJ, Armstrong DP, Maloney RF (2007) Developing the science of reintroduction biology. Conservation Biology 21, 303–312.

Shier DM (2006) Effect of family support on the success of translocated black-tailed prairie dogs. Conservation Biology 20, 1780–1790.

Short J, Smith A (1994) Mammal decline and recovery in Australia. Journal of Mammalogy 75, 288–297.

Short J, Turner B (2000) Reintroduction of the burrowing bettong *Bettongia lesueur* (Marsupialia: Potoroidae) to mainland Australia. Biological Conservation 96, 185–196.

Sigg DP, Goldizen AW, Pople AR (2005) The importance of mating system in translocation programs: reproductive success of released male bridled nailtail wallabies. Biological Conservation 123, 289–300.

Soorae PS (Ed.) (2008) Global Re-introduction Perspectives: Re-introduction Case-studies from Around the Globe. IUCN/SSC Re-introduction Specialist Group, Abu Dhabi.

Soorae PS (Ed.) (2010) Global Re-introduction Perspectives: Additional Case-studies from Around the Globe. IUCN/SSC Re-introduction Specialist Group, Abu Dhabi.

Soorae PS (Ed.) (2011) Global Re-introduction Perspectives 2011: More Case-studies from Around the Globe. IUCN/SSC Re-introduction Specialist Group, Gland, Switzerland, and Environment Agency-Abu Dhabi, Abu Dhabi.

Swaisgood RR (2010) The conservation-welfare nexus in reintroduction programmes: a role for sensory ecology. Animal Welfare 19, 125–137.

Taborsky B, Taborsky M (1999) The mating system and stability of pairs in kiwi *Apteryx* spp. Journal of Avian Biology 30, 143–151.

Towns DR, Broome KG (2003) From small Maria to massive Campbell: forty years of rat eradications from New Zealand islands. New Zealand Journal of Zoology 30, 377–398.

van Heezik Y, Maloney RF, Seddon PJ (2009) Movements of translocated captive-bred and released critically endangered kaki (black stilts) *Himantopus novaezelandiae* and the value of long-term post-release monitoring. Oryx 43, 639–647.

Van Houtan KS, Halley JM, van Aarde R, Pimm SL (2009) Achieving success with small, translocated mammal populations. Conservation Letters 2, 254–262.

Wanless RM, Cunningham J, Hockey PAR, Wanless J, White RW, Wiseman R (2002) The success of a soft-release reintroduction of the flightless Aldabra rail (*Dryolimnas cuvieri aldabranus*) on Aldabra Atoll, Seychelles. Biological Conservation 107, 203–210.

Weeks, AR., Moro, D., Thavornkanlapachai, R., et. al. (2015). Conserving and enhancing genetic diversity in translocvation programs. In Advanves in reintroduction biology of Australian and New Zealand fauna: 127-140. Armstrong, D. P., Hayward, M. W., Moro, D. & Seddon, P. J. (Eds.). Clayton South, VIC, Australia: CSIRO Publishing.

White GC, Garrott RA (1990) Analysis of wildlife radio-tracking data. Academic Press, San Diego, California.</bok>

White TH, Collar NJ, Moorhouse RJ, Sanz V, Stolen ED, Brightsmith DJ (2012) Psittacine reintroductions: common denominators of success. Biological Conservation 148, 106–115.

Wolf CM, Griffith B, Reed C, Temple SA (1996) Avian and mammalian translocations: update and reanalysis of 1987 survey data. Conservation Biology 10, 1142–1154.

Paper III: The effect of pre-release captivity on post-release performance in reintroduced eastern bettongs *Bettongia gaimardi*

Reintroduction outcomes are ultimately determined by the relative forces of recruitment (reintroduction) and loss (mortality and dispersal). Therefore, it is essential to monitor these variables to assess outcomes, and develop appropriate management responses. In this paper, I compare survival, pouch occupancy and body mass between two experimental groups of wild founders to assess whether pre-release captivity influenced establishment. The publication style is consistent with the respective journal's format and therefore there are slight inconsistencies with other parts of this thesis (e.g. the study-site is referred to as 'the Sanctuary' rather than 'MFWS').

Batson, W.G., Gordon, I.J., Fletcher, D.B. & Manning, A.D. (2015) The effect of pre-release captivity on post-release performance in reintroduced eastern bettongs Bettongia gaimardi, Oryx, First View 1:10

Abstract

Reintroductions are used to re-establish populations of species within their indigenous range, but their outcomes are variable. A key decision when developing a reintroduction strategy is whether to include a temporary period of confinement prior to release. Pre-release confinement is primarily used for the purpose of quarantine or as a delayed-release tactic to influence the performance or behaviour of founders post-release. A common difference between these approaches is that quarantine tends to be conducted in ex situ captivity, whereas delayed releases tend to involve in situ confinement at the release site. Although these practices are commonly viewed independently, it may be possible for a single confinement period to be used for both purposes. We tested whether temporarily holding wild eastern bettongs *Bettongia gaimardi* in ex situ captivity for 95–345 days prior to release (delayed release) influenced their body mass, pouch occupancy or survival during the first 1.5 years post-release, compared to founders released without confinement (immediate release). Our results suggest that exposing founders to captivity did not alter their body mass or performance post-release, despite being heavier and having fewer pouch young when released. We conclude that, for this species, ex situ captivity does not represent a tactical opportunity to improve post-release performance but can be used for quarantine without affecting the probability of establishment.

Introduction

The objective of a reintroduction is to re-establish a population of a species within its indigenous range (Seddon, 2010; IUCN/SSC, 2013); globally many reintroductions have taken place but the outcomes of these projects are variable (Soorae, 2008, 2010, 2011, 2013). A variety of tactics can be incorporated into a reintroduction process to improve the performance (e.g. survival, reproduction) and behaviour (e.g. settlement and dispersal) of the founder population post-release (Batson, et al., 2015). Other tactics can be used to manage the ecological risks associated with reintroductions, including quarantine to avoid detrimental disease and co-introductions of pathogens or parasites (Woodford, 2000). Aspects of a reintroduction that are focused at a population level are usually viewed independently from those focused on the ecosystem (Armstrong & Seddon, 2008). However, certain tactics can induce responses across these ecological levels, and improving our understanding of these could improve the quality and efficiency of reintroduction strategies.

The selection of release tactics is usually defined as a choice between a delayed release, when founders are housed in situ at the release site temporarily prior to release, and an immediate release, with no pre-release confinement (Parker et al., 2012). These are described as soft and hard release, respectively (Wanless et al., 2002; Mitchell et al., 2011), but these terms are considered inappropriate unless the effect on the severity of transition into the recipient environment is known (Parker et al., 2012; Moseby et al., 2014, Batson et al., 2015). Delayed release can improve the probability of establishment by allowing founders to recover from the translocation, acclimatize, establish social relationships and become familiar with their surroundings prior to release (Bright & Morris, 1994; Gusset et al., 2006; Mitchell et al., 2011). However, adopting this approach can have a detrimental effect by increasing mortality, stress and injury, especially in wild animals (Christensen & Burrows, 1994; Linklater et al., 2010; Richardson et al., 2015). In other situations the release tactic used has no effect on the probability of establishment (Castro et al., 1994;

Lovegrove, 1996; Hardman & Moro, 2006), which makes immediate release preferable on the grounds of reduced cost (Hardman & Moro, 2006).

The variability of responses to release tactics inhibits the ability to make sweeping recommendations regarding the most appropriate approach when faced with uncertainty (Parker et al., 2012). However, some general recommendations are provided for certain reintroduction contexts, including the use of delayed releases for captive-bred birds, and immediate releases for wild birds, based on their familiarity and reaction to confinement (Jones & Merton, 2012). The ability to make general recommendations will improve through the accumulation of experimental evidence, highlighting the value of reintroductions within experimental frameworks to test the effectiveness of methodological variations (Armstrong et al., 1994; Moseby et al., 2014; Kemp et al., 2015).

All translocations present a risk that novel organisms will be co-introduced to the recipient environment, and managing this risk should be a key consideration when developing translocation strategies (IUCN/SSC, 2013). Quarantine is often used to manage this risk, and is often conducted within specialist captive facilities that provide the required level of isolation (Woodford, 2000). Although quarantine is used primarily to manage ecological risks it can also induce biological, behavioural or physiological responses in founder populations; for example, exposing European rabbits *Oryctolagus cuniculus* to quarantine generally improves their body condition but disrupts female reproduction (Calvete et al., 2005). As quarantine can affect the performance of translocated wildlife, these effects must be considered carefully when developing translocation strategies.

Many reintroductions include both ex situ quarantine and in situ confinement to obtain population and ecosystem benefits (e.g. McClelland & Gummer, 2006; Cid et al., 2014; Kenyon et al., 2014).

However, in certain situations it may be possible to use ex situ captivity to achieve multiple benefits, including managing ecological risk and improving the probability of establishment; for example, wild Canada lynx *Lynx canadensis* showed an improved rate of post-release survival after being held temporarily in ex situ captivity (Devineau et al., 2011), with this period presumably also presenting the opportunity to conduct quarantine if required. The ability to use a single period of confinement to serve both benefits has obvious attractions, as multiple confinement periods invariably increase the financial cost (Karesh, 1993; Henri et al., 2004).

We investigated whether housing wild eastern bettongs *Bettongia gaimardi* in ex situ captivity for 95–345 days prior to release influences their body mass, survival and pouch occupancy during the initial 1.5 years post-release, compared with those exposed to an immediate release. Based on our results we provide practical recommendations regarding the use of ex situ captivity in subsequent reintroductions. We also tested whether the performance of the founders differed from our pre-release expectations, to assess the effect of the reintroduction and to evaluate post-release establishment. This study focused on the founder population at Mulligans Flat Woodland Sanctuary, in the Australian Capital Territory, released during 2012. This reintroduction represents the first attempt to re-establish eastern bettongs on the Australian mainland following a 100-year absence (Short, 1998), and is a component of a large-scale experiment aiming to restore biological integrity and ecological function to a critically threatened woodland community (Manning et al., 2011; Shorthouse et al., 2012).

Methods

Study areas and species

Tidbinbilla Nature Reserve is located in rural Australian Capital Territory and is owned and operated by the territory government. The Reserve is a certified member of the Zoo and Aquarium Association and operates captive breeding programmes for various threatened species, including northern corroboree frogs *Pseudophryne pengilleyi* and southern brush-tailed rock-wallabies

Petrogale penicillata. A permanent insurance population of eastern bettong was also established at the Reserve, which housed the delayed-release group during the pre-release confinement period. Bettongs were predominantly housed within 2.6–9.4 ha enclosures, with small groups (< 5) housed in smaller enclosures (0.5–1 ha) during an initial 30-day quarantine and during trials. The composition of the groups within each enclosure was managed to ensure that reproduction could only occur among individuals from different regions in Tasmania (Figure. 1). A specialized on-site veterinary centre was used to conduct all health assessments (Portas et al., 2014). All enclosures were protected by electrified fences and were not accessible by the public. Food (fruits, vegetables, nuts, seeds and proteins) and water were provided daily ad libitum. All enclosures included natural vegetation suitable for bettongs to make diurnal nests and for natural foraging behaviour.

Mulligans Flat Woodland Sanctuary is a publicly accessible area within Mulligans Flat Nature Reserve, adjacent to the northern suburbs of Canberra, and is co-managed by the Woodland and Wetlands Trust and the Australian Capital Territory government. It is c. 60 km from Tidbinbilla Nature Reserve. The Sanctuary encompasses 485 ha of critically threatened mixed yellow-box *Eucalyptus melliodora* and Blakely's red gum *Eucalyptus blakelyi* grassy woodland (McIntyre et al., 2010), enclosed by a barrier fence against foxes, cats and dogs, which have been eradicated from the internal area. The Sanctuary is considered an outdoor laboratory and is the location of the Mulligans Flat–Goorooyarroo Woodland Experiment (Manning et al., 2011; Shorthouse et al., 2012). The bettong population is treated as wild, with no husbandry management or supplementary resources provided. Bettongs have complete access to the Sanctuary, except for 12 1-ha sites that are fenced to facilitate assessment of the ecological effect of bettong diggings.

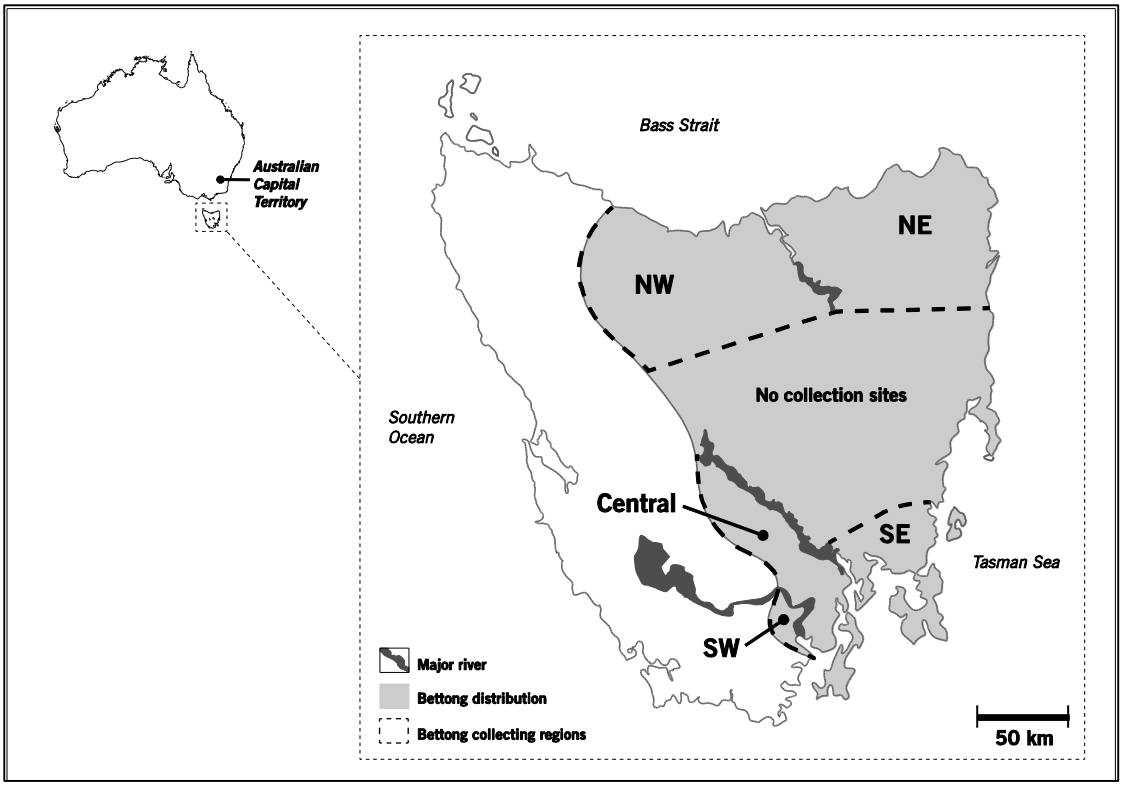

Figure 1. Regions of Tasmania where eastern bettongs Bettongia gaimardi in free-ranging populations were trapped for reintroduction in Australian Capital Territory. As a precaution, each region was assumed to be genetically isolated by geographical barriers (e.g. major rivers).

Eastern bettongs (also known as Tasmanian bettongs) are nocturnal, ground dwelling, mycophagous marsupials that occupy various woodland and forest habitats (Taylor, 1993a,b; Johnson, 1994). Females reach sexual maturity at c. 9 months of age and are capable of near-continuous breeding (Rose, 1987). Once common throughout eastern mainland Australia, their distribution is now restricted to eastern Tasmania (Fig. 1) and the species is categorized as Near Threatened on the IUCN Red List (Menkhorst, 2008). Disease transmission from feral cats has been implicated as a cause of a recent population decline (Fancourt, 2014). Bettongs dig soil when foraging and are therefore considered to be ecosystem engineers, and their reintroduction may help to re-establish diminished ecological processes (Fleming et al., 2014; Manning et al., 2015).

The translocation process

Sixty adults (19 male, 41 female) and their 28 pouch young were translocated from Tasmania to the Australian Capital Territory in four collection events during July 2011–September 2012 (Table 1). Bettongs were collected from wild populations from five geographical areas in Tasmania to increase genetic diversity (Figure. 1). Subadults, females carrying furred pouch young, and females with elongated teats were excluded from the translocation. A female-biased sex ratio was established to increase post-translocation population growth, and the pouches of females carrying pouch young were taped to prevent ejection. Once selected for translocation each individual was weighed and administered diazepam to act as a mild sedative, before being transported by road and air to Tidbinbilla Nature Reserve, where they arrived within 18 hours of acquisition. A second dose of diazepam was administered immediately before air transportation. Upon arrival each individual was anaesthetized, fitted with a passive integrated transponder tag, and given a full health assessment by a qualified veterinarian, which included measurements of body mass, pes (foot) length, tail width, head length and ectoparasite load, and classifications of body condition (using a subjective assessment of fat stores around hips), tooth wear and coat condition. Rectal, urogenital, conjunctival and nasal tract swabs and blood samples were collected to evaluate pathogen history and endoparasite load, and ear biopsies were collected for genetic analyses. The head length and sex of pouch young were also assessed. No food or water was provided during the translocation process but saline was administrated intravenously if required. Portas et al. (2014) provide further details regarding the translocation process and health assessments.

Upon arrival each bettong was assigned at random to a population (Tidbinbilla Nature Reserve or Mulligans Flat Woodland Sanctuary) but those with health conditions were kept permanently at the Reserve. Pouch young stayed with their mothers throughout the translocation. Twenty-eight adults were assigned to the permanent captive population at the Reserve. The remaining 32 adults were assigned to the wild population at the Sanctuary, with 16 (11 female) in the delayed-release

group (i.e. housed at the Reserve prior to release at the Sanctuary), and 16 (10 female) in the immediate-release group. Following the completion of the initial health assessments those assigned to the delayed-release group were released into small enclosures at the Reserve for a 30-day quarantine period. Following a post-quarantine health assessment members of this group were moved to the large enclosures, where they remained until their transfer to the Sanctuary. Upon completion of the 95–345 day confinement period bettongs were transferred to the Sanctuary in similar sized groups as the immediate-release group (Table 1), and released at similar times. Members of the immediate-release group were transferred and released at the Sanctuary following the completion of the heath assessment at the Reserve on the day of translocation. All immediate releases occurred within 24 hours of initial acquisition in Tasmania.

Post-release monitoring

Thirty-one founders were fitted with VHF (V5C_161C; Sirtrack, Hawkes Bay, New Zealand) or global positioning system (GPS)/VHF radio collars (Q4000E; Telemetry Solutions, Walnut Creek, USA) when released. One individual was not collared because of a neck injury. Each collar weighed 28–32 g, which is < 2.5% of the body mass of the lightest individual released. The collars transmitted a continuous VHF pulse, and a mortality signal was activated following 12 hours without movement. The post-release survival of each individual was monitored daily for 1 month post-release, and thereafter at least weekly until the collar was removed after 1 year. If a mortality signal was detected the collar was located immediately to determine the cause. On one occasion a collar was removed because of injury, and four collars detached accidentally. Three of the detached collars were reattached before the completion of the monitoring period. Necropsies were conducted on all deceased individuals (Portas et al., 2014).

Post-release health assessments were scheduled to occur at 1, 3, 6 and 12 months post-release but the timing and frequency varied because of logistical constraints (Table 1). To trap bettongs for a

scheduled health assessment we radio-tracked each individual of interest to its daytime nest and deployed six traps in close proximity. The health assessment included measurements of body mass, pes length and tail width, assessment of body condition, and measurement of the head length of pouch young. The assessments were conducted without sedation but with procedures in place to minimize handling time, which was generally <10 minutes. The pouches of females carrying unfused pouch young were taped to reduce the risk of pouch ejection (the tape detaches within a few hours). Individuals were released at the point of capture upon completion of the health assessment. When non-target individuals were captured they were either given a full health assessment or were weighed and released, depending on the proximity to their scheduled health assessment. In total, 218 capture events were recorded during the monitoring period.

Statistical methods

All statistical analyses were conducted using *SPSS v. 22* (IBM, Armonk, USA), with significance assumed at $P < 0.05$.

Body mass We used body mass as a proxy for body condition (sensu Moseby et al., 2014). We opted not to use a body condition index (e.g. Hardman & Moro, 2006) because of the lack of correlation between pes length and body mass in our data (R^2<0.1). The body mass of females with occupied pouches was adjusted by subtracting the estimated mass of the pouch young. This was calculated using the quadratic equation for estimating the age of a pouch young from its head length and an exponential equation to estimate its mass from its estimated age, as described by Rose (1989). We excluded the body mass of females carrying pouch young from the analysis if the head length of the pouch young was not recorded. The records were divided into the following periods: acquisition, data collected during translocations from Tasmania; release, data collected when individuals were released at the Sanctuary (synonymous with acquisition for the immediate-release group); days 1–60, data collected 1–60 days post-release; days 61–180, data collected 61–180 days post-release; days 181–360, data collected 181–360 days post-release; days 361–540,

data collected 361–540 days post-release. To minimize the effect of repeated measures we used the mean body mass of any individual captured multiple times within a period, which reduced the dataset to 143 samples. We compared the body mass of the two groups using a linear mixed model with time and group as factors (using a compound symmetry correlation structure), with release as the starting point. We conducted randomization tests to assess whether body mass within the two groups was different within each period. This process was similar to that used by Moseby et al. (2014). We did not differentiate between sexes because of the lack of sexual dimorphism (Rose, 1989; Claridge et al., 2007). We compared the post-release body mass of the entire population against our pre-release expectation, using a randomization test. Our expectation was set according to the body mass at acquisition ($\tilde{x}$=1,629 ± SD 176 g).

Pouch occupancy Pouch occupancy was assessed by visually inspecting the pouches of females during health assessments. A pouch was considered occupied if a pouch young was observed in the pouch or in the trap with the adult. The data were organized into the periods described above, with samples excluded if the pouch young had been recorded previously, based on the expected growth rate and a 106-day pouch life (Rose, 1989). It was possible for multiple pouch young to be recorded from a single female within a period when pouch young were replaced between health assessments. The proportions of pouch occupancy of the two groups were compared for each period using Fisher's exact test. This approach was also used to assess whether post-release pouch occupancy for the entire population differed from our pre-release expectation, which was set at 0.71, representing the proportion observed at acquisition. We confirmed that all delayed-released females had access to potential mates at the Reserve within 106 days of release, to ensure that pouch inactivity was not attributable to lack of mating opportunities.

Table 1. The reintroduction history of the founder population of eastern bettongs Bettongia gaimardi, with ID, time at TNR, sex, release group, condition at release, mortality, origin (Figure 1), and the number of times each individual was trapped during acquisition, release, and 1-60, 61-180, 181-360 and 361-540 days post-release.

ID	Days at TNR	Sex	Group	Condition at release	Mortality	Origin	Acquisition	Release	Days 1–60	Days 61–180	Days 181–360	Days 361–540
9607	0	F	Immediate	Good	No	Central	1	1	1	1	0	1
0A3E	0	F	Immediate	Fair	No	NW	1	1	1	1	2	1
1F8A	0	M	Immediate	Good	No	Central	1	1	1	3	1	2
A3F5	0	M	Immediate	Good	No	SW	1	1	2	1	3	6
A5F0	0	M	Immediate	Good	No	SE	1	1	3	1	3	2
BB1E	0	F	Immediate	Good	No	SE	1	1	1	2	1	1
BFD1	0	M	Immediate	Good	No	NE	1	1	1	0	0	1
C1F5	0	F	Immediate	Good	Misadventure (20)*	SW	1	1	1	1	0	0
C38E	0	F	Immediate	Good	No	SE	1	1	2	1	1	2
C5A3	0	M	Immediate	Fair	No	NW	1	1	1	0	3	2
CC1D	0	F	Immediate	Good	No	SW	1	1	2	1	1	3
CD42	0	M	Immediate	Good	No	SW	1	1	2	2	3	4
CFC1	0	F	Immediate	Good	No	NW	1	1	1	0	2	2
DE28	0	F	Immediate	Fair	No	NE	1	1	0	3	1	1
F271	0	F	Immediate	Fair	Health condition (33–35)*	NE	1	1	1	0	0	0
F88D	0	F	Immediate	Good	No	Central	1	1	1	1	0	1
3643	345	F	Delayed	Excellent	No	SW	1	1	1	1	2	2
7E03	95	M	Delayed	Good	No	Central	1	1	0	0	0	1
8906	313	F	Delayed	Excellent	Health condition (0)*	NW	1	1	0	0	0	0
9475	282	F	Delayed	Good	No	NW	1	1	1	0	0	1
895D	190	F	Delayed	Good	No	NW	1	1	1	2	1	4
9F8A	154	F	Delayed	Good	No	NE	1	1	2	1	2	3
B401	284	F	Delayed	Excellent	No	NE	1	1	1	0	0	0
B5F9	188	M	Delayed	Excellent	No	NW	1	1	1	3	3	3
C728	175	F	Delayed	Good	No	NE	1	1	2	1	2	1
CAB1	99	F	Delayed	Excellent	No	Central	1	1	1	1	6	4
D4AC	180	F	Delayed	Good	Misadventure (13)*	NE	1	1	1	0	0	0
E578	303	F	Delayed	Excellent	Health condition (332–336)*	NE	1	1	1	0	1	0
EBAD	96	M	Delayed	Good	No	SW	1	1	1	0	4	2
F4BC	97	M	Delayed	Good	No	SE	1	1	2	0	2	1
F68E	306	M	Delayed	Good	No	NE	1	1	1	1	3	3
F823	190	F	Delayed	Excellent	No	NW	1	1	1	1	2	3

Survival No meaningful statistical comparison of survival between the two groups was possible because of the low number of mortalities, and therefore only descriptive accounts are presented. We tested whether the mortality rate observed during the first year post-release differed from the expected rate of 0.2 per annum using Fisher's exact test. The expected mortality rate was based on the maximum life expectancy of 6 years (Rose, 1987), and the ages of founders randomly falling between 1 and 6 years when released. This assumption was used because the ages at acquisition could not be estimated accurately, with the minimum age being based on the exclusion of non-mature individuals at acquisition. The analysis was restricted to the first year post-release because the status of all individuals was known following completion of this period, although some of the evidence for this was outside the data set used during this study.

Results

The linear mixed effect model indicated there was no significant difference between the body mass of the two groups ($F_{1, 30} = 0.161$, P = 0.691). However, the body mass of founders was influenced by time ($F_{4, 88} = 4.674$, P = 0.002), and there was a significant interaction between time and treatment ($F_{4, 88} = 6.999$, P < 0.001). These results reflect that the delayed-release group was heavier when released, and the extra mass was lost soon after release, before stabilizing, whereas the immediate-release group maintained consistent body mass across the monitoring period. The randomization tests confirmed that the only significant difference between the two groups was at release (P < 0.001), although the difference approached significance at acquisition (P = 0.1). Overall, the post-release body masses recorded at the Sanctuary exceeded our initial expectation (P = 0.005), indicating that the body mass of the whole population increased significantly post-release (Figure 2).

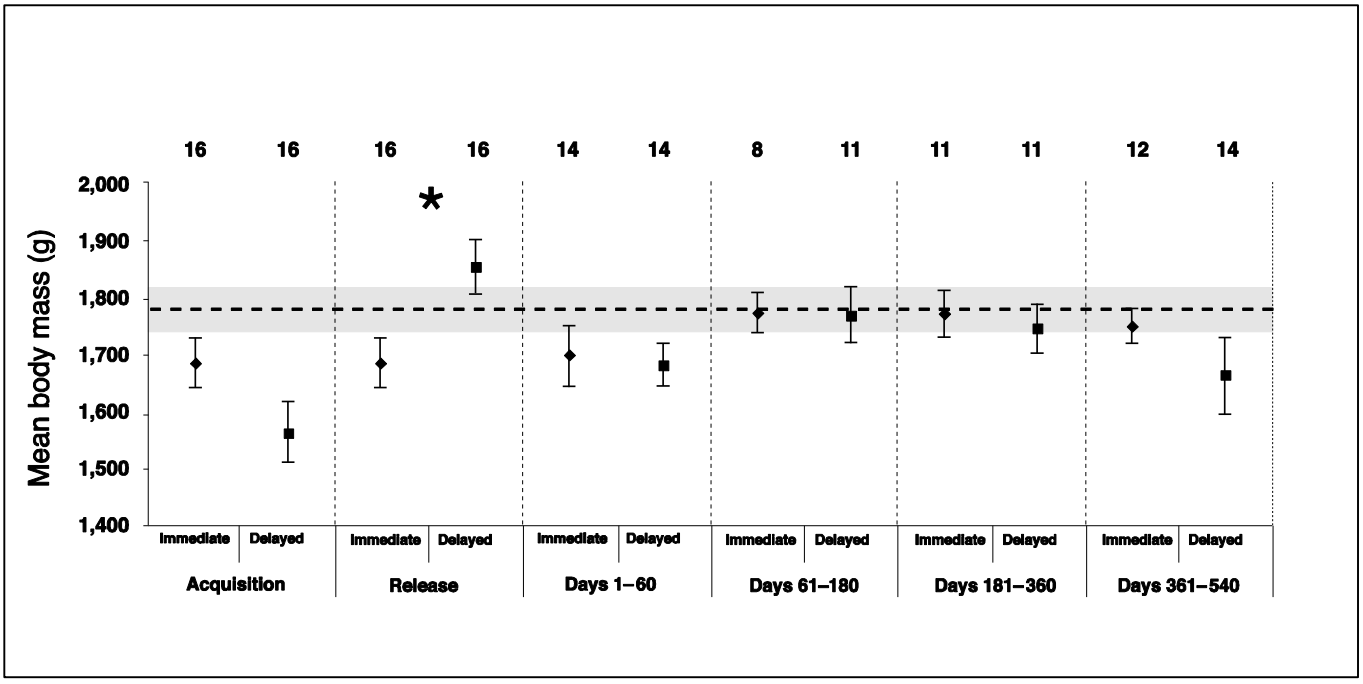

Figure 2. The mean body mass of bettongs in each release group within six sampling periods: acquisition, release, and 1–60, 61–180, 181–360 and 361–540 days post-release. The numbers above the data points represent the number of individuals sampled, and the asterisk represents a significant difference between the groups. Error bars represent ± 1 SE. The horizontal line represents the expected body mass based on that recorded at acquisition, with the shaded area representing ± 1 SE.

The proportion of pouch occupancy was greater in the immediate-release group compared to the delayed-release group at release ($P = 0.03$), with no other significant between-group differences occurring within any other period (Figure 3). Overall, the rate of post-release pouch occupancy differed significantly from the expected rate ($P = 0.01$), indicating that the reproductive activity of females was higher at the Sanctuary compared to the source populations in Tasmania. Two pouch young were known to be lost between sampling events prior to the expected 106 day pouch life, and pouch occupancy was recorded in all surviving females within 6 months of release.

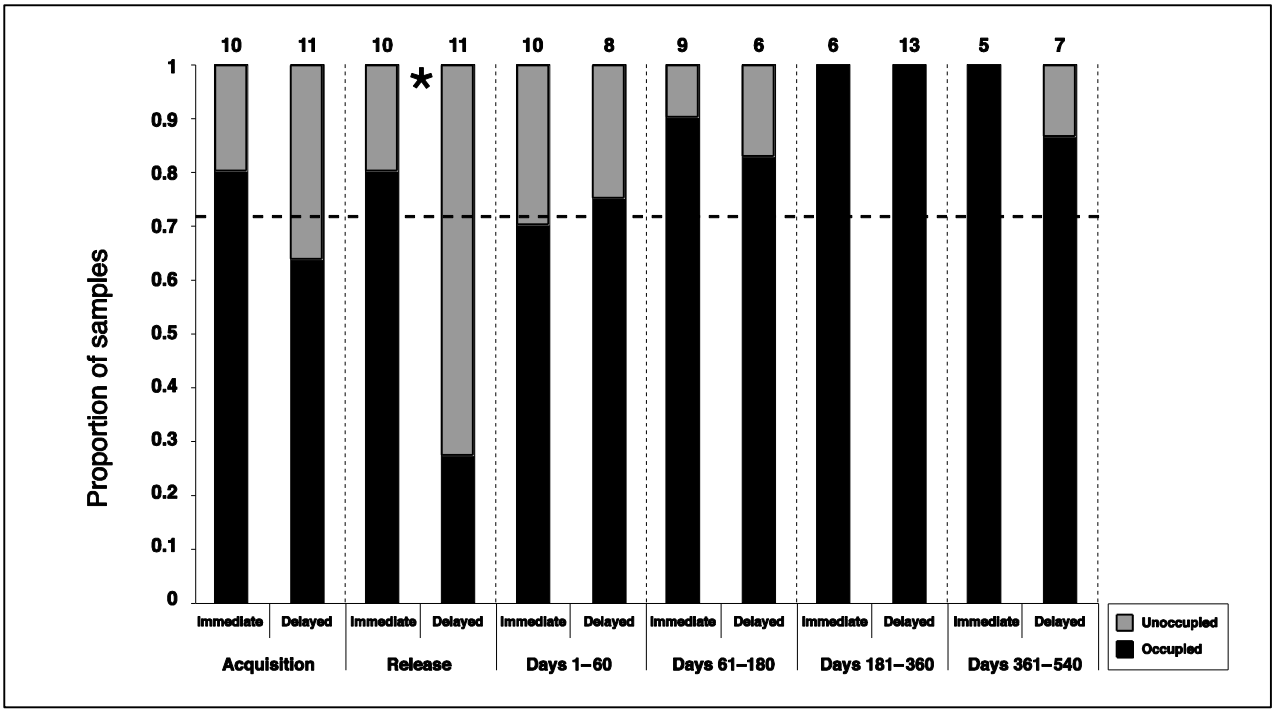

Figure 3. The rate of pouch occupancy recorded in each group within the six sampling periods. The numbers above the bars represent the number of individuals sampled, and the asterisk represents a significant difference between the groups. The horizontal line represents the expected rate of pouch occupancy based on the rate recorded at acquisition.

Five mortalities were recorded during the monitoring period. All deceased bettongs were female; three were members of the delayed-release group (Table 1). Necropsies confirmed that three of the mortalities resulted from pre-existing health conditions (two in the delayed-release group), with the remaining deaths being attributed to misadventure. The timing of two of the mortalities may have been influenced by the reintroduction process, given the temporal proximity to release: a member of the delayed-release group did not recover from being anaesthetized on the day of release, and a member of the immediate-release group died c. 1 month post-release. Overall, the mortality rate observed during the first year post-release was 0.16, which did not differ significantly from the expected mortality rate of 0.2 ($P = 1$).

Discussion

Our results suggest that exposing founders to ex situ captivity did not influence the body mass, pouch occupancy or survival of the founder group within any period post-release for the bettongs released at the Sanctuary. This is despite the delayed-release group being significantly heavier (+ 10%) and having a lower rate of pouch occupancy (27 vs 80%) than the immediate-release group when released. Overall, this indicates pre-release captivity does not represent a viable release tactic for improving the performance of founders post-release, unless it induces a positive behavioural response, which was not assessed in this study.

The lack of a significant effect on post-release survival is consistent with the results of similar studies involving translocated macropods (family Macropodidae); for example, implementing delayed and immediate releases did not affect post-release survival in burrowing bettongs *Bettongia lesueur*, greater bilbies *Macrotis lagotis* (Moseby et al., 2014) or banded *Lagostrophus fasciatus* and rufous hare-wallabies *Lagorchestes hirsutus* (Hardman & Moro, 2006). As these studies involved wild and captive-bred macropods, it appears that the life history of founders does not alter the survival response to various release tactics, which contrasts with the general trend observed in birds, whereby survival is generally higher when captive-bred birds are exposed to a delayed release, and the converse is true for certain species of wild birds (Mitchell et al., 2011; Jones & Merton, 2012; Richardson et al., 2015). As many of the macropod studies have been conducted in the absence of exotic predators (e.g. this study; Moseby et al., 2014), and involve small experimental groups (e.g. Hardman & Moro, 2006; Moseby et al., 2014), the effect of release tactics on predation vulnerability needs to be assessed before robust conclusions regarding reintroductions to wild sites can be drawn.

Our results suggest that captivity had a negative effect on reproduction, although near-continuous breeding has been achieved in another captive population of eastern bettongs (Rose, 1987). The

variability of captive pouch occupancy may indicate that reproduction is primarily affected when wild bettongs are temporarily exposed to captivity, or that there is a specific cause at Tidbinbilla Nature Reserve, with obesity, diet, stress and human-determined mate-choice providing possible explanations (Kleiman et al., 2010; Michel & Bonnet, 2012). The reduction of pouch occupancy at release needs to be considered when developing reintroduction strategies for eastern bettongs because it will increase the lag time to post-release recruitment. However, as every surviving female was observed to be reproductively active within 6 months of release, the initial reduced proportion of pouch occupancy is unlikely to affect the long-term genetic viability (Jamieson & Lacy, 2012).

The body mass advantage of the delayed-release group at release was not maintained, with no significant differences detected post-release. Moseby et al. (2014) observed a similar trend in burrowing bettongs, although the delayed-release group was still relatively heavier 2 weeks after release, partly because the immediate-release group lost weight during that period. Although an immediate weight loss was not detected in the immediate-release animals in our study, it may have occurred without being detected, given the frequency of trapping events. Overall, it appears that the body mass of translocated bettongs (eastern and burrowing) is determined primarily by environmental surroundings, and that the relative body mass at release has only a short-term effect. This also suggests that temporarily exposing wild bettongs to captivity does not influence their ability to acquire resources once released back into the wild.

The body mass and rate of pouch occupancy in the founder group post-release exceeded our expectation, whereas post-release survival was consistent with the expected rate. However, as 80% of the mortalities recorded appear to have been influenced by the translocation process or post-release monitoring, survival at the Sanctuary could also be considered to have exceeded the expected rate. The performance of the founder group reflects the suitability of the habitat, low

levels of competition, and absence of exotic predators at the Sanctuary, and provides evidence that the founder group transitioned successfully through the establishment phase of a reintroduction (Armstrong & Seddon, 2008; IUCN/SSC, 2013). This is also supported by the recruitment of new individuals at the Sanctuary. Given the favourable conditions at the Sanctuary it is likely that the body mass and performance recorded in the founder group were near-optimal for a wild population, which provides a useful comparison to evaluate the condition of other populations.

The lack of a significant biological response to varying release tactics is consistent with the general outcomes of other studies involving reintroduced macropods, using in situ captivity for delayed release (Hardman & Moro, 2006; Moseby et al., 2014). In addition to the effects on body mass, survival and reproduction, release tactics were also found to have no effect on settlement or dispersal in greater bilbies (Moseby et al., 2014) or banded and rufous hare-wallabies (Hardman & Moro, 2006) despite influencing settlement in burrowing bettongs (Moseby et al., 2014). As delayed release did not provide a significant establishment benefit we would recommend immediate release to increase resource efficiency if pre-release quarantine was not required. This conclusion is consistent with the prediction of the conceptual model presented by Moseby et al. (2014), based on the behavioural characteristics (sociality, site fidelity and ranging) of eastern bettongs and the environmental characteristics (fencing and predation risk) of Mulligans Flat Woodland Sanctuary.

Despite the lack of significant effects detected in macropod studies, the popularity of delayed releases appears to be increasing (Clayton et al., 2014). This suggests that the designs of these reintroductions are based on perceived benefits rather than experimental evidence, which is a common feature of reintroductions (Parker et al., 2012). However, implementing a delayed release can provide a number of non-biological benefits. During this reintroduction the delayed release facilitated quarantine, ecological risk assessments (Portas et al., 2014), and equipment trials prior

to release. The use of both release tactics within a structured framework spread the risk of failure by exposing founders to various methods, and facilitated experimental investigation of the responses to these variations. The delayed release also provided an opportunity for the bettongs that were translocated from Tasmania in poor condition to increase their body mass prior to release. Although many of the non-biological benefits could have been provided by in situ confinement, the use of ex situ captivity avoided the need to build new infrastructure, and the delayed-release group could be managed by professional staff as part of the daily operations at the Reserve.

We acknowledge that the strength of our statistical analyses is restricted by the small number of individuals, which is common in reintroduction biology (Seddon et al., 2007). We also accept that the probability of success was high because of the lack of predators at the Sanctuary, and the barrier to dispersal (Short et al., 1992; Clayton et al., 2014). However, low-risk reintroduction often represents the most appropriate environment to test the effectiveness of various methodologies, because predation and dispersal can mask subtle effects. The results of such experiments can then be used to develop new hypotheses and improve the quality of reintroduction strategies for releases into higher-risk environments. One of the strengths of this study is that it assessed the responses to release tactics over a prolonged period, which is sometimes essential to detect an effect (e.g. Richardson et al., 2015).

Based on our results we recommend selecting release tactics based on evaluations of financial cost and ecological risk rather than the assumed effect on establishment. However, effects on stress, settlement, dispersal and vulnerability to predation need to be assessed before a robust conclusion can be drawn. If the risk of detrimental co-introduction is considered high in subsequent reintroductions, we advocate the use of a delayed release involving ex situ captivity as an appropriate form of quarantine, because of its minimal effect on establishment probabilities. We

also recommend this approach when these ecological risks are unknown, as a precaution. However, if the ecological risks are considered low then an immediate release should be used to maximize cost efficiency.

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References

Armstrong, D.P. & Seddon, P.J. (2008) Directions in reintroduction biology. Trends in Ecology & Evolution, 23, 20–25.

Armstrong, D.P., Soderquist, T. & Southgate, R. (1994) Designing experimental reintroductions as experiments. In Reintroduction Biology of Australian and New Zealand Fauna (ed. M. Serena), pp. 27–29. Surrey Beatty & Sons, Chipping Norton, Australia.

Batson, W.G., Abbott, R. & Richardson, K.M. (2015) Release strategies for fauna reintroductions: theory and tests. In Advances in Reintroduction Biology of Australian and New Zealand Fauna (eds D.P. Armstrong, M.W. Hayward, D. Moro & P.J. Seddon), pp. 7-16. CSIRO Publishing, Collingwood, Australia.

Bright, P.W. & Morris, P.A. (1994) Animal translocation for conservation: performance of dormice in relation to release methods, origin and season. Journal of Applied Ecology, 31, 699–708.

Calvete, C., Angulo, E., Estrada, R., Moreno, S. & Villafuerte, R. (2005) Quarantine length and survival of translocated European wild rabbits. The Journal of Wildlife Management, 69, 1063–1072.

Castro, I., Alley, J.C., Empson, R.A. & Minot, E.O. (1994) Translocation of hihi or stitchbird *Notiomystis cincta* to Kapiti Island New Zealand: transfer techniques and comparison of release strategies. In Reintroduction Biology of Australian and New Zealand Fauna (ed. M. Serena), pp. 113–120. Surrey Beatty & Sons, Chipping Norton, Australia.

Christensen, P.E.S. & Burrows, N. (1994) Project desert dreaming: experimental reintroduction of mammals to the Gibson Desert, Western Australia. In Reintroduction Biology of Australian and New Zealand Fauna (ed. M. Serena), pp. 199–207. Surrey Beatty & Sons, Chipping Norton, Australia.

Cid, B., Figueira, L., Mello, A.F.T., Pires, A.S. & Fernandez, F.A.S. (2014) Short-term success in the reintroduction of the red-humped agouti *Dasyprocta leporina*, an important seed disperser, in a Brazilian Atlantic Forest reserve. Tropical Conservation Science, 7, 796–810.

Claridge, A.W., Seebeck, J. & Rose, R.W. (2007) Bettongs, Potoroos and the Musky Rat-Kangaroo. CSIRO Publishing, Collingwood, Australia.

Clayton, J.A., Pavey, C.R., Vernes, K. & Tighe, M. (2014) Review and analysis of Australian macropod translocations 1969–2006. Mammal Review, 44, 109–123.

Devineau, O., Shenk, T.M., Doherty, P.F., White, G.C. & Kahn, R.H. (2011) Assessing release protocols for Canada lynx reintroduction in Colorado. The Journal of Wildlife Management, 75, 623–630.

Fancourt, B.A. (2014) Rapid decline in detections of the Tasmanian bettong (*Bettongia gaimardi*) following local incursion of feral cats (Felis catus). Australian Mammalogy, 36, 247–253.

Fleming, P.A., Anderson, H., Prendergast, A.S., Bretz, M.R., Valentine, L.E. & Hardy, G.E.S. (2014) Is the loss of Australian digging mammals contributing to a deterioration in ecosystem function? Mammal Review, 44, 94–108.

Gusset, M., Slotow, R. & Somers, M.J. (2006) Divided we fail: the importance of social integration for the re-introduction of endangered African wild dogs (*Lycaon pictus*). Journal of Zoology, 270, 502–511.

Hardman, B. & Moro, D. (2006) Optimising reintroduction success by delayed dispersal: is the release protocol important for hare-wallabies? Biological Conservation, 128, 403–411.

Henri, K., Milne, G.R. & Shah, N.J. (2004) Costs of ecosystem restoration on islands in Seychelles. Ocean & Coastal Management, 47, 409–428.

IUCN/SSC (2013) Guidelines for Reintroductions and other Conservation Translocations. IUCN Species Survival Commission, Gland, Switzerland.

Jamieson, I.G. & Lacy, R.C. (2012) Managing genetic issues in reintroduction biology. In Reintroduction Biology: Integrating Science and Management (eds J.G. Ewen, D.P. Armstrong, K.A. Parker & P.J. Seddon), pp. 441–475. Wiley-Blackwell, Oxford, UK.

Johnson, C.N. (1994) Nutritional ecology of a mycophagous marsupial in relation to production of hypogeous fungi. Ecology, 75, 2015–2021.

Jones, C.G. & Merton, D.V. (2012) A tale of two islands: the rescue and recovery of endemic birds in New Zealand and Mauritius. In Reintroduction Biology: Integrating Science and Management (eds J.G. Ewen, D.P. Armstrong, K.A. Parker & P.J. Seddon), pp. 33–72. Wiley-Blackwell, Oxford, UK.

Karesh, W.B. (1993) Cost evaluation of infectious disease monitoring and screening programs for wildlife translocation and reintroduction. Journal of Zoo and Wildlife Medicine, 24, 291–295.

Kemp, L., Norbury, G., Groenewegen, R. & Comer, S. (2015) The roles of trials and experiments in fauna reintroduction programs. In Advances in Reintroduction Biology of Australian and New Zealand Fauna (eds D.P. Armstrong, M.W. Hayward, D. Moro & P.J. Seddon), pp. 73-90. CSIRO Publishing, Collingwood, Australia.

Kenyon, M., Streicher, U., Loung, H., Tran, T., Tran, M., Vo, B. & Cronin, A. (2014) Survival of reintroduced pygmy slow loris *Nycticebus pygmaeus* in South Vietnam. Endangered Species Research, 25, 185–195.

Kleiman, D.G., Thompson, K.V. & Baer, C.K. (eds) (2010) Wild Mammals in Captivity: Principles and Techniques for Zoo Management. The University of Chicago Press, Chicago, USA.

Linklater, W.L., MacDonald, E.A., Flamand, J.R.B. & Czekala, N.M. (2010) Declining and low fecal corticoids are associated with distress, not acclimation to stress, during the translocation of African rhinoceros. Animal Conservation, 13, 104–111.

Lovegrove, T.G. (1996) Island releases of saddlebacks *Philesturnus carunculatus* in New Zealand. Biological Conservation, 77, 151–157.

Manning, A., Wood, J., Cunningham, R., McIntyre, S., Shorthouse, D., Gordon, I. & Lindenmayer, D. (2011) Integrating research and restoration: the establishment of a long-term woodland experiment in south-eastern Australia. Australian Zoologist, 35, 633–648.

Manning, A.D., Eldridge, D.J. & Jones, C.G. (2015) Policy implications of ecosystem engineering for multiple ecosystem benefits. In Advances in Reintroduction Biology of Australian and New Zealand Fauna (eds D.P. Armstrong, M.W. Hayward, D. Moro & P.J. Seddon), pp. 167-184. CSIRO Publishing, Collingwood, Australia.

McClelland, P. & Gummer, H. (2006) Reintroduction of the critically endangered Campbell Island teal *Anas nesiotis* to Campbell Island, New Zealand. Conservation Evidence, 3, 61–63.

McIntyre, S., Stol, J., Harvey, J., Nicholls, A.O., Campbell, M., Reid, A. et al. (2010) Biomass and floristic patterns in the ground layer vegetation of box-gum grassy eucalypt woodland in Goorooyarroo and Mulligans Flat Nature Reserves, Australian Capital Territory. Cunninghamia, 11, 319–357.

Menkhorst, P. (2008) Bettongia gaimardi. In The IUCN Red List of Threatened Species v. 2012.1. Http://www.iucnredlist.org [accessed 24 July 2012].

Michel, C.L. & Bonnet, X. (2012) Influence of body condition on reproductive output in the guinea pig. Journal of Experimental Zoology, 317, 24–31.

Mitchell, A.M., Wellicome, T.I., Brodie, D. & Cheng, K.M. (2011) Captive-reared burrowing owls show higher site-affinity, survival, and reproductive performance when reintroduced using a soft-release. Biological Conservation, 144, 1382–1391.

Moseby, K.E., Hill, B.M. & Lavery, T.H. (2014) Tailoring release protocols to individual species and sites: one size does not fit all. PLoS ONE, 9(6), e99753.

Parker, K.A., Dickens, M.J., Clarke, R.H. & Lovegrove, T.G. (2012) The theory and practice of catching, holding, moving and releasing animals. In Reintroduction Biology: Integrating Science and Management (eds J.G. Ewen, D.P. Armstrong, K.A. Parker & P.J. Seddon), pp. 105–137. Wiley-Blackwell, Oxford, UK.

Portas, T., Fletcher, D., Spratt, D., Reiss, A., Holz, P., Stalder, K. et al. (2014) Health evaluation of free-ranging eastern bettongs (*Bettongia gaimardi*) during translocation for reintroduction in Australia. Journal of Wildlife Diseases, 50, 210–223.

Richardson, K., Castro, I.C., Brunton, D.H. & Armstrong, D.P. (2015) Not so soft? Delayed release reduces long-term survival in a passerine reintroduction. Oryx, 49, 535-541

Rose, R.W. (1987) Reproductive biology of the Tasmanian bettong (*Bettongia gaimardi*: Macropodidae). Journal of Zoology, 212, 59–67.

Rose, R.W. (1989) Age estimation of the Tasmanian bettong (*Bettongia gaimardi*) (Marsupialia, Potoroidae). Wildlife Research, 16, 251–261.

Seddon, P.J. (2010) From reintroduction to assisted colonization: moving along the conservation translocation spectrum. Restoration Ecology, 18, 796–802.

Seddon, P.J., Armstrong, D.P. & Maloney, R.F. (2007) Developing the science of reintroduction biology. Conservation Biology, 21, 303–312.

Short, J. (1998) The extinction of rat-kangaroos (Marsupialia: Potoroidae) in New South Wales, Australia. Biological Conservation, 86, 365–377.

Short, J., Bradshaw, S.D., Giles, J., Prince, R.I.T. & Wilson, G.R. (1992) Reintroduction of macropods (Marsupialia: Macropodoidea) in Australia: a review. Biological Conservation, 62, 189–204.

Shorthouse, D.J., Iglesias, D., Jeffress, S., Lane, S., Mills, P., Woodbridge, G. et al. (2012) The ‘making of’ the Mulligans Flat–Goorooyarroo experimental restoration project. Ecological Management & Restoration, 13, 112–125.

Soorae, P.S. (ed.) (2008) Global Re-introduction Perspectives: Re-introduction Case-Studies from Around the Globe. IUCN/SSC Re-introduction Specialist Group, Abu Dhabi, UAE.

Soorae, P.S. (ed.) (2010) Global Re-introduction Perspectives: Additional Case-Studies from Around the Globe. IUCN/SSC Re-introduction Specialist Group, Abu Dhabi, UAE.

Soorae, P.S. (ed.) (2011) Global Re-introduction Perspectives: 2011. More Case Studies from Around the Globe. IUCN/SSC Re-introduction Specialist Group, Gland, Switzerland and Environment Agency, Abu Dhabi, Abu Dhabi, UAE.

Soorae, P.S. (ed.) (2013) Global Re-introduction Perspectives: 2013 Further Case-Studies from Around the Globe. IUCN/SSC Re-introduction Specialist Group, Gland, Switzerland and Environment Agency, Abu Dhabi, Abu Dhabi, UAE.

Taylor, R.J. (1993a) Habitat requirements of the Tasmanian bettong (*Bettongia gaimardi*), a mycophagous marsupial. Wildlife Research, 20, 699–710.

Taylor, R.J. (1993b) Home range, nest use and activity of the Tasmanian bettong, *Bettongia gaimardi*. Wildlife Research, 20, 87–95.

Wanless, R.M., Cunningham, J., Hockey, P.A.R., Wanless, J., White, R.W. & Wiseman, R. (2002) The success of a soft-release reintroduction of the flightless Aldabra rail (*Dryolimnas [cuvieri] aldabranus)* on Aldabra Atoll, Seychelles. Biological Conservation, 107, 203–210.

Woodford, M.H. (2000) Quarantine and Health Screening Protocols for Wildlife Prior to Translocation and Release into the Wild. Published jointly by the IUCN Species Survival Commission's Veterinary Specialist Group, Gland, Switzerland, the Office International des Epizooties (OIE), Paris, France, Care for the Wild, UK, and the European Association of Zoo and Wildlife Veterinarians, Switzerland.

Paper IV: The effect of pre-release captivity on the stress physiology of a reintroduced population of wild eastern bettongs.

Reintroduction is inherently stressful for the animals involved. Severe physiological responses (e.g. chronic stress) can influence the probability of establishment post-release, and have serious welfare implications. Founders experiencing chronic stress are often more vulnerable to threats including predation, disease and dispersal, and less likely to successfully reproduce. In this paper, I continue to build upon the body of empirical research investigating the effects of pre-release captivity on reintroduced eastern bettongs, focusing on the impacts on stress physiology. I draw statistical comparisons using faecal glucocorticoid metabolite concentrations (FGM) to assess the physiological implications of releasing wild founders with and without pre-release captivity.

Batson, W.G., Gordon, I.J., Fletcher, D.B. Portas, T. & Manning, A.D. (under review) The effect of pre-release captivity on the stress physiology of a reintroduced population of wild eastern bettongs. Journal of Zoology.

Abstract

Stress is important in reintroduction biology because it can influence mortality, dispersal and recruitment and determine establishment success. As stress is unavoidable during reintroduction, it requires deliberate management. Release tactics (e.g. 'delayed- and immediate-release') are often selected specifically based on their presumed effect on physiological stress, yet, the actual physiological effects are seldom tested. Delayed-release involves pre-release confinement (*in-situ*), or captivity (*ex-situ*) which can improve post-release performance in some cases, or induce a detrimental effect in others, especially in wild animals. Quarantine is another common pre-release practice that requires captivity/confinement carrying similar post-release physiological implications. We use faecal glucocorticoid metabolite concentrations (FGMs) to evaluate how a delayed-release involving 95-345 days in captivity influences the stress physiology of wild eastern bettongs (*Bettongia gaimardi*), compared to an immediate-release (within 24 hours of capture), across the initial 18 months post-release. The results suggest that FGMs were relatively higher in the delayed-release group at release, but significantly lower after ca. 2 months of release. We assessed seasonal fluctuations in FGMs, the effect of release tactics on in-trap behaviour, and the relationship between those behaviours and FGMs. We found that FGMs fluctuated seasonally, but release tactics did not influence behaviour, and that behavioural variations had no relationship with FGMs. Overall our results, coupled with previous research, suggest that an immediate-release is preferable when quarantine is not required.

Introduction:

Stress is an unavoidable consequence of fauna reintroductions that needs to be managed because it can influence survival, reproduction and dispersal which ultimately determine establishment success (Teixeira, De Azevedo, Mendl *et al.*, 2007; Dickens, Delehanty & Romero, 2010; Parker, Dickens, Clarke *et al.*, 2012). 'Delayed- and immediate-releases', i.e. translocations with and without pre-release confinement, are common tactics for managing stress (Batson, Abbott & Richardson, 2015a; Batson, Gordon, Fletcher *et al.*, 2015b). Despite stress having a critical influence on reintroduction outcomes (Teixeira, *et al.*, 2007; Dickens, *et al*., 2010) and being an important factor to consider when designing reintroduction processes (IUCN/SSC, 2013), the physiological effects of different methods are rarely tested (Dickens, Delehanty & Romero, 2009). In the absence of empirical evidence of the physiological effects, conclusions are often drawn from indirect proxies including survival, body-condition and reproductive activity (e.g. Richardson, Castro, Brunton *et al.*, 2015; Jenni, Keller, Almasi *et al.*, 2015).

Physiological stress is often classified as 'acute', describing short-term physiological responses to a specific stimulus, or 'chronic', i.e. the accumulated effect of multiple responses to multiple stimuli (Sapolsky, Romero, & Munck, 2000). Acute stress responses are often essential for maintaining the welfare of the animal (e.g. the fight-or-flight response), while chronic stress can have a detrimental effect on an animal's health and well-being, and both forms of stress should to be considered when designing and evaluating reintroduction processes (Teixeira *et al.*, 2007, Dickens *et al.*, 2010). Stress can be assessed using a variety of methods, which need to be carefully considered when designing experiments and interpreting results (Millspaugh & Washburn, 2004). A common approach in wildlife studies is to measure the concentration of glucocorticoids (GCs), or their metabolites, within biological substrates (Sheriff, Dantzer, Delehanty *et al.*, 2011). Glucocorticoids are produced and excreted as part of normal biological function, but the rate of excretion can change during a stress response (Sapolsky, Romero & Munck, 2000). During an acute response GC excretion can increase rapidly, and the strength of this response is often

measured via snapshot assessments of the GCs circulating within blood or saliva. The concentration of GC metabolites within non-circulating substrates such as faeces is often preferred for assessing chronic stress. The main advantage of this approach is that it provides an averaged measure of circulating GCs over a period, which reduces the effects of acute stress responses and natural fluctuations (Sheriff *et al.*, 2011). Behavioural variations can also be used to assess physiological stress in certain situations, if hormonal excretion induces a behavioural response and these complex interactions are understood (Silverin, 1998).

All reintroductions present a risk that parasites and pathogens will be co-introduced to the detriment of the biological community at the release-site (IUCN/SSC, 2013). This risk is often managed by quarantine conducted *ex-situ* to obtain the level of isolation required to minimise the risk of transmission to free-ranging populations (Woodford, 2000). As quarantine involves pre-release confinement, it may provide many of the benefits associated with a delayed-release (e.g. enable pre-release recovery, acclimatisation, and physiological conditioning). The potential benefit is indicated by the improved establishment of wild Canadian lynx (*Lynx canadensis*) when released following pre-release captivity (Devineau, Shenk, Doherty *et al.*, 2011). However, the post-release physiological, behavioural and biological effects of captivity should be evaluated as captivity is often recognised as a factor in reintroduction failure (Kleiman, 1989, Fischer & Lindenmayer, 2000). If successful, *ex-situ* captivity could provide multiple benefits (quarantine and delayed-release) and increase the cost efficiency of reintroduction projects.

Here we compare the concentration of faecal glucocorticoid metabolites (FGMs) between wild eastern bettongs (*Bettongia gaimardi*) reintroduced to a predator-free fenced reserve in south-east Australia using two different release tactics. Half of the founder population (N = 16) was exposed to a ‘delayed-release’ which included 95-345 days in *ex-situ* captivity; with the remainder (N = 16) exposed to an immediate-release within 24 hours of acquisition from the wild. We use the results

to draw comparisons regarding the effects on stress physiology between the groups across the initial 18 months following release. We also investigate the relationships between sex, season, behaviour and FGMs; and test whether the release tactics influenced behaviour. This study builds upon previous research which showed that release tactics did not have a significant effect on post-release survival, female reproduction or body mass, despite the delayed-release group being heavier and having fewer pouch-young when released (Batson, Gordon, Fletcher *et al.* 2015c). Given that this reintroduction was considered 'low-risk' with minimal expected predation and dispersal, we investigated sub-lethal responses to release tactics to develop new hypotheses and make recommendations for reintroductions with greater risk. This project represents the first attempt to reintroduce eastern bettongs to the Australian mainland and was conducted within an experimental framework to provide information regarding the understanding of its reintroduction biology (Kemp, Norbury, Groenewegen *et al.*, 2015, Manning, Eldridge & Jones, 2015).

Methods

Methods and Materials:

Study area:

Two reintroduced populations were established in the Australian Capital Territory (ACT). One population was established at Tidbinbilla Nature Reserve (TNR) (www.tidbinbilla.act.gov.au), which was also the location of the confinement period incorporated into the delayed-release. TNR represents a 'low-intensity captive environment' without public access. The second population was established at Mulligans Flat Woodland Sanctuary (MFWS) (www.mulligansflat.org.au). MFWS is a 485 ha fenced reserve now free of cats (*Felis catus*), foxes (*Vulpes vulpes*), and dogs (*Canis lupus*) (Shorthouse, Iglesias, Jeffress *et al.*, 2012). MFWS is situated within a critically endangered box-gum grassy woodland ecological community (McIntyre, Stol, Harvey *et al.*, 2010), which is

subject to a multifaceted long-term ecological restoration experiment (Manning, Wood, Cunningham *et al.*, 2011, www.mfgowoodlandexperiment.org.au).

Study-species:

The Eastern bettong (or Tasmanian bettong) is a nocturnal, ground dwelling, mycophagous marsupial (Taylor, 1993a; 1993b; Johnson, 1994). Once common throughout south-eastern Australia, this species became extinct on the mainland by the 1920s, primarily due to predation from introduced predators (Short, 1998). It is listed as 'near-threatened' by the IUCN (Menkhorst, 2008), and prior to this project the distribution was restricted to eastern Tasmania (Claridge, Seebeck & Rose, 2007).

The translocation process, trapping and sample collection:

Sixty adults (19♂, 41♀ + 28 pouch-young) were translocated from Tasmania to the ACT during 2011 and 2012. Translocated adults were reproductively mature, but an accurate age estimation was not possible. Twenty-eight adults were used as the founder population for TNR, with the remaining 32 being used as the founder population for MFWS. This study exclusively focuses on the adult founders at MFWS.

Bettongs were collected from free-ranging populations from five geographic regions in Tasmania. Females with furred pouch-young, or young-at-foot were excluded in Tasmania due to the risk to dependent young. Some males were excluded to ensure a female biased sex-ratio at the reintroduction site. Adults were sedated with intramuscular diazepam (1 mg/kg), and transported to TNR arriving within 18 hours of capture. Upon arrival, adults were anaesthetised, given a health assessment and administered a passive integrated transponder. Bettongs were systematically

assigned as founders for MFWS or TNR, maintaining the desired sex-ratio and geographic representation within both populations. Bettongs assigned to MFWS were systematically assigned a release tactic ensuring that both groups contained a similar number of individuals, and contained similar demographic and geographic representations. The delayed-release group contained 11 females and five males, with the immediate-release group containing 10 females and six males.

Upon completion of the health assessments conducted upon arrival, the immediate-release group was transported and released at MFWS within 24 hours of capture in Tasmania. No supplemental food or water was provided at MFWS. With the exception of a delayed-release male, each bettong was fitted with a VHF radio collar (V5C_161C; Sirtrack, Hawkes Bay, New Zealand) or global positioning system (GPS)/VHF collar (Q4000E; Telemetry Solutions, Walnut Creek, USA). Collars were removed approximately one year after release. Each collar weighed between 28-32g which was less than 2.5% of the body-weight.

Members of the delayed-release group were initially released into small enclosures at TNR (0.5-1 ha, ≤ 5 bettongs per-enclosure) for 30 days quarantine. Following quarantine they were transferred to larger enclosures (2.6-9.5 ha, ≤ 20 bettongs per-enclosure), where they remained until transferred to MFWS. The exception being 'bachelor groups' of males (≤ 5 bettongs) that were housed in the small enclosures post-quarantine to facilitate breeding management. During quarantine members of the delayed-release group co-inhabited enclosures with founders of the TNR population who were translocated during the same event, and then with the permanent TNR population post-quarantine until transferred to MFWS. In general, the group within an enclosure was female-biased to restrict breeding opportunities between bettongs from different collection regions in Tasmania. Members of the delayed-release group spent 95-345 days at TNR, with the variation being associated with the irregular intervals between translocation events, and to ensure that the timing and size of release events into MFWS were similar for both groups (i.e. delayed-release and immediate-release groups). Enclosures at TNR included natural vegetation, and food

and water were provided *ad libitum.* Bettongs were trapped at 3 month intervals to conduct health assessments which were repeated when the individual was transferred to MFWS.

All trapping was conducted using baited cage-traps with padded doors, laid on a plastic sheet, and covered with a hessian sack. Traps were cleared within 4 hours after sunset. Trapping was conducted at approximately 1, 3, 6, 9 and 12 months post-release at MFWS to conduct health assessments. All health assessments included measuring the weight, body-condition (subjective assessment of fat stored around rump and tail), and pouch activity. In Tasmania and at MFWS the in-trap behaviour was assessed using a subjective 'in-trap stress rating' (ITSR) using a scale between (1) little movement within the trap and no obvious response to approaching observers, (2) moderate movement within the trap (e.g. scratching at the door, moving from one end to another, moderate jumps), and a detectable reaction to approaching observers, and (3) extensive movement within the trap (e.g. fully extended jumps, frantic movement between each end of the trap, gnawing at trap), immediate, constant and significant response to approaching observers. All observers were trained to standardise these assessments. Bettongs usually defecated while trapped, allowing faecal samples to be collected from the plastic sheet. Faecal samples were collected immediately after the trap was cleared in Tasmania ('release' for immediate-release group), immediately after the trap was cleared at TNR on the night founders were transferred to MFWS ('release' for the delayed-release group), and during health assessments at MFWS (post-release for both groups). Samples of faeces were frozen (-20 °C) within 2-6 hours of collection, apart from those collected in Tasmania which were immediately frozen on arrival at TNR, and remained frozen until laboratory analysis. Each trap was cleaned and the plastic sheet replaced following any animal capture. For further information regarding the translocation, trapping and health assessments (see Portas, Fletcher, Spratt, *et al.* 2014, and Batson, *et al.* 2015c).

Laboratory validation, sample processing, extraction and assay:

FGMs were determined by enzyme immunoassay (EIA) using a polyclonal antibody, R4866, raised in rabbits against cortisol-3-carboxymethyloxime and a horse radish peroxidase (HRP) conjugated label (both provided by C.J. Munro, University of California–Davis, Davis, California, USA). The known cross-reactivities for the antibody are cortisol (100%), prednisolone (9.9%), prednisone (6.3%), cortisone (5%) and <0.1% with androstenedione, androsterone, corticosterone, desoxycorticosterone, 11-desoxycortisol, 21-desoxycortisone and testosterone (Munro & Lasley, 1988). Each faeces sample was manually mixed, with sub-samples of 0.5 g of wet faeces being placed in 5 mls of 80% methanol; briefly vortexed and extracted overnight using gentle mixing; centrifuged at 635 x g for 15 mins; and the supernatant recovered and stored at -20 °C. For the enzyme immunoassay 50 µl of antibody diluted 1:9000 in coating buffer (50 mM sodium bicarbonate, pH 9.6) was added to each well, excluding the first row of a 96-well microtiter plate, and incubated at 4 °C overnight. 70 µl of each extract was air-dried at 60 °C for one hour then reconstituted in 140 µl phosphate buffer. Plates were washed four times to remove unbound antibody, then 50 µL each of diluted faecal extracts, zeros, high and low controls and standards (3.9 – 500 pg/50 µL), were added in duplicate. 50 µL of working dilution cortisol-HRP label (1:20,000) was added to each well, mixed and incubated for 1 hour at room temperature. The plates were washed four times to remove unbound antigen and 100 µL ABTS substrate (0.5 M H2O2, 40 mM azino-bis [3-ethylbenzthiazoline-6-sulfonic acid] in 0.05 M citrate buffer, pH 4.0) was added, mixed, and incubated at room temperature until the optical density of the 0 standard reached between 0.8 and 1.0. Optical density of the plates was read at 405 nm on a microplate reader. The optical densities were recorded and cortisol concentrations calculated. All faeces data is expressed (ng/g) net dry faeces weight basis. The assay was validated for eastern bettong faeces by demonstrating parallelism between serial dilutions of pooled faecal extractions and the respective cortisol standard curves; and detection of a cortisol peak following a physiological challenge (capture and transfer). Intra-assay coefficients of variation were 7.8% and 3.3%, and inter-assay coefficients of variation were 3.5% and 4.5% for low and high controls, respectively.

The assay method used in this study was chosen on the basis of the work by Fanson *et al*. (2015) who found that it was the most effective of five possible approaches.

Statistical method:

All statistical analyses were conducted using Genstat (VSN International, 2014) unless otherwise stated, and significance was assumed at $P<0.05$. The dataset included FGMs from 105 samples, with one sample being excluded from all analyses due to the extreme FGM attributed to a serious health condition. Normality was tested using a Shapiro-Wilk Test, and a log transformation was used to normalise the data. We did not differentiate between sexes based on the outcome of a Student's t.test indicating that FGM was not significantly different between males and females ($t_{88} = 1.545$, $P = 0.126$). To account for the irregularity of the trapping events, the samples were organised into the following periods (as per Batson *et al.*, 2015c): Release (at MFWS), 1-60 days post-release, 61-180 days post-release, 181-360 days post-release, and 360-540 days post-release. If multiple samples were collected from an individual within a period, only the first sample collected was included in the analysis, as a consequence the dataset was reduced to 90 samples. We conducted a linear mixed model, with FGM included as the dependent variable; release tactic, season (summer, autumn, winter and spring), period and the interaction between tactic and period as fixed factors; and the individual as a random factor. The mean FGMs were compared between tactics within each period using randomisation tests with 999 replications. As the trend of the change appeared to differ between the groups post-release, we conducted a simple linear regression including the interaction between groups and period in the model (excluding data from the date of release) to assess whether the observed difference was significant. As season and period showed significant effects we conducted one-way ANOVAs and post-hoc Tukey's Tests to identify the location of the differences.

A dataset of 105 samples were used to compare the ITSR between the two groups post-release. Using the periods described above we compared the ITSR between the two groups within each

period using Fisher exact 2 x 3 contingency tables using SPSS (IBM Corp., 2013). Again, we excluded all but the first record from any individual within a given period. To test the relationship between ITSR and FGM we conducted a one-way ANOVA using the ITSR to differentiate the groups. As some records lacked an ITSR or FGM measure this was conducted using 62 samples.

Results

The linear mixed model indicated that all factors we tested had a significant effect on FGM - release tactic ($F_{1, 17} = 5.254$, P = 0.03), period ($F_{4, 70} = 4.610$, P = 0.002), season ($F_{3, 71} = 4.853$, P= 0.004) and the interaction between release tactic and period ($F_{4, 66} = 8.145$, P < 0.001). This suggests that each variable influenced FGMs, and that the trend across the monitoring period differed between the groups. In the delayed-release group, FGMs were relatively high at release, before decreasing to the first post-release sampling period, and then gradually increasing across the remainder of the monitoring period. This contrasted with the FGMs in the immediate-release group which remained fairly consistent across the monitoring period (Fig. 1.). The simple linear regression model suggested that the change in FGMs differed significantly between the groups post-release ($t_{69} = 2.72$, P = 0.008). The randomisation tests confirmed that the FGMs in the delayed-release group was higher than the immediate-release group at release (P = 0.001), but the converse difference was observed between 1-60 day post-release (P = 0.014).

The ANOVAs and Tukey's Tests indicated that FGMs were lower in spring than autumn and winter (Fig. 2), and were lower between 1-60 days post-release compared to 361-540 days post-release. The results of the contingency tables indicated that the ITSR differed between the two groups during the period 181-360 days post-release (P = 0.02) (Fig. 3). The ANOVA displayed no relationship between the ITSRs and FGMs ($F_{2, 59} = 2.002$, P = 0.144) (Fig. 4).

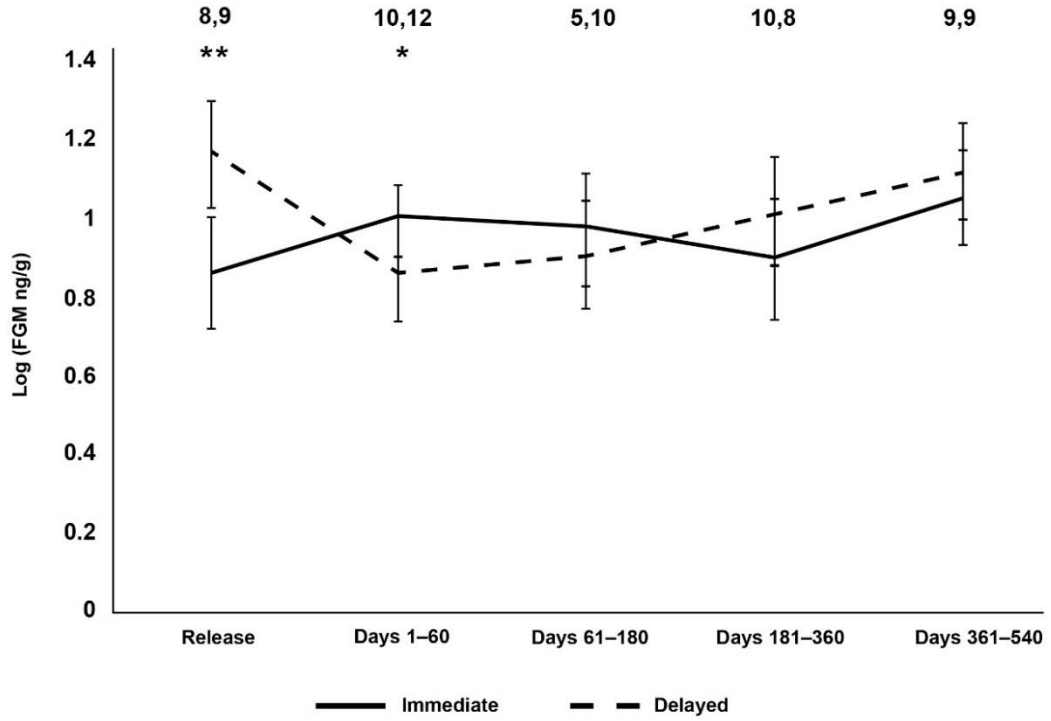

Figure 1. The concentrations of faecal glucocorticoid metabolites (FGMs) in a reintroduced population of eastern bettongs released using delayed- and immediate-release tactics across the first year post-release. Error-bars indicate mean ± 1 SD, upper numbers indicate sample sizes (immediate, delayed), a between group difference within a sampling period is indicated by ** when P<0.001, and * when P<0.05.

The ANOVAs and Tukey's Tests indicated that FGMs were lower in spring than autumn and winter (Figure 2), and were lower between 1-60 days post-release compared to 361-540 days post-release. The results of the contingency tables indicated that the ITSR differed between the two groups during the period 181-360 days post-release (P = 0.02) (Figure 3). The ANOVA displayed no relationship between the ITSRs and FGMs ($F_{2, 59}$ = 2.002, P = 0.144) (Figure 4).

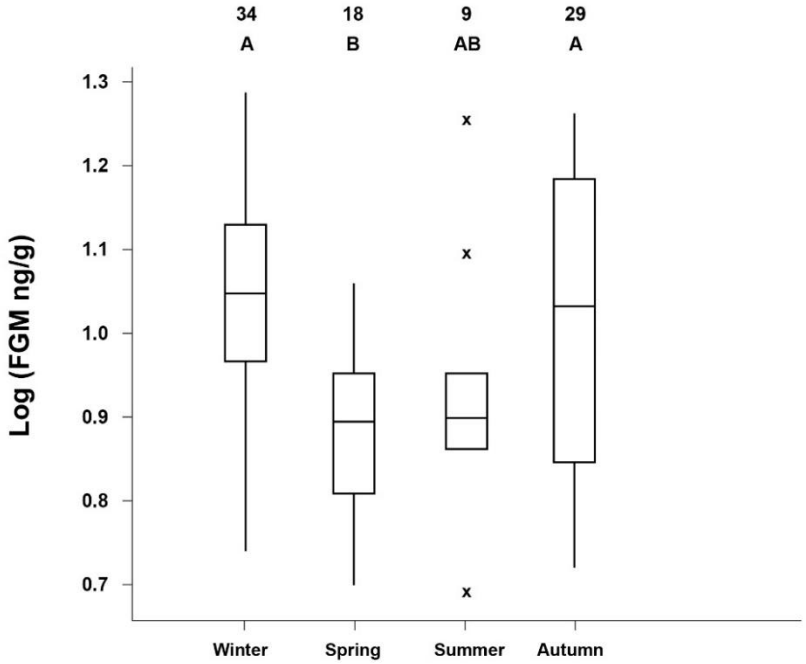

Figure 2. Comparing the concentration of faecal glucocorticoid metabolites (FGMs) among seasons within a reintroduced population of eastern bettongs (immediate- and delayed-release groups combined) established at Mulligans Flat Woodland Sanctuary in south-eastern Australia. Upper numbers represent sample size, and matching upper letters indicate non-significant difference. Boxes indicate median to quartile range, whiskers indicate quartiles to extremes, and 'x' indicate statistical outliers.

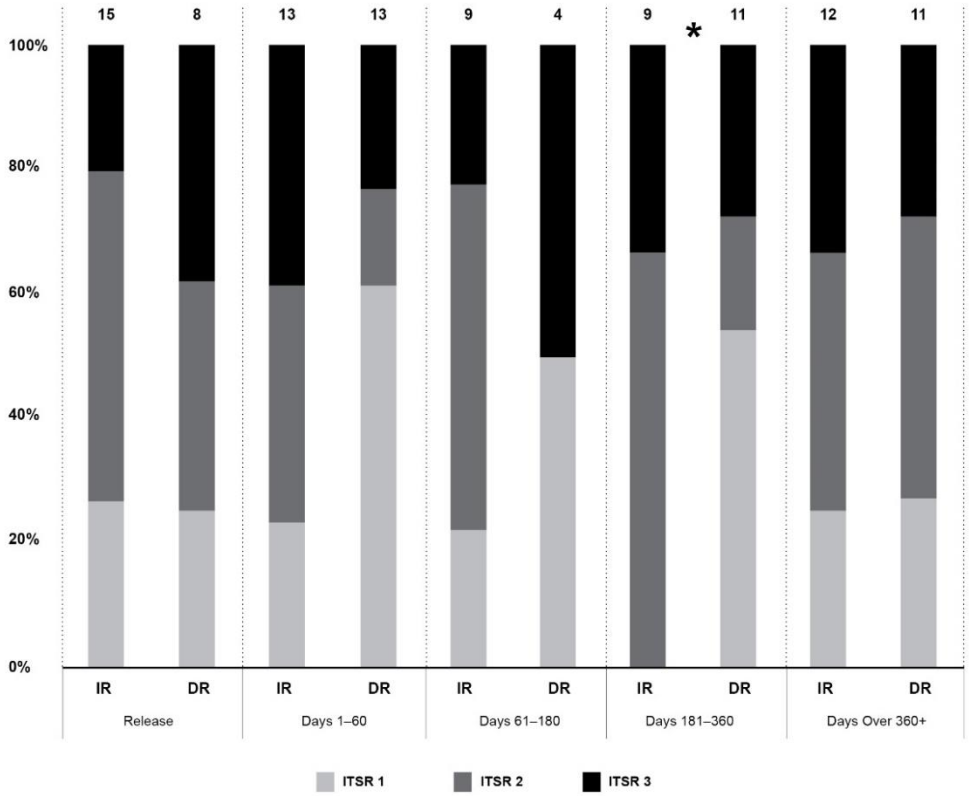

Figure 3. The in-trap stress ratings (ITSR) of the reintroduced population of eastern bettongs (immediate- and delayed-release groups combined) established at Mulligans Flat Woodland Sanctuary in south-eastern Australia. The ITSR reflects the behavioural assessment using a 1 (calm) - 3 score (highly stressed) based on the movement of the animal while in the trap. Upper number represent sample size. Boxes indicate median to quartile range, whiskers indicate quartiles to extremes, and 'x' indicate statistical outliers.

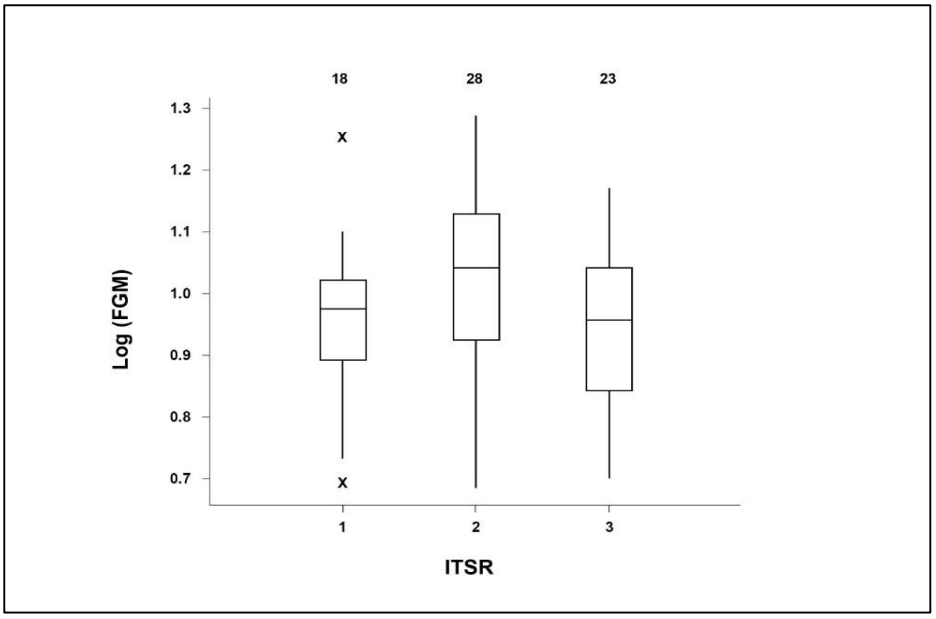

Figure 4. The relationship between the in-trap stress behaviours (ITSR) and the concentration of faecal glucocorticoid metabolites (FGMs) in a reintroduced population of eastern bettongs at Mulligans Flat Woodland Sanctuary in south-eastern Australia. The ITSR reflects the behavioural assessment using a 1 (calm) - 3 (highly stressed) score based on the movement of the animal while in the trap. Upper numbers represent sample size, and * indicates a significant difference between the groups when $P \leq 0.05$.

Discussion

To the best of our knowledge, this is the first study to quantitatively assess the effect of pre-release captivity on the stress physiology of a reintroduced population post-release. However, it builds upon a substantial body of research investigating the impact of translocation on stress (e.g. Franceschini, Rubenstein, Low *et al.*, 2008, Dickens, Delehanty & Romero, 2009, Bosson, Palme & Boonstra, 2013), and release tactics on establishment (e.g. Bright & Morris, 1994, Mitchell, *et al.*, 2011, Richardson, *et al.*, 2015). The absence of similar studies is surprising given that experimental assessments of release tactics have been a prominent feature of reintroduction biology (Seddon, Armstrong & Maloney, 2007), and the emphasis placed on stress in recommendations governing the design of reintroductions (IUCN/SSC, 2013). Accumulating empirical evidence of the physiological effects of release tactics is essential for identifying and selecting an appropriate approach to reintroducing species into their native range (Batson *et al.*, 2015b).

The use of FGMs to assess chronic stress appears to be growing in popularity, mainly because it can often be conducted without invasive sampling techniques, and because it can provide a metric that is resistant to the confounding effects of acute stress (Sheriff *et al.*, 2011). The complexity of physiological processes restricts the ability to draw robust conclusions from a single physiological assessment; However, FGMs that differ significantly from normal levels can indicate a physiological stress response in certain situations (Millspaugh & Washburn, 2004). FGMs provide a measure of stress over a temporal period that is determined by the hormonal pathway involving excretion, metabolism and defecation. The maximum duration of this period in eastern bettongs is likely to be similar to the 25-30 hour gut retention times in rufus rat-kangaroo (*Aepyprymnus rufescens*) and brush-tailed bettong (*B. penicillata*) (Wallis, 1994). However, this period may be reduced in our study as trapping appeared to induce defecation (WGB. *pers obs*.).

The difference in FGMs between the two groups at release, suggests that the delayed-release group was experiencing long-term chronic stress in TNR. This may be because the delayed-release group did not acclimatise to captivity despite the extensive confinement period. This contrasts the 1-2 months taken for wild Tasmanian devils (*Sarcophilus harrisii*) to acclimatise to captivity based on the re-establishment of pre-capture plasma cortisol concentrations (Jones, Lockhart & Rose, 2005). The maintenance of chronic stress may be a factor in the reduction in female reproductive activity observed in the delayed-release group and the permanent population at TNR (Batson *et al.*, 2015c). Pre-release captivity has been associated with physiological stress in translocated species including Grevy's zebra (*Equus grevyi*) (Franceschini, Rubenstein, Low *et al.*, 2008), mantled howlers (*Alouatta palliata*) (Aguilar-Cucurachi, Dias, Rangel-Negrin *et al.*, 2010), and chukar partridge (*Alectoris chukar*) (Dickens, Delehanty & Romero, 2009), and was presented as a potential cause of reproductive disruption in translocated rhinoceros (*Diceros bicornis* and *Ceratotherium simum*) (Linklater *et al.*, 2010). Differences in diet between the wild in Tasmania and TNR could also contribute to the difference in FGM concentrations between the two groups at release, which could

be a factor responsible for the difference observed between the two groups prior to release (Von Der Ohe & Servheen, 2002).

FGMs were higher in the delayed-release group than the immediate-release group at release, but this trend reversed during the initial 2 months of release, with no other significant differences occurring during the remainder of the monitoring period. This trend was primarily driven by changes within the delayed-release group which suggests that this group was experiencing a stronger physiological response to the translocation. The difference at release suggests that FGMs were higher in captivity than in Tasmania, which contrasts with the indication that captive-bred populations of greater bilbies (*Macrotis lagotis*) have lower GCs, compared to 'semi-wild' populations within fenced reserves similar to MFWS (Narayan, Evans & Hero, 2014). These results suggest that origin (wild-bred vs captive-bred), and environmental familiarity can have a strong influence on stress physiology, reinforcing the need to tailor reintroduction processes to specific projects (Parker, Dickens, Clarke *et al.*, 2012, Moseby, Hill & Lavery, 2014). The difference between the groups post-release, may reflect different intensities of physiological stress at that time, or that the delayed-release group was suffering from adrenal exhaustion which reduced FGMs over the short-to-medium term post-release (Kock, Toit, Kock *et al.*, 1990). However, adrenal exhaustion would likely have a detrimental impact on an animal's health which was not supported by the outcomes of the health assessments (Portas *et al.*, 2014; Batson *et al.*, 2015c). It could be considered that the FGMs recorded throughout this study are acceptable because they do not appear to have an impact on the health or biological performance (Portas *et al.*, 2014; Batson *et al.*, 2015c). However, in addition to other important factors including financial cost, the potential impact on stress physiology should still be considered when designing future reintroductions to avoid compromising animal welfare, and to reduce the risk that stress will induce detrimental response when the precise effects are unknown.

Behaviour can provide an indication of physiological stress in certain situations (Cook, Ingram, Mellor *et al.*, 2000). However, we have no evidence that in-trap behaviour was influenced by relative FGMs, nor that the release tactics used influenced those behaviours. The indication that pre-release captivity did not influence in-trap behaviour, is similar to the conclusions drawn by Batson et al. (2015c) that these release tactics did not influence a founder's ability to find and obtain resources based on a the similarity of body-weight between the two groups post-release. These findings ease concerns regarding the behavioural effects of exposing wild bettongs to pre-release captivity. We acknowledge the statistical power of our analysis is restricted by small sample size throughout this study, and suspect that the significant difference in ITSRs observed between 181-360 days reflect a sampling anomaly, rather than a real effect.

FGMs appear to be lower during spring compared to winter and autumn. Seasonal fluctuations in stress hormones have been observed in other mammal species including deer mice (*Peromyscus maniculatus*), and red-backed voles (*Clethrionomys gapperi*) (Harper & Austad, 2001). Seasonal fluctuations are often associated with seasonal breeding (Millspaugh & Washburn, 2004), but this is an unlikely cause in eastern bettongs which breed aseasonally (Rose, 1987). Near-constant breeding was observed during the post-release monitoring period at MFWS (Batson *et al.*, 2015c). Seasonal resource availability, and the corresponding changes to diet provide a more plausible explanation (Taylor, 1992; Johnson, 1994). However, this presents a possibility that the relative changes in FGMs reflect a bias caused by changes to another physiological processes such as hormone metabolism (Sheriff *et al.*, 2011). As a precaution we recommend that future reintroductions of bettongs to be scheduled in early spring to minimise the potential cumulative effects of physiological stress.

This project was deliberately designed to minimise stress on the animals involved (e.g. short-transit times, low-intensity captivity and absence of predators). However, our results suggest that exposing bettongs to a delayed-release may influence their stress physiology which may have negative influences post-release, especially in unfenced environments where stress could make

founders increasingly vulnerable to threats including predation and dispersal. Physiological stress could reduce the probability of establishment by affecting decision-making and behaviour, including space-use, refuge choice and activity (Lima, 1998; Yoder et al., 2004). Based on the results to date, we recommend an immediate-release for reintroductions that do not require quarantine to minimise cost and the severity of physiological stress, but accept that *ex-situ* captivity may be deemed appropriate for quarantine to reduce risk of detrimental co-introductions.

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References

Aguilar-Cucurachi, M.A., Dias, P.A., Rangel-Negrin, A., Chavira, R., Boeck, L. & Canales-Espinosa, D. (2010). Preliminary evidence of accumulation of stress during translocation in mantled howlers. American Journal of Primatology 72, 805-810.

Batson, W., Abbott, R. & Richardson, K.M. (2015a). Release strategies for fauna reintroductions: theory and tests. In Advances in Reintroduction Biology of Australian and New Zealand Fauna: 7-16. Armstrong, D.P., Hayward, M.W., Moro, D. & Seddon, P.J. (Eds.). Collingwood, Australia: CSIRO Publishing.

Batson, W.G., Gordon, I.J., Fletcher, D.B. & Manning, A.D. (2015b). Translocation tactics: A framework to support the IUCN Guidelines for wildlife translocations and improve the quality of applied methods. Journal of Applied Ecology. Early View, 1-10.

Batson, W.G., Gordon, I.J., Fletcher, D.B. & Manning, A.D. (2015c). The effect of pre-release captivity on post-release performance in reintroduced eastern bettongs (*Bettongia gaimardi*). Oryx. First View, 1-10.

Bright, P.W. & Morris, P.A. (1994). Animal translocation for conservation - performance of dormice in relation to release methods, origin and season. Journal of Applied Ecology. 31, 699-708.

Claridge, A.W., Seebeck, J. & Rose, R.W. (2007). Bettongs, potoroos and the musky rat-kangaroo. Collingwood, Australia: CSIRO Publishing.

Cook, C.J., Ingram, J.R., Mellor, D.J., Harris, P.J. & Matthews, L.R. (2000). Hands-on and hands-off measurement of stress. In The biology of animal stress: basic principles and implications for animal welfare: 123-146. Moberg, G.P. & Mench, J.A. (Eds.). Wallingford, UK: CABI Publishing.

Devineau, O., Shenk, T.M., Doherty, P.F., White, G.C. & Kahn, R.H. (2011). Assessing release protocols for Canada lynx reintroduction in Colorado. Journal of Wildlife Management. 75, 623-630.

Dickens, M.J., Delehanty, D.J. & Romero, L.M. (2009). Stress and translocation: alterations in the stress physiology of translocated birds. Proceedings of the Royal Society B: Biological Sciences. 276, 2051-2056.

Dickens, M.J., Delehanty, D.J. & Romero, L.M. (2010). Stress: An inevitable component of animal translocation. Biological Conservation. 143, 1329-1341.

Fanson, KV, Best, EC, Bunce, A., Fanson, B.G., Hogan, L.G., Keeley, T., Narayan, E.J., Palme, R., Parrott, M.L., Sharp, T.M., Skogvold, K., Tuthill, L., Webster, K.N., Bashaw, M. (*accepted*). One size does not fit all: Monitoring faecal glucocorticoid metabolites in marsupials. General and Comparative Endocrinology.

Fischer, J. & Lindenmayer, D.B. (2000). An assessment of the published results of animal relocations. Biological Conservation. 96, 1-11.

Franceschini, M.D., Rubenstein, D.I., Low, B. & Romero, L.M. (2008). Fecal glucocorticoid metabolite analysis as an indicator of stress during translocation and acclimation in an endangered large mammal, the Grevy's zebra. Animal Conservation. 11, 263-269.

Harper, J.M. & Austad, S.N. (2001). Effect of capture and season on fecal glucocorticoid levels in deer mice (*Peromyscus maniculatus*) and red-backed voles (*Clethrionomys gapperi*). General and Comparative Endocrinology. 123, 337-344.

IBM Corp. (2013). IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY.

IUCN/SSC (2013). Guidelines for Reintroductions and Other Conservation Translocations. Version 1.0. Gland, Switzerland: IUCN Species Survival Commission.

Jenni, L., Keller, N., Almasi, B., Duplain, J., Homberger, B., Lanz, M., Korner-Nievergelt, F., Schaub, M. & Jenni-Eiermann, S. (2015). Transport and release procedures in reintroduction programs: stress and survival in grey partridges. Animal Conservation. 18, 62-72.

Johnson, C.N. (1994). Nutritional ecology of a mycophagous marsupial in relation to production of hypogeous fungi. Ecology. 75, 2015-2021.

Jones, S.M., Lockhart, T.J. & Rose, R.W. (2005). Adaptation of wild-caught Tasmanian devils (*Sarcophilus harrisii*) to captivity: evidence from physical parameters and plasma cortisol concentrations. Australian Journal of Zoology. 53, 339-344.

Kemp, L., Norbury, G., Groenewegen, R. & Comer, S. (2015). The roles of trials and experiments in fauna reintroduction programs In Advances in reintroduction biology of Australian and New Zealand fauna: 73-90. Armstrong, D. P., Hayward, M. W., Moro, D. & Seddon, P. J. (Eds.). Collingwood, Australia: CSIRO Publishing.

Kleiman, D.G. (1989). Reintroduction of Captive Mammals for Conservation. Bioscience. 39, 152-161.

Kock, M.D., Toit, R.D., Kock, N., Morton, D., Foggin, C. & Paul, B. (1990). Effects of Capture and Translocation on Biological Parameters in Free-Ranging Black Rhinoceroses (Diceros bicornis) in Zimbabwe. Journal of Zoo and Wildlife Medicine 21, 414-424.

Linklater, W.L., Macdonald, E.A., Flamand, J.R.B. & Czekala, N.M. (2010). Declining and low fecal corticoids are associated with distress, not acclimation to stress, during the translocation of African rhinoceros. Animal Conservation. 13, 104-111.

Manning, A.D., Eldridge, D.J. & Jones, C.G. (2015). Policy implications of ecosystem engineering for multiple ecosystem benefits. In Advances in reintroduction biology of Australian and New Zealand fauna: 167-184. Armstrong, D. P., Hayward, M. W., Moro, D. & Seddon, P. J. (Eds.). Clayton South, Australia: CSIRO Publishing.

Manning, A., Wood, J., Cunningham, R., Mcintyre, S., Shorthouse, D., Gordon, I. & Lindenmayer, D. (2011). Integrating research and restoration: the establishment of a long-term woodland experiment in south-eastern Australia. Australian Zoologist 35, 633-648.

McIntyre, S., Stol, J., Harvey, J., Nicholls, A.O., Campbell, M., Reid, A., Manning, A.D. & Lindenmayer, D. (2010). Biomass and floristic patterns in the ground layer vegetation of

box-gum grassy eucalypt woodland in Goorooyarroo and Mulligans Flat Nature Reserves, Australian Capital Territory. Cunninghamia. 11, 319-357.

Menkhorst, P. (2008). *Bettongia gaimardi.* In IUCN 2014. IUCN Red List of Threatened Species. Version 2014.1.

Millspaugh, J.J. & Washburn, B.E. (2004). Use of fecal glucocorticoid metabolite measures in conservation biology research: considerations for application and interpretation. General and Comparative Endocrinology. 138, 189-199.

Mitchell, A.M., Wellicome, T.I., Brodie, D. & Cheng, K.M. (2011). Captive-reared burrowing owls show higher site-affinity, survival, and reproductive performance when reintroduced using a soft-release. Biological Conservation. 144, 1382-1391.

Munro, C.J. & Lasley, B.L. (1988). Non-radiometric methods for immunoassay of steroid hormones. Progress in Clinical and Biological Research. 285, 289-329.

Narayan, E.J., Evans, N. & Hero, J.M. (2014). Monitoring physiological stress in semi-free ranging populations of an endangered Australian marsupial, the Greater Bilby (*Macrotis lagotis*). European Journal of Wildlife Research. 60, 727-735.

Parker, K.A., Dickens, M.J., Clarke, R.H. & Lovegrove, T.G. (2012). The theory and practice of catching, holding, moving and releasing animals. In Reintroduction biology: Integrating science and management: 105-137. Ewen, J.G., Armstrong, D.P., Parker, K.A. & Seddon, P.J. (Eds.). Oxford, UK: Wiley-Blackwell Publishing Ltd.

Portas, T., Fletcher, D., Spratt, D., Reiss, A., Holz, P., Stalder, K., Devlin, J., Taylor, D., Dobroszczyk, D. & Manning, A. (2014). Health evaluation of free-ranging eastern bettongs (*Bettongia gaimardi*) during translocation for reintroduction in Australia. Journal of Wildlife Diseases. 50, 210-223.

Richardson, K., Castro, I.C., Brunton, D.H. & Armstrong, D.P. (2015). Not so soft? Delayed release reduces long-term survival in a passerine reintroduction. Oryx, 49, 535-541.

Rose, R.W. (1987). Reproductive biology of the Tasmanian bettong (*Bettongia gaimardi*: Macropodidae). Journal of Zoology 212, 59-67.

Sapolsky, R.M., Romero, L.M. & Munck, A.U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocrine Reviews. 21, 55-89.

Seddon, P.J., Armstrong, D.P. & Maloney, R.F. (2007). Developing the science of reintroduction biology. Conservation Biology. 21, 303-312.

Sheriff, M.J., Dantzer, B., Delehanty, B., Palme, R. & Boonstra, R. (2011). Measuring stress in wildlife: techniques for quantifying glucocorticoids. Oecologia 166, 869-887.

Short, J. (1998). The extinction of rat-kangaroos (Marsupialia:Potoroidae) in New South Wales, Australia. Biological Conservation. 86, 365-377.

Shorthouse, D.J., Iglesias, D., Jeffress, S., Lane, S., Mills, P., Woodbridge, G., Mcintyre, S. & Manning, A.D. (2012). The 'making of' the Mulligans Flat – Goorooyarroo experimental restoration project. Ecological Management & Restoration. 13, 112-125.

Silverin, B. (1998). Behavioural and hormonal responses of the pied flycatcher to environmental stressors. Animal Behaviour. 55, 1411-1420.

Taylor, R.J. (1992). Seasonal-changes in the diet of the Tasmanian bettong (*Bettongia gaimardi*), a mycophagous marsupial. Journal of Mammalogy. 73, 408-414.

Teixeira, C.P., De Azevedo, C.S., Mendl, M., Cipreste, C.F. & Young, R.J. (2007). Revisiting translocation and reintroduction programmes: the importance of considering stress. Animal Behaviour. 73, 1-13.

VSN International. (2014). GenStat for Windows, 17th Edition, Hertfordshire, UK.

Wallis, I.R. (1994). The rate of passage of digesta through the gastrointestinal tracts of potoroine marsupials - more evidence about the role of the potoroine foregut. Physiological Zoology. 67, 771-795.

Woodford, M.H. (2000). Quarantine and health screening protocols for wildlife prior to translocation and release into the wild. Gland, Switzerland: IUCN/SSC Veterinary Specialist Group, Paris, France: the Office International des Epizooties, UK: Care for the Wild, and Switzerland: the European Association of Zoo and Wildlife Veterinarians.

Yoder, J.M., Marschall, E.A. & Swanson, D.A. (2004). The cost of dispersal: predation as a function of movement and site familiarity in ruffed grouse. Behavioral Ecology, 15, 469-476.

Paper V: Evaluating the behavioural effects of captivity to improve the outcomes of eastern bettong (*Bettongia gaimardi*) reintroductions

The risk to founders is often elevated during period immediately following release because their behaviour is effected in response to the translocation. The risks associated with undesirable behaviours are often expected to be greater in founders with pre-release captive experience. In this paper, I continue to build upon the body of empirical research investigating the effects of pre-release captivity on reintroduced eastern bettongs, focusing on the impacts on movement and nesting behaviour. I draw statistical comparisons using location and observational data to assess the behavioural implications of releasing founders with different levels of captive experience. As this paper includes an additional experimental group (captive-bred founders) the terminology used to describe the experimental groups differs from Papers III & IV.

Batson, W.G., Gordon, I.J., Fletcher, D.B. & Manning, A.D. (under review) Evaluating the behavioural effects of captivity to improve the outcomes of eastern bettong (Bettongia gaimardi) reintroductions. Wildlife Research.

Abstract

Reintroductions frequently fail because founders cannot become established post-release. Pre-release captivity is considered to reduce the probability of success primarily through behavioural adaptations that reduce founder performance. The behavioural implications of captivity are usually considered by comparing the performance of captive-bred and wild-bred founders, but many reintroductions also include a temporary period of captivity for wild founders which could influence their behaviour post-release. Behaviours are usually considered important when there is evidence that they impact on survival, reproduction or dispersal, but detecting these effects may require exposing founders to a level of risk (e.g. predation) which is inappropriate given their demographic, genetic and economic value. A preferable approach could be to initially conduct behavioural experiments during a low-risk reintroduction (e.g. within a fenced area), evaluate the sub-lethal effects, and use the results to develop reintroduction protocols for subsequent reintroductions of greater risk (e.g. outside a fence). Here we compare movement and nesting behaviours of three experimental treatment groups (1) wild-wild, (2) wild-captive-wild, (3) captive-wild of eastern bettong (*Bettongia gaimardi*) reintroduced to south-eastern Australia. Our results suggest that founders with pre-release captive experience tend to make wider exploratory movements and spend a greater proportion of the night active, which could increase predation vulnerability beyond-the-fence; but captive-bred founders tended to settle closer to the release-site over the medium-term. Temporary exposure to captivity also appeared to reduce the construction quality of nests which could further increase predation risk. Based on our results we recommend captivity is avoided where possible, except when dispersal from the release-site is considered the primary threat to establishment, and for our results to be used to guide reintroduction experiments beyond-the-fence.

Introduction

Reintroductions aim to re-establish self-sustaining populations of focal species within their native range (IUCN/SSC 2013), yet many fail, especially during the 'establishment phase' immediately following release (Armstrong and Seddon 2008; Bennett et al. 2012; Fischer and Lindenmayer 2000). Pre-release captivity is often implicated as having a detrimental effect on the probability of establishment and can exaggerate the severity of challenges faced by founders within their new environment (Letty et al. 2007). A common problem associated with captivity is that it induces behavioural adaptations that increase the vulnerability of founders post-release (Beck et al. 1994; Griffith et al. 1989; Jule et al. 2008). Developing a better understanding of the behavioural implications of captivity is critical to managing the detrimental effects which are often amplified by the small size of founder populations (Sutherland 1998). Evidence of behavioural responses can then be used to identify and develop tactics to reduce the risk of failure, and can also be used as parameters in systematic decision-making models (Batson et al. 2015c; Ebrahimi et al. 2015; Moseby et al. 2014).

The effects of captivity have been the subject of substantial research including many assessments of the performance of wild and captive founders (Fischer and Lindenmayer 2000; Griffith et al. 1989), which essentially evaluate the effect of multigenerational captivity (Mathews et al. 2005; McPhee 2004). However, many reintroductions require wild founders to be temporarily housed in captivity prior to release for purposes such as quarantine, which can influence post-release behaviour, ultimately affect establishment probabilities (Batson et al. 2015b; Calvete et al. 2005; DeGregorio et al. 2013; Devineau et al. 2011). Temporary captivity shares many characteristics (e.g. limited movement, supplementary resources, increased population density) of a 'delayed-release' which has been frequently tested and found to induce behavioural responses that influence reintroduction outcomes (Batson et al. 2015a; Mitchell et al. 2011; Richardson et al. 2015a). As

such, the effects of both forms of captivity need to be assessed and considered when developing reintroduction protocols.

Animal behaviour is primarily factored into the design of reintroduction protocols when there is evidence that it induces a population-level effect by influencing survival, reproduction or dispersal (Biggins et al. 1999; Bremner-Harrison et al. 2004). Gaining empirical evidence of these effects often requires the loss of individuals, or exposes founders to a level of risk that may be inappropriate given their demographic, economic and genetic value, especially when attempting to reintroduce species of high conservation concern. Therefore, a preferred approach could be to conduct behavioural experiments during low-risk reintroductions, and use the results to make predictions regarding their likely effects during reintroductions of greater risk (Mathews et al. 2005; Vickery and Mason 2003).

Mainland islands represent reserves protected by a barrier fence within which specific pests have been eradicated and, have become popular reintroduction sites in many regions of the world including Australia (Dickman 2012; Saunders and Norton 2001). The popularity of re-establishing populations within mainland islands is largely derived from the improved probability of success, when the founder population is released in the absence of introduced predators, namely foxes (*Vulpes Vulpes*) and cats (*Felis catus*) in Australia which represent the greatest threat to native fauna (Moseby et al. 2011; Short 2009; Woinarski et al. 2015). A common objective of reintroducing threatened species to mainland islands is to establish a viable source population for subsequent reintroductions *beyond-the-fence*, which refers to unfenced areas usually subject to active predator control management. However, the increased risk of failure can deter beyond-the-fence reintroductions, and can bring the conservation value of mainland islands into question (Scofield et al. 2011). A significant conservation benefit of reintroductions into mainland islands is to conduct experiments and exploit the release-site as an *outdoor laboratory* which provides a

level of control that is unattainable in an unfenced environment, without exposing founders to unnecessary risk (Kemp et al. 2015; Manning et al. 2011; Manning et al. 2015). This information can then be used to improve the probability of reintroduction success in subsequent reintroductions beyond-the-fence.

This study focuses on an experiment conducted during the reintroduction of eastern bettongs (*Bettongia gaimardi*) to Mulligans Flat Woodland Sanctuary (MFWS), a mainland island and outdoor laboratory in the Australian Capital Territory (ACT) (Manning et al. 2011; Shorthouse et al. 2012). This reintroduction represents the first attempt to re-establish eastern bettongs to mainland Australia after an absence of approximately 100 years, which highlights the need to undertake research to better understand the reintroduction biology of the species. More specifically, we assess the behavioural implications of variable experience with captivity prior to release by drawing comparison among three experimental treatments groups (1) 'captive-wild' incorporating captive-bred founders; (2) 'wild-captive-wild' incorporating wild founders temporarily housed in *ex-situ* captivity prior to release; (3) 'wild-wild' incorporating wild founders released without captivity. We focus on the effect on movement and nesting behaviors, and interpret the results according to the expected impact on the risk of predation, and the spatial disconnection among founders (e.g. no opportunity to breed due to distance between individuals) which is equivalent to mortality in terms of its effect of establishment. We also use relative behaviors to assess where captivity influenced post-release settlement.

This study builds upon previous studies conducted during this reintroduction that have suggested that temporary captivity, did not influence survival, female reproduction or body-mass post-release, despite increasing body-mass, and reducing pouch occupancy at release (Batson et al. 2015b). The absence of a population-level effect may reflect the favorable conditions at MFWS (e.g. absence of predators and competitors), which could mask sub-lethal effects which could carry

serious implications beyond-the-fence. For example, temporarily exposing the wild founders to captivity prior to release did influence their stress physiology over the medium-term post-release (Batson et al. in review). The central objective of these associated studies, is to accumulate a body of empirical evidence which can be used to guide future reintroduction methods, and develop new hypotheses to be experimentally tested.

Methods

Study species

Eastern bettong (also known as Tasmanian bettong) is a small, mainly solitary, nocturnal macropod marsupial (family: Macropodidae, e.g. kangaroos and wallabies) (Claridge et al. 2007). This species was once common throughout south-eastern Australia including the island of Tasmania, by suffered dramatic declines in range and numbers following European colonization and the introduction of mammalian predators including foxes and cats, and was extinct on the mainland by the 1920s (Burbidge and McKenzie 1989; Short 1998). Populations persist in Tasmania which is absent of foxes, and eastern bettongs are currently classified as 'near-threatened' in the IUCN Red-list (Menkhorst 2008). Eastern bettongs have large home-ranges relative to their body-size (males 47-85 ha, females 38-63 ha), and display *nomadic* nesting behaviour which includes the use of multiple nests which they build from vegetative material (e.g. bark, grass) collected from the surrounding area, with nests usually built within available structures (e.g. tussocks, coarse woody debris) (Claridge et al. 2007; Taylor 1993).

Study areas

Tidbinbilla Nature Reserve

Two populations were established in the ACT during this reintroduction. A captive population was established at Tidbinbilla Nature Reserve (TNR) which implements captive-breeding programs for a number of native species (www.tidbinbilla.act.gov.au). This population was the source of the captive-bred founders, and was the location of the captive period within the wild-captive-wild

release regime. Food (fruit, vegetables and kangaroo pellets) and water were provided daily. Bettongs were trapped at 3 month intervals for health and population monitoring. Four small enclosures (0.5-1 ha) were used for quarantine, to house excess males, and to trial equipment. Bettongs were housed in two large enclosures (2.6-9.4 ha) outside these times. The enclosures were not publicly accessible, and contained natural vegetation structures suitable for foraging and nesting. Group compositions within enclosures were manipulated to retain genetic diversity by ensuring breeding interactions between individuals from different collection regions in Tasmania.

Mulligans Flat Woodland Sanctuary

This study focusses on the second population established MFWS which is a 485 ha mainland island and outdoor laboratory located within a box-gum grassy woodland (www.mulligansflat.org.au, McIntyre et al. 2010). Foxes, cats and dogs (*Canis lupus*) are eradicated, and rabbits (*Oryctolagus cuniculus*) are intensively controlled (Shorthouse et al. 2012). MFWS is subject to a large-scale ecological experiment, with this reintroduction representing one of several experimental treatments (www.mfgowoodlandexperiment.org.au, Manning et al. 2011). Bettongs were not provided with supplementary food or water, and breeding interactions were not managed. For more detailed descriptions see Portas et al. (2014) and Batson et al. (2015b).

The translocation process, experimental groups, and post-release monitoring

Sixty bettongs (carrying 28 pouch young) were translocated from wild populations from five geographically isolated regions in Tasmania during 2011-12. Thirty-two of the translocated groups were originally included in the founder population at MFWS, with the remainder used as founders at TNR. This includes the 'immediate- and delayed-release' groups as described by Batson et al. (2015b, in review). However, in 2013 a cohort of 20 were transferred from TNR to MFWS to manage density at both sites. This cohort included 13 individuals born at TNR, and 7 of the group was transferred from Tasmania.

The wild-captive-wild group (5 M, 13 F) were housed at TNR for between 95-566 days prior to being released at MFWS. The duration of captivity varied due to the timing of collection and release events, the desire to synchronize the releases of the wild-wild and wild-captive-wild groups, and because two females released in 2013 were retrospectively included in this group to replace individuals with missing GPS-collar data. One wild-captive-wild female died prior to release. The wild-wild group (6 M, 10 F) was released directly into MFWS within 24 hours of capture in Tasmania, and the captive-wild group (2 M, 4 F) was transferred directly to MFWS from TNR.

Bettongs selected for translocation in Tasmania were mildly sedated, transported by air and road to TNR arriving within 18 hours of capture. Upon arrival, bettongs were anesthetized, given a health assessment, and allowed to recover (~ 1 hour). The wild-wild group was then driven to MFWS (~1 hour drive) and released within 24 hours of capture in Tasmania. The wild-captive-wild group were released into the small enclosures at TNR for 30 days quarantine, before being relocated to the large enclosures, and managed as part of the captive population until transfer to MFWS. As the captive-wild group were not translocated from Tasmania, they were trapped, driven to MFWS and released (total process < 4 hours). Prior to transfer to MFWS, the wild-captive-wild and captive-wild groups were anesthetized and received full health assessments akin to those undertaken on arrival from Tasmania.

Bettongs included within the three experimental groups were fitted with VHF (V5C_161C; Sirtrack, Hawkes Bay, New Zealand) or global positioning system (GPS)/VHF radio-collars (Q4000E; Telemetry Solutions, Walnut Creek, USA) prior to release. Collars included a mortality function activated after 12 hours without movement. The collars weighed between 28-32g which was < 2.5% of the weight of the lightest individual released. The GPS/VHF-collars were replaced

with a VHF-collars after 1 month, and VHF-collars were removed after 1 year. Founders were released from 10 randomly selected points within MFWS in mixed groups in an attempt to create an even spatial distribution. For a details regarding of the translocation process see Portas et al. (2014) and Batson et al. (2015b).

The initial cohorts of the wild-captive-wild group (2 M, 6 F) and wild-wild (4 M, 6 F) groups were released with VHF-collars and monitored using the VHF-method. This monitoring regime involved each individual being radio-tracked to their diurnal nest-site daily for the first 30 days at MFWS. To minimize disturbance the observer maintained a distance of 10m from the estimated nest location. If the bettong was flushed from the nest, the nest was immediately located, otherwise the location of the nest was estimated using triangulation, and marked using flags (Figure. 1). Once a bettong had moved nests the flags were used to locate the unoccupied nest. If the nest was confidently identified its location was recorded using a handheld GPS (Garmin: GPDMap 64); but if the nest could not be identified the estimated location was recorded as indicated by the flags. Identified nests were given a construction score of (1) nest located within a vegetative structure (e.g. tussock) without altering its form or adding foreign material (e.g. bark or dead grass); (2) nest located within a vegetative structure including altering its form (e.g. digging shallow indentation, pulling over tussock), but without adding foreign material; or (3) nest located within a vegetative structure by altering its form and by adding foreign material.

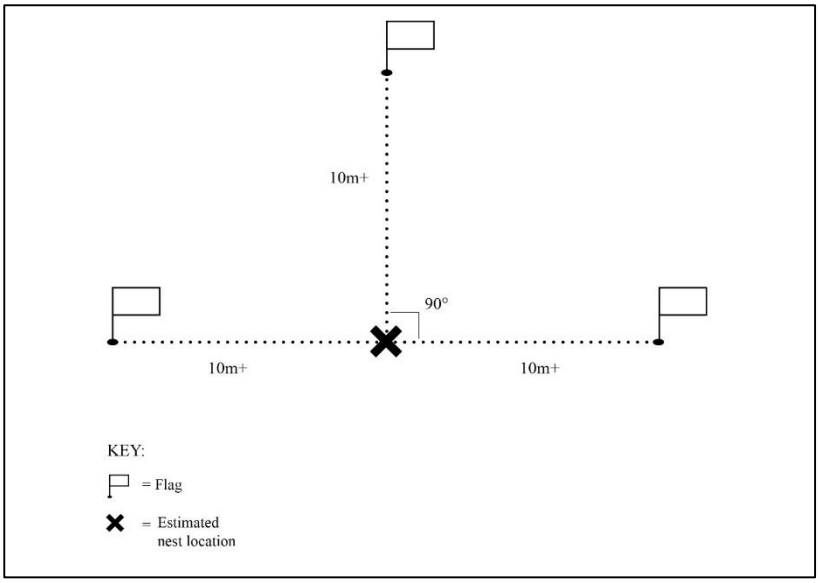

Figure 1. The flagging arrangement established by an observer using the VHF-method (involving radio-telemetry and triangulation) to estimate and mark the nest location for target founders daily for the initial 30 days post-release. Observers maintained 10m+ from the estimated nest to reduce disturbance. Following nest vacation, the flags were used to search for the nest. If the nest could not be confidently identified then the estimated location was recorded.

Seven of the wild-captive-wild group (5 F, 2 M), 6 of the wild-wild group (4 F, 2 M), and 6 captive-wild group (4 F, 2 M) were fitted with GPS-collars and monitored using the 'GPS-method'. The GPS-collars were programmed to record a fix at noon and every 20 minutes between sunset and sunrise across Period 1 (nights 1-5 post-release) and Period 2 (nights 20-24 post-release). Bettongs were not radio-tracked to their nest, but survival was monitored daily until the collars were removed.

Behavioural assessments

Movement behaviours

Activity was calculated for all three groups using GPS-method for nights within Periods 1 and 2. This measure represents the proportion of the night (release/sunset to sunrise) a bettong spent moving. Inactivity was assumed if the distances between consecutive fixes were < 20m for more than one continuous hour; with activity assumed outside these times.

Exploration was calculated for all three groups using GPS-method for nights within Periods 1 and 2. This measure represents the mean distance from the nightly start-point (release-site or nest) of the upper quartile of fixes from that point. This approach minimized any potential bias caused by fixes with significant error, and fixes from periods of inactivity were excluded.

Dispersal was calculated for all three groups using GPS-method and VHF-method for nights within Periods 1 and 2 which also included the 19th night. This measure represents the distance between a nest and that bettongs respective release-site. Nests were estimated from the GPS-method by averaging the x and y coordinates of fixes recorded while the bettongs was inactive within that nest.

Nesting behaviours

Nest occupancy was calculated for the wild-captive-wild and wild-wild groups using the VHF-method. This measure represents the mean number of nights nests were occupied during the monitoring period which was 30 days, except for two that died within that period.

Nest reuse was calculated for the wild-captive-wild and wild-wild groups using the VHF-method. This measure represents the number of consecutive or non-consecutive nights an individual reused a nest site.

Nest construction was calculated for the wild-captive-wild and wild-wild groups using the VHF-method. This measure represents the proportion of nest construction scores recorded during the monitoring period.

Statistical methods

All statistical analyses were conducted using the statistical package Genstat (VSN International. 2013) unless otherwise stated. Significance was assumed at $p \leq 0.05$. Prior to analysis any fixes recorded prior to release, with horizontal variation values > 10, and those more than 20 m outside the fence were excluded. The GPS-collars varied in performance (e.g. battery life), and nights with < 30 fixes from an individual were excluded for analysing *activity* and *exploration*.

Activity and *exploration* were analysed using Linear Mixed-Effect Models (LMM) with GROUP, PERIOD, GROUP*PERIOD and SEX, as fixed effects; BETTONG and NIGHT as random effects. Rainfall was estimated using data from the Australian Bureau of Meteorology weather station #070351 at Canberra Airport 15 km from MFWS (www.bom.gov.au), but was not included as a covariate rain (2+ mm) occurred on a single night. Instead, that night was excluded from the analysis which represented the 24th night for three of the wild-captive-wild group. Time at TNR, and release-site (nor microhabitat characteristics) were not included as covariates due to the small sample size. Normality of the response variable was assumed when skewness and kurtosis were between -1 and 1, and confirmed by a visual assessment of the distribution of residuals. The same model structure was used to analyse *dispersal* but COLLAR-TYPE was added included as a fixed effect.

Normality was achieved for *activity* using a reflected square-root transformation, *exploration* using a Log^{10} transformation, and *dispersal* using a square-root transformation. If SEX, PERIOD or COLLAR-TYPE were significant they were compared using a Student's T. Test with the assumption of equal variance based on the results of an F-Test. If GROUP or COLLAR-TYPE were significant they were compared using One-way Analysis of Variance (ANOVA) and a Tukey's HSD Tests. *Nest occupancy* and *nest reuse* were compared using randomization tests, and

nest construction via a Fisher's Exact 2 x 3 contingency table using the statistical package SPSS (IBM Corporation. 2013). A Fisher's Exact Test was used to test whether there was a difference in the ability to confidently identifying nests between the wild-captive-wild and wild-wild groups.

Results

The LMMs indicated that *activity* ($F_{1, 9} = 10.22$, $p = 0.012$) and *dispersal* ($F_{1, 16} = 103.47$, $p < 0.001$) were significantly affected by PERIOD; whereas, *exploration* was effected by GROUP ($F_{2, 14} = 5.03$, $p = 0.022$) and SEX ($F_{1, 14} = 9.56$, $p = 0.008$). Student's T. Tests ($t_{146} = -6.22$, $p < 0.001$) indicated that *activity* was lower during Period 1 ($\overline{x} = 0.69 \pm 0.18$ SD) than Period 2 ($\overline{x} = 83\% \pm 7$ SD); and that *dispersal* ($t_{361} = -3.69$, $p < 0.001$) was also lower in Period 1 ($\overline{x} = 563$m ± 447 SD) than Period 2 ($\overline{x} = 741$ m ± 472 SD). ANOVA supported the between group difference in *exploration* ($F_{2, 63} = 11.83$, $p < 0.001$), and the Tukey's HSD Test suggested that *exploration* was lower in the wild-wild group ($\overline{x} = 395$m ± 222 SD) than the wild-captive-wild ($\overline{x} = 581$m ± 186 SD) and captive-wild groups ($\overline{x} = 560$ m ± 377 SD). The Student's T. Test ($t_{158} = -5.34$, $p < 0.001$) indicated that *exploration* was greater in males ($\overline{x} = 649$m ± 306m SD) than females ($\overline{x} = 425 \pm$ 231 SD).

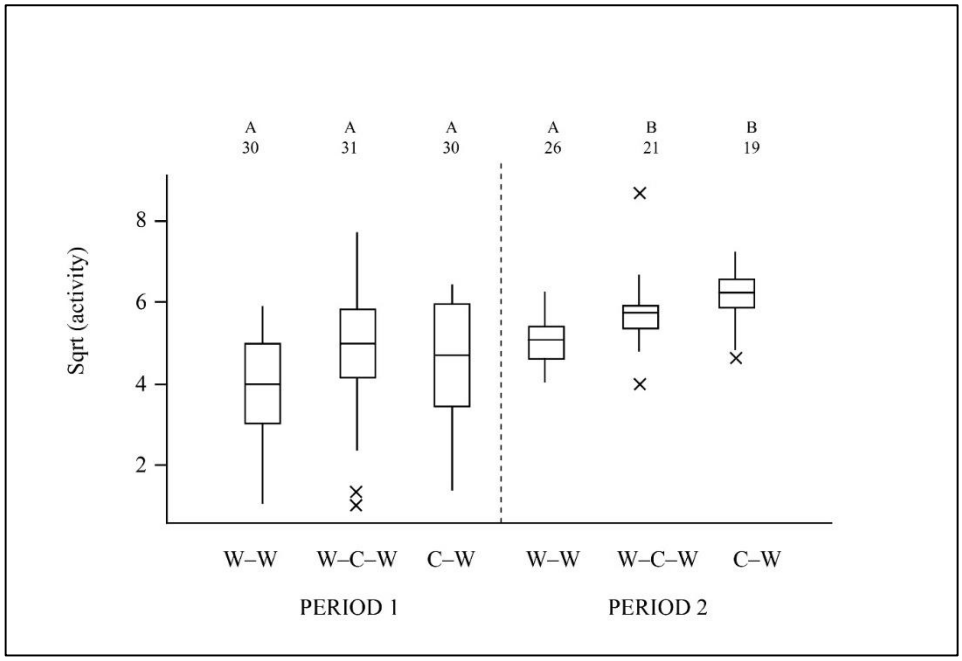

Figure 2. Activity was measured using the GPS-method for all three experimental groups: wild-wild (W-W), wild-captive-wild (W-C-W), captive-wild (C-W). Activity represents the percentage of the night (sunset/release-sunrise) the target founder moved around landscape. The number above bars represent sample size, and letters indicate significant between group differences within the respective period ($p \leq 0.05$). Period 1 represents nights 1-5 post-release, and Period 2 represents nights 20-24 post-release. 'x' indicate statistical outliers.

ANOVA indicated a between group difference in *activity* ($F_{2, 63} = 11.83$, $p < 0.001$), and *dispersal* ($F_{2, 179} = 4.85$, $p = 0.009$) during Period 2; whereas, *exploration* differed between the groups during Period 1 ($F_{2, 88} = 8.27$, $p < 0.001$) and Period 2 ($F_{2, 66} = 3.44$, $p = 0.038$). The Tukey's HSD Tests suggested that *activity* was lower in the wild-wild group ($\overline{x}$ = 78% ± 6 SD) than the wild-captive-wild ($\overline{x}$ = 84% ± 6 SD) and captive-wild groups ($\overline{x}$ = 87% ± 5 SD) during Period 2 (Figure. 2). *Exploration* was lower in the wild-wild group ($\overline{x}$ = 388m ± 258 SD) than the wild-captive-wild ($\overline{x}$ = 606m ± 203 SD) and captive-wild groups ($\overline{x}$ = 560m ± 347 SD) during Period 1, and lower in the wild-wild group ($\overline{x}$ = 403m ± 179 SD) than the wild-captive-wild group ($\overline{x}$ = 545 ± 157 SD) during Period 2 (Figure. 3). *Dispersal* was greater in wild-wild group ($\overline{x}$ = 853 m ± 531 SD), than the captive-wild group ($\overline{x}$ = 542 m ± 409 SD) during Period 2, with no statistical difference between the wild-captive-wild group ($\overline{x}$ = 656 m ± 363 SD) and the other groups (Figure. 4).

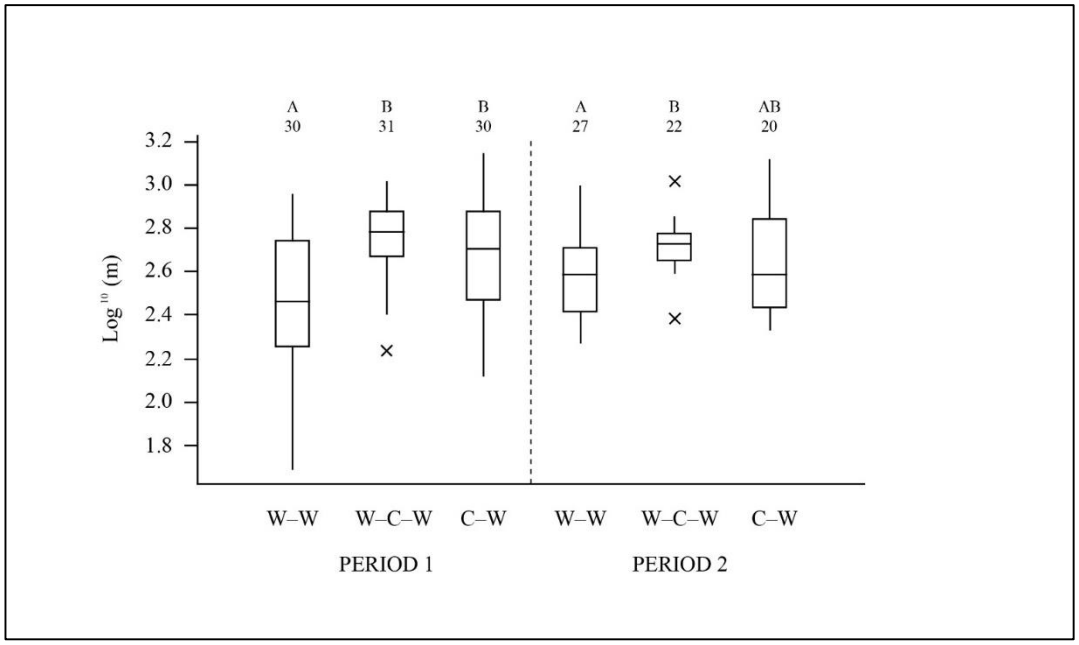

Figure 3. Exploration was measured using the GPS-method for all three experimental groups: wild-wild (W-W), wild-captive-wild (W-C-W), captive-wild (C-W). Exploration represents the mean distance of the upper quartile of fixes in terms of distance from start-point (release-site or nest-site) for target founders. The number above bars represent sample size, and letters indicate significant between group differences within the respective period ($p \leq 0.05$). Period 1 represents nights 1-5 post-release, and Period 2 represents nights 20-24 post-release. 'x' indicate statistical outliers.

The randomization tests found no difference in nest reuse ($\overline{x}$ = 4th night ± 2.6 SD, P = 0.85), or nest occupancy between groups ($\overline{x}$ = 3 nights ± 1 SD, P = 0.929). The Fisher's Exact Test indicated no difference between the groups in regard to the probability of identifying a nest (p = 0.88), but a significant difference in nest construction ($p < 0.001$). This reflected a tendency for the wild-wild group to add foreign material to their nests (55% of nests, observed in 90% of individuals); whereas, the wild-captive-wild group tended not to add foreign material (10% of nests, observed in 43% of individuals) (Figure. 5).

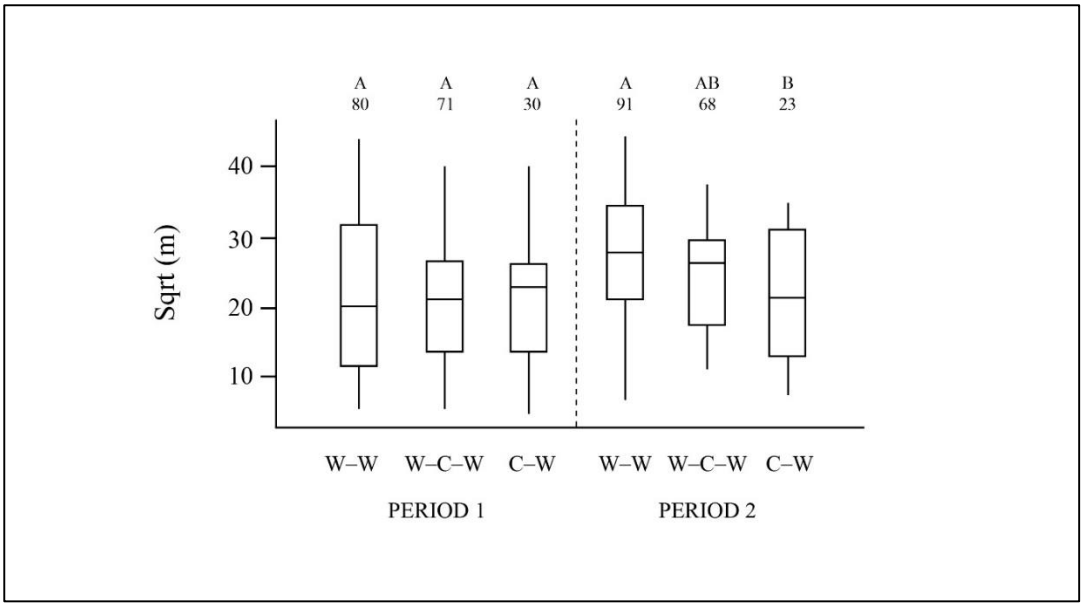

Figure 4. Dispersal was measured using the GPS-method for all three experimental groups: wild-wild (W-W), wild-captive-wild (W-C-W), captive-wild (C-W). Dispersal represents the distance between a founder's release-site and daily nest-site. The number above bars represent sample size, and letters indicate significant between group differences within the respective period ($p \leq 0.05$). Period 1 represents Nights 1-5 post-release, and Period 2 represents Nights 19-24 post-release.

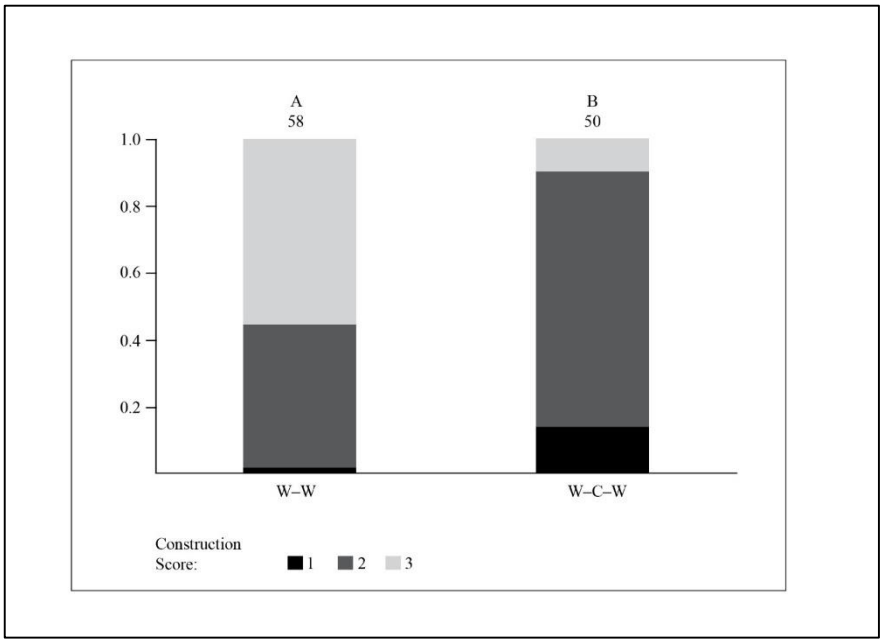

Figure 5. Nest construction was measured using the *VHF-method* for two experimental groups: wild-wild (W-W), wild-captive-wild (W-C-W). Nest construction represents the type of nests built by target founders during the initial 30 days post-release. Construction scores: 1 = nest located within a vegetative structure (e.g. tussock) without altering its form or adding foreign material (e.g. bark or dead grass), 2 = nest located within a vegetative structure including altering its form (e.g. digging shallow indentation, pulling over tussock), but without adding foreign material, 3 = nest located within a vegetative structure by altering its form and by adding foreign material. The number above bars represent sample size, and letters indicate significant difference in the ratios of the two groups ($p \leq 0.05$).

Discussion

Our results suggest that differences in captive experience can influence specific movement and nesting behaviours post-release for eastern bettongs. Despite the variations in post-release behaviour not inducing a direct impact on establishment at MFWS (a mainland island with reduced risk), they could carry serious implications for founder populations released beyond-the-fence. Therefore, we interpret the results according to their assumed effect on the risk of predation, spatial disconnection, and settlement. This study builds upon the substantial research assessing the effects of translocation, pre-release experience (including origin), behaviour and reintroduction success (Biggins et al. 1999; Blythe et al. 2015; Bright and Morris 1994). However, further experimentation is required to improve our understanding of these factors because the complexity of behavioural interactions confounds the ability to predict their effects (Kemp et al. 2015). Exploiting the opportunity to conduct reintroduction experiments within mainland islands reduces the risk to valuable individuals, and provides a level of experimental control that is unattainable in a wild setting (Manning et al. 2011; Manning et al. 2015).

Bettongs with pre-release captive experience (both groups) tended to display larger exploration movements than the wild-wild group across both periods assessed using the GPS-method. Increased movement is usually considered undesirable in the context of reintroduction because it increases the probability of encountering predators (Banks et al. 2002; Eastridge and Clark 2001; Moehrenschlager and Macdonald 2003; Warren et al. 1996). Therefore, we predict that founders released following permanent or temporary captivity may be more vulnerable to predation and this may have to be managed through the design of release protocols (e.g. increasing the number of founders, or predator avoidance training) (Batson et al. 2015c). A similar prediction could be made about sex-biased predation due to the increased movement of males, consistent with their larger home-range (Taylor 1993); however, this would contradict the higher mortality in female macropods during reintroductions, despite movements generally being larger in males (Richards and Short 2003; Short et al. 1992). Any difference in the risk between sexes may need to be

managed through sex-specific reintroduction protocols as recommended for other species including rabbits (Letty et al. 2000).

Pre-release captive experience seems to induce a similar, but weaker effect on activity which was significantly lower in the wild-wild group during Period 2. This absence of an effect during Period 1 reflects the substantial variability within each group immediately following release, suggesting that short-term responses are dependent on the individual. The lower rates of activity observed in Period 1 also suggest that the effects of the translocation were stronger immediately following release and lessened with time. Increased activity can be expected to increase the risk of predation by increasing the time spent outside nests that provide visual camouflage (Lima and Dill 1990; Martin et al. 2003; Zimmer et al. 2011). This behavioural shift is most likely to affect the threat of predation by nocturnal avian predators, including the sooty owl (*Tyto tenebricosa*) which known to prey upon bettongs (Bilney et al. 2010). As time-budgets essentially represent trade-offs among behavioural states, it is also possible that the apparent increase in activity in bettongs with captive experience could come at the expense of another important behaviour including rest or predator avoidance. Comparable changes to time-budgets have been observed in other translocated species including Przewalski's horse (*Equus ferus przewalskii*) which could be to the detriment of the welfare of the animal (Boyd 1998).

The wild-captive-wild group tended to display exploration and activity behaviours that were more similar to the captive-bred founders, than founders translocated directly from the wild. This suggests that temporarily exposing wild bettongs to captivity induces a behavioural adaptation within those individuals, which is similar to the adaptations that occur across the first generation of the captive population. Although the drivers of the behavioural adaptations are unclear, they could reflect an alteration in how founders perceive the risk of predation post-release. This theory is encapsulated by a change in the ‘landscape of fear’ which predicts that animal behaviour is

influenced by perceived risks, in addition to direct interactions with those threats (Laundre et al. 2001; Lima and Dill 1990; Manning et al. 2009). Another plausible explanation is that supplementary feeding in captivity had a negative impact on foraging capabilities and increased the time and space required to obtain resources. Captivity has been found to reduce foraging efficiency within wild individuals (DeGregorio et al. 2013), and within multigenerational captive populations which is frequently acknowledged as a cause of reduced reintroduction success (Beck et al. 1994; Kleiman 1989; Mathews et al. 2005).

Limiting dispersal can be vital to reintroduction success because long-range dispersal can be equivalent to mortality, and often requires specific tactics to manage its effects (Batson et al. 2015c; Le Gouar et al. 2012; Richardson et al. 2015b). Our results suggest that captive-bred founders may be less prone to dispersing away from the release-site. However, as the effect of the barrier fence on dispersal is difficult to assess, and the between group difference only became apparent during Period 2, dispersal may still need to be actively managed immediately following release to obtain this benefit especially given the ability of bettongs to move long distances within short periods (Taylor 1993). A reduction in dispersal has been observed in other captive-bred animals, including Apennine chamois (*Rupicapra pyrenaica*), which settle closer to the release-site than the wild-bred conspecifics over the long-term post-release (Bocci et al. 2014). If this trend is maintained beyond-the-fence then using captive-bred founders may be appropriate for small reintroduction-site with intensive predator control.

Animals invest nest building to obtain a range of benefits including thermoregulation and protection from predators (Gaskill et al. 2013). From our results, it appears that temporary captivity effected nest construction across the first month post-release. This may reflect the erosion of wild nesting behaviour at TNR, or could be associated with the increased body-mass of the wild-captive-wild group at release reducing their thermal insulation requirements (Batson et al.

2015b; Gaskill et al. 2013). Nocturnality and nest building in macropods presumably reflect evolutionary responses to a range of pressures including predation (Fisher et al. 2001; Withers et al. 2004). Therefore, we assume that the absence of added foreign material could increase predation, especially by diurnal raptors including wedge-tailed eagles (*Aquila audax*) (Richards and Short 1998; Withers et al. 2004). A similar effect has been observed in captive-bred greater stick-nest rats (*Leporillus conditor*) which build nests in less complex vegetation and suffer greater predation than wild conspecifics following translocation (Moseby et al. 2014).

Temporary captivity did not appear to influence nest reuse or nest occupancy which provide behavioural proxies for settlement in related species including burrowing bettong (*Bettongia lesueur*) (Moseby et al. 2014). However, these proxies may not be appropriate for eastern bettong due to their nomadic use of multiple nests (Taylor 1993). The potential increase in nest occupancy was evaluated as longer nest use could cause the accumulation of scent and increase detectability to olfactory predators including foxes and cats (Banks et al. 2002).

Based on the results across this study, we predict that captive experience could have a negative effect on predation avoidance in both captive and wild founders. We therefore, generally recommend for captivity to be avoided whenever possible for subsequent release beyond-the-fence as a precaution. A possible exception could be reintroductions into areas where dispersal is expected to be the primary threat to founders. The avoidance of captivity is consistent with the conclusions of Batson *et al.* (2015b, in review) based on physiological responses, and financial cost. However, this goes against the apparent increase in success when captive founders are used for macropods translocations, but this trend is more likely to reflect an inherence difference in the release-site rather than an intrinsic differences within the founders (e.g. captive macropods are more commonly used for reintroduction into mainland islands) (Clayton et al. 2014).

Despite our best efforts to develop precautionary recommendations, it is important to acknowledge that they are founded on imperfect knowledge. We therefore, also recommend that these predictions are tested in subsequent reintroductions and that strategic monitoring regimes are developed to enable robust evaluations. This information can then also be used to develop a better understanding regarding the interactions and over-riding effects of various factors on behavioural responses and reintroduction success. Whenever, founders are exposed to increased risk those responsible for the project must consider the tactical options available to mitigate those threats (Armstrong and Seddon 2008; Batson et al. 2015c). By assessing the sub-lethal effects within a low-risk environment it is possible to begin to manage many of the potential threats that founders will be exposed to beyond-the-fence without exposing highly valuable individuals to unnecessary risk.

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References

Armstrong DP, Seddon PJ (2008) Directions in reintroduction biology. Trends Ecology and Evolution. 23:20-25.

Banks PB, Norrdahl K, Korpimaki E (2002) Mobility decisions and the predation risks of reintroduction. Biological Conservation. 103:133-138.

Batson WG, Abbott R, Richardson KM (2015a) Release strategies for fauna reintroductions: theory and tests. In: Armstrong DP, Hayward MW, Moro D, Seddon PJ (eds) Advances in Reintroduction Biology of Australian and New Zealand Fauna. CSIRO Publishing, Clayton South, Australia, pp 7-16

Batson WG, Gordon IJ, Fletcher DB, Manning AD (2015b) The effect of pre-release captivity on post-release performance in reintroduced eastern bettongs (*Bettongia gaimardi*). Oryx FirstView:1-10.

Batson WG, Gordon IJ, Fletcher DB, Manning AD (2015c) Translocation tactics: A framework to support the IUCN Guidelines for wildlife translocations and improve the quality of applied methods. Journal of Applied Ecology. Early View:1-10.

Batson WG, Gordon IJ, Fletcher DB, Portas T, Manning AD (in review) The effect of pre-release captivity on the stress physiology of a reintroduced population of wild eastern bettongs. Animal Conservation.

Beck BB, Rapaport LG, Price MRS, Wilson AC (1994) Reintroduction of captive-born animals. In: Olney PJS, Mace GM, Feistner ATC (eds) Creative Conservation. Chapman & Hall, London, UK, pp 265-286

Bennett VA, Doerr VAJ, Doerr ED, Manning AD, Lindenmayer DB (2012) The anatomy of a failed reintroduction: a case study with the Brown Treecreeper. Emu 112:298-312.

Biggins DE, Vargas A, Godbey JL, Anderson SH (1999) Influence of prerelease experience on reintroduced black-footed ferrets (*Mustela nigripes*). Biological Conservation. 89:121-129.

Bilney RJ, Cooke R, White JG (2010) Underestimated and severe: Small mammal decline from the forests of south-eastern Australia since European settlement, as revealed by a top-order predator. Biological Conservation. 143:52-59.

Blythe RM, Smyser TJ, Johnson SA, Swihart RK (2015) Post-release survival of captive-reared Allegheny woodrats. Animal Conservation. 18:186-195.

Bocci A, Menapace S, Alemanno S, Lovari S (2014) Conservation introduction of the threatened Apennine chamois *Rupicapra pyrenaica ornata*: post-release dispersal differs between wild-caught and captive founders. Oryx. FirstView:1-6.

Boyd L (1998) The 24-h time budget of a takhi harem stallion (*Equus ferus przewalskii*) pre- and post-reintroduction. Applied Animal Behaviour Science 60:291-299.

Bremner-Harrison S, Prodohl PA, Elwood RW (2004) Behavioural trait assessment as a release criterion: boldness predicts early death in a reintroduction programme of captive-bred swift fox (*Vulpes velox*). Animal Conservation. 7:313-320.

Bright PW, Morris PA (1994) Animal translocation for conservation - performance of dormice in relation to release methods, origin and season. Journal of Applied Ecology. 31:699-708.

Burbidge AA, McKenzie NL (1989) Patterns in the modern decline of western Australia's vertebrate fauna: Causes and conservation implications. Biological Conservation. 50:143-198.

Calvete C, Angulo E, Estrada R, Moreno S, Villafuerte R (2005) Quarantine length and survival of translocated European wild rabbits. Journal of Wildlife Management. 69:1063-1072.

Claridge AW, Seebeck J, Rose RW (2007) Bettongs, potoroos and the musky rat-kangaroo. CSIRO Publishing, Collingwood, Australia.

Clayton JA, Pavey CR, Vernes K, Tighe M (2014) Review and analysis of Australian macropod translocations 1969-2006. Mammal Review. 44:109-123.

DeGregorio BA, Weatherhead PJ, Tuberville TD, Sperry JH (2013) Time in captivity affects foraging behavior of ratsnakes: implications for translocation. Herpetological Conservation and Biology 8:581-590.

Devineau O, Shenk TM, Doherty PF, White GC, Kahn RH (2011) Assessing release protocols for Canada lynx reintroduction in Colorado. Journal of Wildlife Management. 75:623-630.

Dickman C (2012) Fences or Ferals? Benefits and Costs of Conservation Fencing in Australia. In: Somers MJ, Hayward M (eds) Fencing for conservation: Restriction of evolutionary potential or a riposte to threatening processes? Springer, New York, pp 43-63.

Eastridge R, Clark JD (2001) evaluation of 2 soft-release techniques to reintroduce black bears. Wildlife Society Bulletin. 29:1163-1174.

Ebrahimi M, Ebrahimie E, Bull CM (2015) Minimizing the cost of translocation failure with decision-tree models that predict species' behavioral response in translocation sites. Conservation Biology. 29:1208-1216.

Fischer J, Lindenmayer DB (2000) An assessment of the published results of animal relocations. Biological Conservation. 96:1-11.

Fisher DO, Owens IPF, Johnson CN (2001) The ecological basis of life history variation in marsupials. Ecology 82:3531-3540.

Gaskill BN, Gordon CJ, Pajor EA, Lucas JR, Davis JK, Garner JP (2013) Impact of nesting material on mouse body temperature and physiology. Physiology and Behaviour. 110:87-95.

Griffith B, Scott JM, Carpenter JW, Reed C (1989) Translocation as a species conservation tool - Status and strategy. Science 245:477-480.

IBM Corp. (2013) IBM SPSS Statistics for Windows, Version 22.0. New York.

IUCN/SSC (2013) Guidelines for reintroductions and other conservation translocations. IUCN Species Survivial Commission, Gland, Switzerland.

Jule KR, Leaver LA, Lea SEG (2008) The effects of captive experience on reintroduction survival in carnivores: A review and analysis. Biological Conservation. 141:355-363.

Kemp L, Norbury G, Groenewegen R, Comer S (2015) The roles of trials and experiments in fauna reintroduction programs In: Armstrong DP, Hayward MW, Moro D, Seddon PJ (eds) Advanves in reintroduction biology of Australian and New Zealand fauna. CSIRO Publishing, Clayton South, Australia, pp 73-90

Kleiman DG (1989) Reintroduction of captive mammals for conservation. BioScience 39:152-161.

Laundre JW, Hernandez L, Altendorf KB (2001) Wolves, elk, and bison: reestablishing the "landscape of fear" in Yellowstone National Park, USA. Canadian Journal of Zoology. 79:1401-1409.

Le Gouar P, Mihoub J, Sarrazin F (2012) Dispersal and habitat selection: Behavioral and spatial constraints for animal translocations In: Ewen J, Armstrong DP, Parker KA, Seddon PJ (eds) Reintroduction biology: Integrating science and management. Blackwell Publishing Ltd., Oxford, UK, pp 138-164

Letty J, Marchandeau S, Aubineau J (2007) Problems encountered by individuals in animal translocations: Lessons from field studies. Ecoscience 14:420-431.

Letty J, Marchandeau S, Clobert J, Aubineau J (2000) Improving translocation success: an experimental study of anti-stress treatment and release method for wild rabbits. Animal Conservation. 3:211-219.

Lima SL, Dill LM (1990) Behavioral decisions made under the risk of predation - A review and prospectus. Canadian Journal of Zoology 68:619-640.

Manning A, Wood J, Cunningham R, McIntyre S, Shorthouse D, Gordon I, Lindenmayer D (2011) Integrating research and restoration: the establishment of a long-term woodland experiment in south-eastern Australia. Australian Zoology 35:633-648.

Manning AD, Eldridge DJ, Jones CG (2015) Policy implications of ecosystem engineering for multiple ecosystem benefits. In: Armstrong DP, Hayward MW, Moro D, Seddon PJ (eds) Advances in reintroduction biology of Australian and New Zealand fauna. CSIRO Publishing, Clayton South, Australia, pp 167-184

Manning AD, Gordon IJ, Ripple WJ (2009) Restoring landscapes of fear with wolves in the Scottish Highlands. Biological Conservation 142:2314-2321.

Martin J, Lopez P, Cooper WE (2003) When to come out from a refuge: Balancing predation risk and foraging opportunities in an alpine lizard. Ethology 109:77-87.

Mathews F, Orros M, McLaren G, Gelling M, Foster R (2005) Keeping fit on the ark: assessing the suitability of captive-bred animals for release. Biological Conservation. 121:569-577.

McIntyre S et al. (2010) Biomass and floristic patterns in the ground layer vegetation of box-gum grassy eucalypt woodland in Goorooyarroo and Mulligans Flat Nature Reserves, Australian Capital Territory. Cunninghamia 11: 319-357.

McPhee ME (2004) Generations in captivity increases behavioral variance: considerations for captive breeding and reintroduction programs. Biological Conservation .115:71-77.

Menkhorst P (2008) *Bettongia gaimardi.* In: IUCN Red List of Threatened Species.

Mitchell AM, Wellicome TI, Brodie D, Cheng KM (2011) Captive-reared burrowing owls show higher site-affinity, survival, and reproductive performance when reintroduced using a soft-release. Biological Conservation. 144:1382-1391.

Moehrenschlager A, Macdonald DW (2003) Movement and survival parameters of translocated and resident swift foxes *Vulpes velox.* Animal Conservation 6:199-206.

Moseby KE, Hill BM, Lavery TH (2014) Tailoring release protocols to individual species and sites: One size does not fit all. PLoS One 9:1-12.

Moseby KE, Read JL, Paton DC, Copley P, Hill BM, Crisp HA (2011) Predation determines the outcome of 10 reintroduction attempts in arid South Australia. Biological Conservation. 144:2863-2872.

Portas T et al. (2014) Health evaluation of free-ranging eastern bettongs (*Bettongia gaimardi*) during translocation for reintroduction in Australia. Journal of Wildlife Diseases. 50:210-223.

Richards JD, Short J (1998) Wedge-tailed eagle (*Aquila audax*) predation on endangered mammals and rabbits at Shark Bay, Western Australia. Emu 98:23-31.

Richards JD, Short J (2003) Reintroduction and establishment of the western barred bandicoot *Perameles bougainvill*e (Marsupialia : Peramelidae) at Shark Bay, Western Australia. Biological Conservation. 109:181-195.

Richardson K, Castro IC, Brunton DH, Armstrong DP (2015a) Not so soft? Delayed release reduces long-term survival in a passerine reintroduction. Oryx 49:535-541.

Richardson KM, Doerr V, Ebrahimi M, Lovegrove TG, Parker KA (2015b) Considering dispersal in reintroduction and restoration planning. In: Armstrong DP, Hayward MW, Moro D, Seddon PJ (eds) Advances in reintroduction biology of New Zealand and Australian fauna. CSIRO Publishing, Clayton South, Australia, pp 59-72

Saunders A, Norton DA (2001) Ecological restoration at mainland islands in New Zealand. Biological Conservation. 99:109-119.

Scofield RP, Cullen R, Wang M (2011) Are predator-proof fences the answer to New Zealand's terrestrial faunal biodiversity crisis? New Zealand Journal of Ecology. 35:312-317.

Short J (1998) The extinction of rat-kangaroos (Marsupialia:Potoroidae) in New South Wales, Australia. Biological Conservation. 86:365-377.

Short J (2009) The characteristics and success of vertebrate translocations within Australia. Australian Government Department of Agriculture, Fisheries and Forestry, Canberra, Australia.

Short J, Bradshaw SD, Giles J, Prince RIT, Wilson GR (1992) Reintroduction of macropods (Marsupialia, Macropodoidea) in Australia - A review. Biological Conservation 62:189-204.

Shorthouse DJ et al. (2012) The 'making of' the Mulligans Flat – Goorooyarroo experimental restoration project. Ecological Management and Restoration. 13:112-125.

Sutherland WJ (1998) The importance of behavioural studies in conservation biology. Animal Behaviour. 56:801-809.

Taylor RJ (1993) Home range, nest use and activity of the Tasmanian bettong, *Bettongia gaimardi*. Wildlife Research. 20:87-95.

Vickery SS, Mason GJ (2003) Behavioral persistence in captive bears: Implications for reintroduction. Ursus 14:35-43.

VSN Inter. (2013) GenStat for Windows. 16th Edition. Hemel Hempstead, UK

Warren CD, Peek JM, Servheen GL, Zager P (1996) Habitat use and movements of two ecotypes of translocated caribou in Idaho and British Columbia. Conservation Biology 10:547-553.

Withers PC, Cooper CE, Buttemer WA (2004) Are day-active small mammals rare and small birds abundant in Australian desert environments because small mammals are inferior thermoregulators? Australian Mammalogy 26:117-124

Woinarski JCZ, Burbidge AA, Harrison PL (2015) Ongoing unraveling of a continental fauna: Decline and extinction of Australian mammals since European settlement. Proceedings of the National Academy of Science. 112:4531-4540.

Zimmer C, Boos M, Bertrand F, Robin JP, Petit O (2011) Behavioural adjustment in response to increased predation risk: A study in three duck species. PLoS ONE 6:9.

Appendix I: Reintroduction of eastern bettong to a critically endangered woodland habitat in the Australian Capital Territory, Australia.

This paper presents the ACT bettong reintroduction project as a case-study to be included in the next edition of the IUCN Global Reintroduction Perspectives Series. It is included as an appendix as it is non-science focused, but provides valuable background information regarding the overall project.

Batson, W.G., Fletcher, D.B, Portas, T., Crisp, H., Ryan, S., Wimpenny, C., Gordon, I.J. and Manning, A.D. (Accepted). Reintroduction of eastern bettong to a critically endangered woodland habitat in the Australian Capital Territory, Australia. IUCN Global Reintroduction Perspectives Series.

Introduction

The eastern (or Tasmanian) bettong (*Bettongia gaimardi*) is a 1–2 kg mycophagous marsupial. Once common throughout south-eastern Australia, the species went extinct on the mainland by the 1930s due to fox (*Vulpes vulpes*) and cat (*Felis catus*) predation, habitat modification and human persecution (Short, 1998). Wild populations are now restricted to eastern Tasmania, and the species is listed as 'near-threatened' by the IUCN (Menkhorst, 2008). This reintroduction was intended to re-establish bettongs on mainland Australia to stock future reintroductions. Two populations were established in the Australian Capital Territory (ACT), one as part of a captive breeding programme at Tidbinbilla Nature Reserve (TNR) (http://www.tidbinbilla.act.gov.au), and one as a wild population within the fox and cat free Mulligans Flat Woodland Sanctuary (MFWS) (http://www.mulligansflat.org.au). The MFWS is part of a larger woodland restoration project which aims to restore ecological function to a critically endangered woodland ecosystem, including research focused on the species' role as an 'ecosystem engineer' (Manning, Wood, Cunningham et al., 2011, Shorthouse, Iglesias, Jeffress et al., 2012, http://www.mfgowoodlandexperiment.org.au). The reintroduction was undertaken through a partnership between the ACT Government, the Australian National University, CSIRO, and the James Hutton Institute; with support from the Tasmanian Government, the Australian Research Council and the Capital Woodland and Wetlands Conservation Trust.

Goals & success indicators

Goal 1: Establish two geographically isolated, healthy and genetically diverse populations in the ACT to provide a sustainable source for future reintroductions on the mainland, and provide insurance in case of further declines in Tasmania.

Goal 2: Develop trapping and translocation protocols that minimize the risks to source population, and maximises the probability of long-term persistence in reintroduced populations.

Goal 3: Research the behavioural and biological responses to different reintroduction techniques and environmental conditions.

Goal 4: Research the species' ecological function as an ecosystem engineer derived through its foraging and digging behaviours.

Goal 5: Capture and maintain the genetic diversity present in the wild Tasmanian populations, whilst maintaining wild behaviours.

Indicator 1: 75% survival rate of adults and pouch-young from acquisition in Tasmania to their arrival in the ACT.

Indicator 2: A 75% adult survival during the initial 3 months post-release, and 20% per annum thereafter.

Indicator 3: Reproductive activity in all surviving females within 6 months of release.

Indicator 4: Population growth within both populations (no time limit placed on this due to the use of multiple translocation events over a prolonged period).

Indicator 5: Maintenance of 95% of the genetic diversity present in founder population in both reintroduced populations after 2 generations.

Description of main stages of re-introduction project

Feasibility

As predation was recognised as the primary threat to reintroduction success, this project was initiated following the construction of the fox, cat and rabbit proof fence, and the eradication of foxes and cats from MFWS in 2009. The eastern bettong was selected as a priority species, due to its function as an ecosystem engineer, and the environmental suitability of habitat. The subfossil record confirmed historic accounts that this species was previously present in the ACT. Environmental suitability was assessed through bioclimatic modelling and expert opinion. The arrangements for the project commenced in August 2010 when contact was established between

the ACT's Conservation Research Unit, and the Tasmanian Department of Primary Industries, Parks, Water and Environment (DPIPWE). A license to undertake a sedation trial was granted in April 2011, then successive licenses for a trial translocation, and each collection trip until a total of 60 adults were translocated from Tasmania. Suitable source populations were selected from outside nature reserves and national parks. To minimize the impact on source populations, the number of bettongs taken from any site was never more than one third of the number trapped. The trapping was targeted in five regions separated by geographic barriers. This protocol was based on a previous genetic study by DPIPWE that indicated some genetic differentiation either side of major rivers and between northern and southern Tasmania.

Implementation

In May 2011 a sedation trial was undertaken with four individuals to determine an appropriate dosage of the benzodiazepine diazepam for transportation. The aim was to establish a level of sedation that calmed the animal to reduce its flight response, whilst avoiding excessive sedation e.g. unconsciousness and the risk of an occluded airway. The bettongs used in the sedation trial were returned to the point of capture. In July 2011 three bettongs were translocated from Tasmania to the ACT to trial the translocation protocols. Once the translocation protocols were approved, an additional 57 individuals were translocated over three events between October 2011 and September 2012. In total, 60 adults (19 Male, 41 Female) and 28 pouch-young were translocated to the ACT. As this species is known to readily throw large pouch young when stressed, females observed to be carrying furred pouch young were excluded from the translocation. Females with an elongated teat were also excluded due to the likelihood that they had a dependent young-at-foot which was not trapped. Twenty-eight of the adults were housed permanently at TNR (captive group), 16 were temporarily housed at TNR for between 95 to 345 days before being transferred to MFWS (delayed-release group), and 16 were released directly into MFWS within 24 hours of initial capture (immediate-release group). Twenty adults were also transferred from the captive group to MFWS during 2013 to manage the population density at TNR, and increase population

growth at MFWS. The captive group and the delayed-release group underwent a 30 day quarantine period at TNR remote from other animals. All individuals underwent anaesthesia for complete health evaluation and disease screening upon arrival in the ACT. At TNR, all individuals were provided with their daily requirements of food and water, and mating interactions are controlled to ensure genetic mixing among individuals from the five collection areas. At MFWS the population received no supplementary resources, and mating interactions were not controlled.

Post-release monitoring

At TNR: Capture events are scheduled every 3 months for each individual to conduct full health and physiological assessments. All founders were monitored using remote cameras when released at TNR to conduct behavioural assessments and to test protocols and equipment. Any new animals encountered are pit-tagged, and DNA samples are taken for genetic analysis. In November, 2014 the population at TNR was estimated to be 51 individuals.

At MFWS: With the exception of one individual, every founder was fitted with a VHF or GPS/VHF radio-collar when released, and these were removed at approximately 1 year post-release. The remaining individual was not collared due to a neck injury. Each founder was monitored daily for the first 30 days, and then at least weekly until the collar was removed to evaluate survival using the radio-collar's mortality function. Each founder was scheduled to be trapped a 1, 3, 6, 9 and 12 months post-release and given full health and physiological assessments; however, the actual timing of these events varied due to logistic constraints. Faecal and hair samples were collected during health assessments for dietary and hormonal analyses (e.g. cortisol). Following the removal of all of the collars the population will be monitored at least annually using Capture-Mark-Recapture. Any new animals encountered are pit-tagged, and DNA samples are taken for genetic analysis. In November, 2014 the population at MFWS was estimated to be 179 individuals.

The DNA samples taken from both populations are being analysed to assess genetic diversity and genetic progression.

Major difficulties faced

- Two pouch-young died after being evicted from the pouch either in the trap, or during trapside handling in Tasmania. The risk to the pouch-young was significantly reduced through changes to trapping protocols such as clearing traps before midnight, and approaching the trap rapidly. Four additional adults died within 1 month of release at MFWS due to pre-existing health conditions or misadventure with radio collars. The design of the collars was modified in-house to reduce the risk of future collar in response to these incidences misadventure.

- Lower than expected capture rates at certain locations in Tasmania. This was attributed to lower than expected population densities at these locations. This impacted on the ability to obtain the desired number of founders especially given one third harvesting rule, the exclusion of females with large pouch-young and young-at-foot, and the desired 2:1 sex-ratio. We improved the efficiency of subsequent events by undertaking prospective surveys.

- Difficulty designing and fitting radio-collars that did not cause injury or interfere with foraging ability. Multiple prototypes were tested at TNR to identify a suitable design and fitting method.

- Logistic difficulties relating to the translocation of wildlife interstate. Obtaining the relevant approvals and licenses was a lengthy process and required a long lead-in time for the project.

- Releasing bettongs at MFWS impacted on other on-site management activities at MFWS. For example, the presence of bettongs made broad-scale poisoning and trapping unacceptable options for controlling rabbits and resulted in the use of less cost efficient methods.

Major lessons learned

- Baseline health and disease data were determined for this species and can be used for the conservation management of the source and translocated populations. Administration of diazepam at 1 mg/kg appeared to effectively mitigate the effects of capture myopathy.

- Trapping, transport and monitoring protocols must be specifically designed, and tested within an adaptive and experimental frameworks. Without pre-release trials the probability of success would have been substantially reduced. Many of these trials would not have been possible without access to the captive facilities at TNR. All individuals fitted with radio collars must be regularly captured to reduce the risk of injury.

- The probability of successful establishment is high when this species is released into suitable, fenced and predator-free environments following the protocols developed during this project. The risk of inbreeding can be considered low given the high rates of pouch-occupancy, and lack of genetic assortment at MFWS.

- Uninjured pouch-young can be successfully taped back into the pouch, or alternatively hand-raised and returned to the wild following a pouch-eviction. Wild founders can also perform favourably when released after a temporary period in captivity for quarantine.

- Wild-sourced bettongs assimilate well into captivity, but with supplementary feeding captive bettongs have shown a tendency to become overweight. Quantity of food, animal condition and stress needs to be monitored as it may impact on the breeding success.

Success of re-introduction project

Verdict: Highly successful

Reasons for success

- All indicators of success relating to survival and reproduction were met or exceeded in both populations. This can be attributed to the development and testing of management protocols with adaptive and experimental frameworks. As of November 2014 the ACT population was estimated to be 230.

- The successful establishment of population at MFWS indicates that the habitat at the site can be considered as high quality for this species. The environmental characteristics that are assumed to have contributed to success include the absence of foxes and cats, and the abundance and diversity of vegetation and mycorrhiza.

- The successful collaboration of multiple stakeholders including government, academic and community organisations. The group also included experts from diverse array of disciplines including scientist, wildlife veterinarians, captive breeders, and environmental

practitioners. Those involved shared a willingness to adopt adaptive approaches to problem-solving which was critical to success.

- Housing animals in specialized captive facilities enabled quarantine, and equipment trials to be conducted within a controlled environment before conducting large translocations and releases into the unmanaged site. This reduced the risk of post-release mortality and disease/pathogen/parasite co-introductions.

References

Manning, A., Wood, J., Cunningham, R., McIntyre, S., Shorthouse, D., Gordon, I. , Lindenmayer, D. (2011). Integrating research and restoration: the establishment of a long-term woodland experiment in south-eastern Australia. Australian Zoologist 35, 633.

Menkhorst, P. (2008). *Bettongia gaimardi.* In IUCN 2014. IUCN Red List of Threatened Species. Version 2014.1.

Short, J. (1998). The extinction of rat-kangaroos (Marsupialia:Potoroidae) in New South Wales, Australia. Biological Conservation 86, 365.

Shorthouse, D. J., Iglesias, D., Jeffress, S., Lane, S., Mills, P., Woodbridge, G., McIntyre, S. , Manning, A. D. (2012). The 'making of' the Mulligans Flat – Goorooyarroo experimental restoration project. Ecological Management & Restoration 13, 112.

Appendix II: Changes to eastern bettong *bettongia gaimardi* health parameters following reintroduction to the Australian mainland.

Reintroduction carry obvious health and welfare implications for founders. Health assessments can also be used as a viable alternative to assess reintroduction outcomes, especially when more traditional measures (e.g. survival) are inaccessible, or uninformative. This paper, was led by the project veterinarian Dr Portas. It present the results of a range of blood chemistry and other health variables used to draw comparison within individuals pre- and post-release to evaluate the responses to translocation, and between the two reintroduced populations in the ACT. This paper is included as an appendix as I was not the main researcher, but I was involved in many aspects of its development. It is relevant to the main research theme that runs throughout this thesis, as it highlights a value of developing alternative measures to assess the outcomes of low-risk reintroductions.

Portas, T.J, Cunningham, R.B., Spratt, D., Devlin, J., Holz, P., Batson, W.G., Owens, J, and Manning, A.D. (under review). Changes to eastern bettong bettongia gaimardi health parameters following reintroduction to the Australian mainland. Oryx.

Abstract

The eastern bettong *Bettongia gaimardi*, a potoroid marsupial, has been extinct on the Australian mainland for approximately 100 years. Recently 60 adult bettongs were reintroduced from the island of Tasmania to two predator-free fenced reserves on mainland Australia. We examined baseline health parameters (body weight, haematology and biochemistry, parasites and infectious disease exposure) in a subset of 30 (13 male, 17 female) bettongs at translocation and again at 12–24 post-reintroduction. The mean body weight of bettongs increased significantly post-reintroduction but there were no significant differences between weights of bettongs at the two reintroduction sites or between sexes in response to reintroduction. Differences were evident in multiple haematological and biochemical variables post-reintroduction but there were few differences between the two reintroduced populations or between sexes in response to reintroduction. Ectoparasite assemblages differed with five of 13 ectoparasites failing to persist while an additional four species were identified post-reintroduction. Post-reintroduction none of the bettongs had detectable antibodies to the alphaherpesviruses Macropodid herpesvirus 1 and 2, including one individual that was seropositive at translocation. Similarly, the novel gammaherpesvirus potoroid herpesvirus 1 was not detected by PCR in any of the bettongs post-reintroduction, including one individual that was PCR-positive at translocation. None of the bettongs had detectable antibodies to *Toxoplasma gondii* at either time point. Our data demonstrate changing baseline health parameters in eastern bettongs following reintroduction to the Australian mainland, are suggestive of improved health in the reintroduced populations, and provide additional metrics for assessing the response of macropodoids to reintroduction.

Introduction

The underlying health status of individual animals can influence survival during translocation and establishment in reintroduction programs and disease may influence the persistence of populations in the longer term (Cabezas, Calvete & Moreno, 2011; Kock, Soorae & Mohammed, 2007; Clarke *et al*., 2013). Additionally disease has the potential to impact sympatric species at reintroduction sites and detailed recommendations for assessing and managing disease risk in wildlife translocations have been published (Leighton, 2002; Travis *et al*. 2006; Jakob-Hoff *et al*., 2014). Despite this, comprehensive health evaluations are not routinely undertaken and the potential for disease to influence outcomes is often not considered nor adequately managed in reintroduction programs (Deem *et al*., 2012; Matthews *et al*., 2006). Various health parameters in free-ranging wildlife species, including hematology and biochemistry and parasite assemblages, are influenced by a range of environmental and host factors and could be expected to change when species are reintroduced to environments from which they have been extirpated (Schultz et al., 2011; Robert & Schwanz, 2013; Webster *et al*., 2014). Evaluation of baseline health parameters pre- and post-reintroduction could potentially be used to assess the longer term physiological response of species to reintroduction and may be a useful adjunct to traditional metrics (survival, dispersal and reproductive success) used to assess reintroduction outcomes (Ewen *et al*., 2012; Nichols & Armstrong, 2012; Maceda-Veiga *et al*., 2015).

The eastern bettong *Bettongia gaimardi* is a small, nocturnal, predominantly mycophagous, potoroid marsupial that has been extinct on the Australian mainland for approximately 100 years (Claridge, Seebeck & Rose, 2007). Recently 60 adult eastern bettongs were reintroduced from the island of Tasmania to two predator-free fenced reserves, Tidbinbilla Nature Reserve (TNR) and Mulligan's Flats Woodland Sanctuary (MFWS), in the Australian Capital Territory (ACT) (Batson *et al*., in press). Comprehensive health assessments were undertaken during translocation and baseline health and disease parameters were established for this species (Portas *et al*., 2014). We hypothesised that baseline health parameters of eastern bettongs would change post-reintroduction

and might therefore be useful measures for assessing the effect of reintroduction on bettong health beyond direct observations of morbidity and mortality. In this study we compare body weight, haematology and biochemistry, parasite assemblages, and disease exposure (*Toxoplasma gondii*, macropodoid herpesviruses) of a subset (13 males, 17 females) of the population pre- and post-reintroduction. We also compare differences between haematology and biochemistry of the populations at the two reintroduction sites and differences between sexes in response to reintroduction.

Methods

Study area

Collection sites for free-ranging bettongs in Tasmania have been described previously and included eight locations representing a mix of remnant native forest, forestry plantations and agricultural land (Batson *et al*., in press; 2015; Portas *et al*. 2014). Mulligan's Flat Woodland Sanctuary, in the ACT, is a 400 ha remnant critically endangered box-gum grassy woodland that is the subject of intensive experimental restoration efforts (Manning *et al*., 2011; Shorthouse *et al*., 2012). Tidbinbilla Nature Reserve, also in the ACT, incorporates a fenced sanctuary in an area of wet sclerophyll forest and open grassland for the conservation management of threatened Australian species.

Translocation and health assessments

We previously established base line health and disease parameters in 60 adult eastern bettongs (19 male, 41 female) live-trapped in July– October 2011 and April–September 2012 in Tasmania and reintroduced to two reserves, (TNR and MFWS) in the ACT (Portas *et al*., 2014). Bettongs released into MFWS (8 males, 10 females) were monitored via GPS/VHF collars and live trapping

approximately every three months. Bettongs at MFWS received no supplemental food and the population was unmanaged. Bettongs at TNR (5 males, 7 females) were housed in small groups in natural bushland enclosures (2.6–9.4 ha); managed to maximise genetic diversity of offspring; and received supplemental food, including fresh locally available produce and a commercially available pellet, at least weekly. Monitoring was limited to remote observation of feed stations via camera and live-trapping approximately every three months.

Trapping, sedation and anaesthetic procedures at translocation have been described previously (Portas *et al*. 2014). Between 12–24 months post-reintroduction (May–November 2013) bettongs were captured at MFWS and TNR at night using padded cage traps baited with a mixture of oats and peanut butter. Traps were set at dusk and checked between 1–4 hr later. Bettongs were removed from traps, placed in cloth bags before being processed on site. Anaesthesia was induced and maintained using isoflurane in oxygen delivered via mask. Post anaesthesia bettongs were allowed to recover in cloth bags for up to 1 hr before being released at the capture site.

A detailed account of the physical examination process, sample and data collection, and sample processing has been described previously (Portas *et al*., 2014). Briefly the following were performed: physical examination; body weight; pes length measurement; ticks and fleas were collected using forceps and fixed in 70% ethanol; mites and lice were collected using skin and hair scrapings in glycerine; blood was collected from the lateral coccygeal vein for assessment of haematological and biochemical parameters and serology (*Toxoplasma gondii*, Macropodid herpesviruses 1 and 2 [MaHV-1, MaHV-2]); pooled swabs from the conjunctival, nasal and urogenital mucosa were collected for the detection of herpesvirus DNA using polymerase chain reaction (PCR); and faecal samples were collected and assessed using the sodium nitrate floatation technique for endoparasite ova. Bettongs were weighed to the nearest gram using electronic scales. Weights for female bettongs with pouch young present were adjusted by subtracting the estimated

weight of the pouch-young (Batson *et al*., 2015). We calculated body condition index (BCI) using the residuals of a linear regression of body weight against pes length (Johnson, 1994).

Haematological and biochemical analyses were performed within 24 hr of collection by Vetnostics, North Ryde, Australia. Haematocrit, haemoglobin, red blood cell count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count, neutrophils, lymphocytes, monocytes, eosinophils, and basophils were measured. Sodium, potassium, chloride, bicarbonate, anion gap, urea, creatinine, glucose, bilirubin, aspartate amino transferase (AST), alanine transaminase (ALT), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), total protein, globulin, albumin, albumin/globulin ratio, calcium, phosphate, creatine kinase, cholesterol, and triglyceride were also measured. Any haematological and biochemical variables that were significantly different post reintroduction, and potentially influenced by nutrition, were reassessed after statistically adjusting for body weight.

Toxoplasma gondii serology was performed at the Department of Primary Industries, Water and Environment, Mount Pleasant Laboratories, Launceston, Australia using the direct (DAT) and modified agglutination tests (MAT) for antibodies to *T. gondii* (Johnson *et al*., 1989). Both tests were performed on all sera at translocation, after which time the laboratory ceased to offer the DAT, and sera collected post-reintroduction were assayed using the MAT only. Herpesvirus serology and detection of herpesvirus DNA by PCR were performed at the Faculty of Veterinary Science, University of Melbourne, Parkville, Australia using previously described techniques (Vaz *et al*., 2012).

Haematological and biochemical values (response variables) varied at two levels; between animals and within animals. Candidate explanatory variables such as weight varied at both the animal and

within animal level whereas the factor sex and site (TNR versus MF) varied only at the animal level. The design variable reintroduction (pre- versus post-) varied only at the within animal level. Given the above multilevel sampling design and data structure, these data were analysed within the framework of general linear mixed models using restricted maximum likelihood with significance assumed at F pr. < 0.05. For overall inference information was combined across the two levels. Statistical computation was performed using GenStat 17th Edition (VSN International Ltd., Hemel Hempstead, UK).

Results

We attempted to calculate BCI in eastern bettongs but found no significant relationship between body weight against pes length (Figure 1). The body weight of eastern bettongs increased (F pr. < .001) from a mean (± SEM) of 1.69 (0.024) kg at translocation to 1.83(0.024) kg post-reintroduction. There were no differences between mean body weights of bettongs at the two reintroduction sites or between males and females in response to reintroduction.

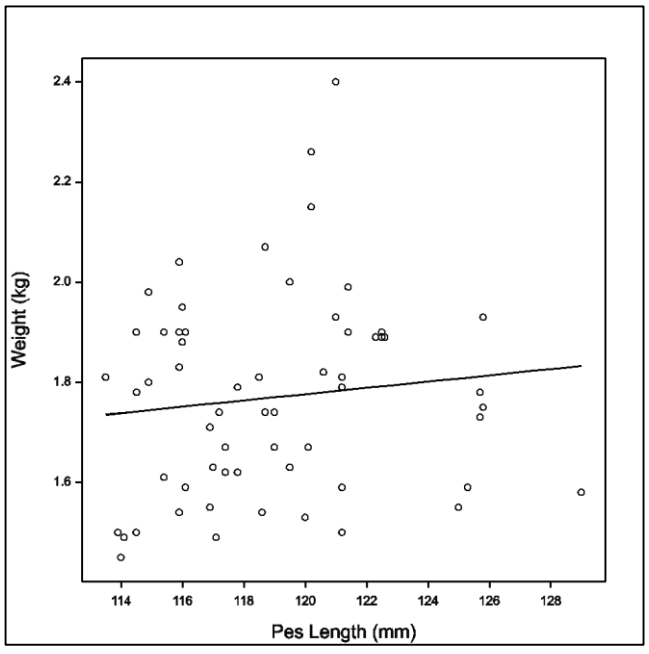

Figure 1. Fitted and observed relationship from regression analysis of body weight versus pes length in eastern bettongs *Bettongia gaimardi*.

Haematocrit, haemoglobin, red blood cell count, MCH and platelets increased while white blood cell count, neutrophils, lymphocytes and monocytes decreased post-reintroduction (Table 1). There were fewer differences between haematological parameters at the two reintroduction sites with platelets, white blood cell count, neutrophils and monocytes higher in the TNR population compared with the MFWS population (Table 1). There were limited differences in response to reintroduction between sexes other than for platelets which increased more (F pr. 0.034) and white blood cell count which decreased more (F pr. 0.046) in males. Potassium, anion gap, urea, creatinine, glucose, globulin and triglyceride were all higher in bettongs post-reintroduction. Sodium, chloride, bicarbonate, AST, ALT, GGT, albumin/globulin ratio, phosphate, creatine kinase and cholesterol were all lower in bettongs post-reintroduction (Table 2). There were few differences between biochemical parameters at the two reintroduction sites with sodium and bicarbonate lower and anion gap, creatinine and ALP higher in the TNR population compared with the MFWS population (Table 2). There only differences in response to reintroduction between sexes was for phosphate (F pr. 0.046) which decreased more in females. Haematocrit (F pr. < 0.001), haemoglobin (F pr. 0.005), red cell count (F pr. 0.001), creatinine (F pr. < 0.001) and triglycerides (F pr. 0.004) were positively related to body weight while MCH (F pr. 0.280), urea (F pr. 0.622) and globulin (F pr. 0.155) were not.

Sera from all 30 bettongs were negative for antibodies to *T. gondii* as at both time points. A single female bettong had detectable antibodies to alphaherpesviruses MaHV-1 and MaHV-2 as determined by serum neutralisation assay at translocation but had no detectable antibodies 19 mo later. The remaining 29 bettongs were seronegative at translocation and post-reintroduction. A pooled nasal/conjunctival/urogenital tract swab from a single male bettong was positive for herpesvirus DNA (potoroid herpesvirus 1, PotHV-1) using PCR at translocation but was negative 12 mo later. The remaining 29 bettongs were negative for herpesvirus DNA using PCR at both time points.

Fleas, lice, mites, and ticks recovered from bettongs were deposited in the Australian National Wildlife Collection CSIRO, Canberra. Five ectoparasite species (*Haemolaelaps hatteni*, *Ixodes cornuatus*, *I. tasmani*, *Pygiopsylla zethi*, *Stephanocircus harrisoni*) present at translocation were not recovered post-reintroduction while an additional four ectoparasite species (*Paraheterodoxus erinaceus*, *Hetrodoxus* cf. *ualabti*, *Eutrombicula macropus*, *Guntheria* cf. *shareli*) were recovered post-reintroduction (Table 3). The prevalence of endo- and ectoparasites recovered from bettongs at both time points is detailed in Table 4.

Discussion

Our data demonstrate changing baseline health parameters in eastern bettongs following reintroduction to the Australian mainland and are suggestive of improved health in the reintroduced populations. Despite recommendations for ongoing health monitoring in reintroduction programs there are few reports of continued monitoring beyond translocation and establishment (Kock *et al*., 2007; Work *et al*., 2010). Furthermore post-reintroduction health monitoring is often limited to direct observations of morbidity and mortality with changes at a physiological level receiving scant attention. Our data demonstrate the value of ongoing comprehensive health evaluations for assessing the response of individuals and populations to reintroduction and can be used as an adjunct to traditional measures for assessing reintroduction outcomes.

Body condition index, usually calculated as the residuals of a linear regression of body weight against a linear morphometric measure, has been used to assess body condition in a number of macropodoid species (Stirrat, 2003; Robert & Schwanz, 2013). Despite previous validation of the use of body weight and pes length for calculating BCI, through calculating total body water and hence proportion of body fat via isotope dilution, in eastern bettongs we found no relationship in this study (Johnson, 1994). Consequently we assessed changes in body weight in response to

reintroduction. Despite the fact that bettongs at TNR received supplemental food there was no difference between mean body weight of the two reintroduced populations and body weight of bettongs at both sites increased. This, coupled with haematological and biochemical data, suggests the source populations in Tasmania were experiencing suboptimal nutrition.

Body condition has been positively correlated with availability of hypogeous fungi in both eastern and northern bettongs Bettongia tropica (Johnson, 1994; Johnson & McIlwee, 1997). We made no attempt to quantify dietary hypogeous fungi intake or assess the nutritional quality of the diet at the source habitat or reintroduction sites. However bettongs were collected from agricultural land and disturbed and fragmented habitat in Tasmania; sites which may have represented suboptimal habitat and afforded poorer quality diets. Alternatively lower population densities and lack of competition at the reintroduction sites may have resulted in greater resource availability, improved nutrition and hence greater body weights for reintroduced bettongs.

Comparisons of haematological and biochemical variables from eastern bettongs at translocation and post-reintroduction revealed significant differences in a range of variables. Some species specific variability in the response of haematological and biochemical parameters to changing environmental conditions has been demonstrated in a range of macropodoids (Ealey, & Main, 1967; Shield, 1971; Algar, Arnold & Grassia, 1988; Stirrat, 2003; Pacioni *et al*., 2013; Robert & Schwanz, 2013). Of particular interest in eastern bettongs were increases in haematocrit, haemoglobin, red cell count, creatinine and triglycerides post-reintroduction. These variables were positively correlated with weight (rather than reintroduction per se) suggesting they may be useful measures of nutritional status in eastern bettongs.

Haemoglobin concentration in eastern bettongs post-reintroduction was comparable to reference values reported for the closely related woylie *Bettongia penicillata* in Western Australia

suggesting post-reintroduction haemoglobin concentration maybe more representative of normal values for the eastern bettong in optimal environments (Pacioni *et al*., 2013). Lower haemoglobin concentrations in response to seasonal declines in diet quality have been demonstrated in western grey kangaroos *Macropus fuliginosus*, common wallaroos *M. robustus* and quokkas *Setonix brachyurus* (Ealey & Main, 1967; Shield, 1971; Algar *et al*., 1988). Haemoglobin concentration was also shown to have a positive association with rainfall in woylies; presumably mediated via changing nutritional content of forage species (Pacioni *et al*., 2013). In contrast seasonal changes in nutrition did not influence haemoglobin concentration in agile wallabies *Macropus agilis* or allied rock wallabies *Petrogale assimilis* and there was no significant difference in haemoglobin concentration between three sub-populations of tammar wallabies *Macropus eugenii* living in separate habitats of variable nutritional quality (Spencer & Speare, 1992; Stirrat, 2003; Robert & Schwanz, 2013).

Increases in red cell count and haematocrit in eastern bettongs post-reintroduction may also be explained by improved nutrition at the reintroduction sites. Red cell count and haematocrit have been shown to have a positive association with rainfall in woylies (Pacioni *et al*., 2013). Haematocrit but not red cell count in agile wallabies from the wet-dry tropics was lower during the dry season and poor nutrition was postulated as the cause (Stirrat, 2003). Haematocrit can increase in response to haemoconcentration but eastern bettongs at the source locations and the reintroduction sites had free access to water making haemoconcentration secondary to dehydration unlikely. Additionally in dehydration total protein, albumin and haematocrit are all increased and in this study neither total protein nor albumin values increased significantly post-reintroduction.

We interpreted the neutrophilia, lymphocytosis and monocytosis observed in bettongs at translocation as a physiological leukocytosis in response to the prolonged period of confinement and transportation prior to sampling (Stockham & Scott, 2008). The higher white blood cell,

neutrophil and monocytes count observed in the TNR population compared with the MFWS population may reflect variable levels of physiological stress at the two sites. Eastern bettongs at TNR have higher faecal corticosteroid metabolites than those at MFWS (Batson *et al*., in review), which could support the possibility of an unknown environmental stressor at TNR.

Creatinine is influenced by muscle mass and renal function (Stockham & Scott, 2008). We attributed the increased creatinine observed in bettongs post-reintroduction to the greater body mass of the animals rather than dehydration. Reduced creatinine concentrations have been observed in malnourished white-tailed deer *Odocoileus virginianus* and in tammar wallabies subjected to nutritional stress (Delgiudice, Mech & Seal, 1990; Robert & Schwanz, 2013). In contrast creatinine concentrations were not significantly different, despite significant changes in body mass, in agile wallabies experiencing seasonal nutritional fluctuations (Stirrat, 2003). Urea was also significantly higher in bettongs post-reintroduction and previous studies in wallaroos, western grey kangaroos, agile wallabies and tammar wallabies have demonstrated a relationship between poor quality diets or reduced protein intake and low urea concentrations (Ealey & Main, 1967; Algar *et al*., 1988; Stirrat, 2003; Robert & Schwanz, 2013). However urea was not positively related to weight in this study and its usefulness as a potential measure of diet quality or protein intake in eastern bettongs is unclear.

An association between triglycerides and kidney fat, a traditional measure of body condition in ungulate species, has been demonstrated in Iberian wild goats *Capra pyrenacica* (Serrano *et al*., 2008). In macropodoids triglycerides have been positively correlated with BCI in tammar wallabies but not agile wallabies (Stirrat, 2003; Robert & Schwanz, 2013). The positive correlation between triglycerides and body weight in this study suggests that triglycerides may be a useful indicator of body condition in eastern bettongs.

Somewhat unexpectedly, given the observed changes in other parameters, neither total protein nor albumin differed significantly in eastern bettongs post-reintroduction. However globulins were significantly higher post-reintroduction but were not positively correlated with weight. Total protein and albumin have been used as indicators of body condition in ungulates (Bahnak *et al*., 1979; Caldeira *et al*., 2007). Albumin is positively correlated with BCI in tammar wallabies (Robert & Schwanz, 2013) and total protein and albumin are associated with protein intake in agile wallabies (Stirrat, 2003). The relationship between total proteins, globulins and albumin and body weight in eastern bettongs requires further investigation.

Creatine kinase values were approximately 20-fold and AST values four-fold lower post-reintroduction. These differences can be explained by the prolonged period of confinement and transport (up to 18 hr) that bettongs underwent prior to sampling at translocation compared with the relatively short period of confinement in traps for sampling post-reintroduction. The initial values likely reflect exertional myopathy and the values obtained post-reintroduction likely reflect more normal values for this species (Portas *et al*., 2014). Post-reintroduction creatine kinase values are comparable with values obtained for another potoroid marsupial, Gilbert's potoroo *Potorous gilbertii* (Vaughan *et al*., 2009). Significant changes in a range of other biochemical parameters including electrolytes are less readily explained and are of unknown clinical significance.

Parasite species are frequently lost when host species colonise a new environment. The host-parasite factors that resulted in five ectoparasites failing to persist on reintroduced bettongs are unknown but could include environmental factors, changes to host physiology and immune function, host density, transmission efficiency or the lack of intermediate hosts (MacLeod *et al*., 2010). Twelve of the bettongs included in this study were treated with ivermectin (200 µg/kg) subcutaneously at translocation to reduce (but not eliminate) gastrointestinal nematode burdens (Portas *et al*., 2014). However the ectoparasites that failed to persist were also found on untreated

conspecifics at translocation making treatment with ivermectin an unlikely explanation for their disappearance. Additionally two of the ectoparasites that failed to persist were fleas against which ivermectin is ineffective.

Of the four novel parasites detected post-reintroduction *Paraheterodoxus erinaceus* has been previously described from long-nosed potoroos *Potorous tridactylus* in Tasmania; *Hetrodoxus ualabati* has been recorded from the swamp wallaby *Wallabia bicolor* in Victoria, New South Wales and Queensland; *Eutrombicula macropus* has been previously reported from macropodids in the Northern Territory, Queensland, Victoria and South Australia; and *Guntheria shareli* has been reported from the red-legged pademelon *Thylogale stigmatica* and the bush rat *Rattus fuscipes* in north Queensland (von Kéler, 1971; Domrow & Lester, 1985; Portas, Crowley & Hufschmid, 2009). Sympatric macropodoids were the most likely source of *Paraheterodoxus erinaceus*, and *Eutrombicula macropus*; the macropodoid host species for these parasites are present in the ACT including a population of long-nosed potoroos at TNR. Of the two previously recorded hosts for *Guntheria shareli* only the bush rat occurs in the ACT and represents the most likely source of this ectoparasite. However, identification was based solely on morphological features of the scutum, which may have been damaged in preparation of skin scrapings, hence our designation *G.* cf. *shareli.* Similarly, variation in the distribution of setae on females of the *Heterodoxus* species recovered compared with that described in the published key for the genus (von Kéler, 1971) may or may not represent normal anatomical variation; hence our designation *H.* cf. *ualabati*. The swamp wallaby, sympatric at both reintroduction sites, is the most likely source of this louse species.

Serum-virus neutralisation assays were used to detect antibodies against MaHV-1 and MaHV-2 (alphaherpesviruses) in serum samples, whereas PCR was used to detect any herpesvirus DNA (including the gammaherpesvirus, PotHV-1) in swab samples. The changing antibody and PCR

status observed in a small number of bettongs is consistent with observations in other species, where herpesvirus shedding has been detected intermittently following periodic reactivation from latency (Roizman & Pellet, 2001). Similarly, one animal was seropositive at translocation but seronegative post-reintroduction. Serum antibodies to herpesviruses generally persist over time, however antibody levels can decrease in some individuals and fall below detectable levels, particularly in the absence of reinfection or reactivation (Kaashoek, Rijsewijk & Van Oirschot, 1996; Van Der Peel *et al*., 1995; Whitley, 2001). Reactivation of latent herpesvirus infections often occur during periods of host immune-compromise or stress, with a recent study in Australian marsupials identifying poor body condition score as a risk factor for herpesvirus shedding (Stalder *et al*., 2015). The higher body weights and changes to haematological and biochemical parameters suggestive of improved nutritional status could therefore help to explain why these viruses were not detected post-reintroduction. However further studies are required to investigate this hypothesis, particularly as the rate of herpesvirus infection detected at translocation was low.

In conclusion we found that body weight of eastern bettongs increased significantly post-reintroduction but was not significantly different between reintroduction sites or between sexes in response to reintroduction. A wide range of haematological and biochemical parameters changed post-reintroduction but there were few differences between reintroduction sites or between sexes in response to reintroduction. Specifically haemoglobin, red cell count, haematocrit, creatinine and triglycerides increased and were positively related to weight providing potentially useful proxies for assessing the nutritional status of eastern bettongs and hence habitat suitability. Given that all bettongs in this study survived post-reintroduction we were unable to establish if any of the variables might be useful predictors of survival. Of the infectious diseases and parasites considered none impacted overall translocation success or individual animal survival. However ongoing monitoring is recommended as these diseases and parasites could be expected to be of greater importance in sub-optimal habitats. The health of eastern bettongs appeared to improve following reintroduction from Tasmania to the ACT. This study demonstrates the value of comprehensive

and ongoing health evaluation during reintroduction programs. The health parameters assessed allowed for the physiological response of eastern bettongs to reintroduction to be documented and provided additional information not attained with standard measures of reintroduction success such as survival and fecundity. We recommend that comprehensive and ongoing health evaluations be incorporated in to future eastern bettong reintroductions and wildlife reintroductions more broadly to allow for more accurate assessment of reintroduction outcomes.

Table 1. Comparison of haematological variables (mean ± SEM) in 30 (13 male, 17 female) eastern bettongs *Bettongia gaimardi* reintroduced from Tasmania to Mulligan's Flat Woodland Sanctuary and Tidbinbilla Nature Reserve in the Australian Capital Territory, Australia pre- and post-reintroduction and between reintroduction sites. TNR, Tidbinbilla Nature Reserve; MFWS, Mulligan's Flat Woodland Sanctuary; Hb, haemoglobin; RBC, red blood cell count, MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular hemoglobin concentration; WBC, white blood cell count [a] denotes significant difference between values.

	Translocation	Post-reintroduction		Reintroduction site		
	Tasmania	ACT	F pr.	MFWS	TNR	F pr.
Measure						
Haematocrit (%)	0.399 (0.006)	0.456 (0.006)	<.001[a]	0.445 (0.009)	0.475 (0.011)	0.106
Hb (g/L)	133.4 (2.24)	154.9 (2.24)	<.001[a]	152.5 (2.8)	159.0 (3.6)	0.337
RBC ($\times 10^{12}$/L)	9.52 (0.156)	10.80 (0.156)	<.001[a]	10.56 (0.24)	11.22 (0.3)	0.161
MCV (fL)	41.50 (0.53)	42.50 (0.53)	0.195	41.86 (0.96)	43.61 (1.17)	0.275
MCH (pg)	14.13 (0.10)	14.46 (0.10)	0.029[a]	14.65 (0.21)	14.14 (0.25)	0.104
MCHC (g/L)	335.7 (1.99)	339.6 (1.99)	0.177	342.1 (3.6)	335.4 (4.4)	0.206
Platelets ($\times 10^9$/L)	387.9 (13.13)	436.9 (13.13)	0.016[a]	405.9 (28.9)	490.4 (3.7)	0.014[a]
WBC ($\times 10^9$/L)	3.58 (0.15)	2.64 (0.15)	<.001[a]	2.09 (0.24)	3.59 (0.03)	0.002[a]
Neutrophils ($\times 10^9$/L)	2.20 (0.13)	1.34 (0.13)	<.001[a]	0.96 (0.19)	2.00 (0.24)	0.010[a]
Lymphocytes ($\times 10^9$/L)	1.38 (0.09)	1.15 (0.09)	0.026[a]	1.00 (0.14)	1.34 (0.17)	0.216
Monocytes ($\times 10^9$/L)	0.15 (0.08)	0.09 (0.08)	0.004[a]	0.06 (0.017)	0.14 (0.021)	0.040[a]
Eosinophils ($\times 10^9$/L)	0.03 (0.01)	0.05 (0.01)	0.446	0.035 (0.014)	0.075 (0.018)	0.278

Table 2. Comparison of biochemical variables (mean ± SEM) in 30 (13 male, 17 female) eastern bettongs *Bettongia gaimardi* reintroduced from Tasmania to Mulligan's Flat Woodland Sanctuary and Tidbinbilla Nature Reserve in the Australian Capital Territory, Australia pre- and post-reintroduction and between reintroduction sites.

	Translocation	Post-reintroduction		Reintroduction site		
	Tasmania	ACT	F pr.	MFWS	TNR	F pr.
Measure						
Sodium (mmol/L)	147.20 (0.56)	143.30 (0.56)	<.001 [a]	144.92 (0.8)	140.50 (1.0)	0.012 [a]
Potassium (mmol/L)	3.67 (0.32)	4.74 (0.32)	0.028 [a]	4.78 (0.47)	4.67 (0.56)	0.914
Chloride (mmol/L)	106.87 (0.63)	101.30 (0.63)	<.001 [a]	102.77 (0.9)	98.77 (1.1)	0.041
Bicarbonate (mmol/L)	29.10 (0.62)	23.60 (0.62)	<.001 [a]	25.48 (0.77)	20.36 (0.99)	0.009 [a]
Anion gap (mmol/L)	15.03 (0.80)	22.38 (0.80)	<.001 [a]	20.26 (1.04)	26.05 (1.26)	0.021 [a]
Urea (mmol/L)	6.05 (0.40)	8.26 (0.40)	<.001 [a]	7.52 (0.54)	9.52 (0.68)	0.105
Creatinine (µmol/L)	41.7 (1.78)	57.1 (1.78)	<.001 [a]	50.4 (2.72)	68.7 (3.43)	0.002 [a]
Glucose (U/L)	11.33 (0.67)	17.80 (0.67)	<.001 [a]	14.62 (1.2)	23.29 (1.42)	<.001 [a]
Bilirubin (U/L)	1.03 (0.08)	1.13 (0.08)	0.409	1.20 (0.11)	1.019 (0.14)	0.473
AST (U/L)	176.2 (5.91)	46.2 (5.91)	<.001 [a]	50.1 (9.27)	40.1 (11.63)	0.572
ALT (U/L)	84.7 (3.82)	32.6 (3.82)	<.001 [a]	34.3 (5.41)	29.7 (6.87)	0.689
GGT (U/L)	16.47 (0.91)	14.61 (0.91)	0.021 [a]	13.26 (1.15)	13.37 (1.46)	0.968
ALP (U/L)	1679 (164.1)	1421 (164.1)	0.275	1821 (392.2)	728 (443.6)	0.033 [a]
Protein (g/L)	55.13 (0.75)	56.80 (0.75)	0.129	56.88 (1.21)	56.66 (1.51)	0.923

Albumin (g/L)	40.56 (0.76)	39.78 (0.76)	0.138	40.13 (1.22)	37.13 (1.52)	0.189
Globulin (g/L)	14.47 (0.66)	17.77 (0.66)	0.002 [a]	16.57 (1.04)	19.53 (1.31)	0.166
Albumin/Globulin ratio	4.03 (0.41)	2.37 (0.41)	0.013 [a]	2.37 (0.56)	2.07 (0.72)	0.807
Calcium (mmol/L)	2.29 (0.02)	2.30 (0.02)	0.793	2.27 (0.03)	2.36 (0.04)	0.147
Phosphate (mmol/L)	2.51 (0.13)	2.01 (0.13)	0.009 [a]	2.214 (0.18)	1.66 (0.21)	0.148
Creatine kinase (U/L)	24018 (1463.9)	1005 (1463.9)	<.001 [a]	118 (2015)	2537 (2565)	0.580
Cholesterol (mmol/L)	3.894 (0.13)	3.050 (0.13)	0.002 [a]	2.94 (0.20)	2.96 (0.25)	0.952
Triglyceride (mmol/L)	0.867 (0.10)	1.51 (0.10)	<.001 [a]	1.46 (0.13)	1.58 (0.16)	0.704

AST, aspartate amino transferase; ALT, alanine transaminase; GGT, gamma glutamyl transferase; ALP, alkaline phosphatase

[a] denotes statistically significant difference between values

Table 3. Ectoparasite assemblages from eastern bettongs *Bettongia gaimardi* reintroduced from Tasmania to the Australian Capital Territory, Australia pre- and post-reintroduction.

Parasite Order, Family	Species	Present at translocation	Present post-reintroduction	Accession number(s)
ACARI				
Listrophoridae	*Paraheterodoxus erinaceus*	No	Yes	AR 1594
	Paraheterodoxus? n. sp.	Yes	Yes	AR 1572, 1574
	Hetrodoxus cf. *ualabati*	No	Yes	AR 1590
Trombiculidae	*Eutrombicula macropus*	No	Yes	AR 1593
	Guntheria cf. *pertinax*	Yes	Yes	AR 1579
	Guntheria cf. *shareli*	No	Yes	AR 1614
Laelapidae	*Haemolaelaps hatteni*	Yes	No	AR 1575
	Thadeua greeni	Yes	Yes	AR 1585
Atopomelidae	*Cytostethum (Metacytostethum) intermedium*	Yes	Yes	AR 1576
	Cytostethum (*Metacytostethum*) *tasmaniense*	Yes	Yes	AR 1576
	Cytostethum (*Metacytostethum*) *thetis*	Yes	Yes	AR 1576
	Cytostethum (*Metacytostethum*) *wallabia*	Yes	Yes	AR 1576
Ixodidae	*Ixodes cornuatus*	Yes	No	AR 1586
	Ixodes tasmani	Yes	No	AR 1587
	Ixodes trichosuri	Yes	Yes	AR 1571

PHTHIRAPTERA				
Pygiopsyllidae	*Pygiopsylla zethi*	Yes	No	AR 1573
Stephanocercidae	*Stephanocircus harrisoni*	Yes	No	AR 1588

Table 4. Prevalence of parasites from eastern bettongs *Bettongia gaimardi* reintroduced from Tasmania to the Australian Capital Territory, Australia pre- and post-reintroduction.

Parasite	At translocation	Post-reintroduction
Endoparasites		
Strongylid eggs	13/30 (43.3%)	22/30 (73.3%)
Strongylid larvae	2/30 (6.7%)	6/30 (20%)
Capillariid-like eggs	1/30 (3.3%)	0/30 (0%)
Eimeria gaimardi	3/30 (10%)	5/30 (16.7%)
Ectoparasites		
Ticks	23/30 (76.7%)	2/30 (6.7%)
Lice	7/30 (23.3%)	11/30 (36.7%)
Fleas	13/30 (43.3%)	0/30 (0%)
Mites	28/30 (93.3 %)	13/30 (43.3%)

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References

Algar, D., Arnold, G.W., & Grassia A. (1988). Effects of nitrogen and season on western grey kangaroo hematology. Journal of Wildlife Management, 52, 616–619.

Bahnak, B.R., Holland, J.C., Verme, L.J., & Ozoga, J.J. (1979). Seasonal and nutritional effects on serum nitrogen constituents in white-tailed deer. Journal of Wildlife Management, 43, 454–460.

Batson, W., Fletcher, D., Portas, T., Crisp, H., Ryan, S., Wimpenny, C., Gordon, I & Manning, A. (In press) Reintroduction of eastern bettong to a critically endangered woodland habitat in the Australian Capital Territory, Australia. IUCN Global Re-introduction Perspectives Series.

Batson, W. G., Gordon, I. J., Fletcher, D. B., & Manning A. (2015). The effect of pre-release captivity on post-release performance in reintroduced eastern bettongs (*Bettongia gaimardi*). Oryx. First View, 1-10.

Batson, W. G., Gordon, I. J., Fletcher, D. B., Portas, T., & Manning A. (In review). The effect of pre-release captivity on the stress physiology of a reintroduced population of wild eastern bettongs. Journal of Zoology.

Cabezas, S., Calvete, C., & Moreno, S. (2011). Survival of translocated wild rabbits: importance of habitat, physiological and immune condition. Animal Conservation, 14, 665–675.

Caldeira, R.M., Belo, T.M., Santos, C.C., Vazques, M.I., & Portugal, A.V. (2007). The effect of long-term feed restriction and over-nutrition on body condition score, blood metabolites and hormonal profiles in ewes. Small Ruminant Research, 68, 242–255.

Claridge, A.W., Seebeck, J. & Rose, R.W. (2007). Bettongs, potoroos and the musky rat-kangaroo. CSIRO Publishing, Collingwood, Australia.

Clarke, J., Warren, K., Calver, M., de Tores, P., Mills, J. & Robertson, I. (2013). Hematologic and serum biochemical reference ranges and an assessment of exposure to infectious diseases prior to translocation of the threatened western ringtail possum (*Pseudocheirus occidentalis*). Journal of Wildlife Diseases, 49, 831–40.

Delgiudice, G.D., Mech, L.D., & Seal, U.S. (1990). Effects of winter undernutrition on body composition and physiological profiles of white-tailed deer. Journal of Wildlife Management, 54, 539–550.

Deem, S. L., Cruz, M. B., Higashiguchi, J. M. & Parker, P. G. (2012). Diseases of poultry and endemic birds in Galapagos: implications for the reintroduction of native species. Animal Conservation, 15, 73–82.

Domrow, R. & Lester, L. N. (1985). Chiggers of Australia (Acari: Trombiculidae): an annotated checklist, keys and bibliography. Australian Journal of Zoology Supplement Series, 33, 1–111.

Ealey, E.H.M., & Main, A.R. (1967). Ecology of the euro, *Macropus robustus* (Gould), in north-western Australia. III. Seasonal changes in nutrition. Wildlife Research, 12, 53–65.

Ewen, J.G., Acevedo-Whitehouse, K., Alley, M. R., Carraro, C., Sainsbury, A. W., Swinnerton, K. & Woodroffe, R. (2012). Empirical consideration of parasites and health in reintroduction. In Reintroduction biology integrating science and management (eds Ewen, J. G., Armstrong, D. P., Parker, K. A. & Seddon, P. J.) pp. 290–335. Wiley-Blackwell, Oxford, UK.

Jakob-Hoff, R.M., MacDiarmid, S.C., Lees, C., Miller, P. S., Travis, D. & Kock, R. (2014). Manual of Procedures for Wildlife Disease Risk Analysis. World Organisation for Animal Health. Paris, 160 pp. Published in association with the International Union for Conservation of Nature and the Species Survival Commission.

Johnson, A.M., Roberts, H., Statham, P., & Munday, B.L. (1989). Serodiagnosis of acute toxoplasmosis in macropods. Veterinary Parasitology, 34, 25–33.

Johnson, C.N. (1994). Nutritional ecology of a mycophagous marsupial in relation to production of hypogeous fungi. Ecology, 75, 2015–2021.

Johnson, C.N., & McIlwee, P. *(*1997*)* Ecology of the northern bettong, *Bettongia tropica*, a tropical mycophagist. Wildlife Research, **24,** 549–559.

Kaashoek, M.J., Rijsewijk, F.A.M., & Van Oirschot, J.T. (1996). Persistence of antibodies against bovine herpesvirus 1 and virus reactivation two to three years after infection. Veterinary Microbiology, 53,103–110.

Kock, R.A., Soorae, P.S., & Mohammed, B. (2007). Role of veterinarians in re-introductions. International Zoo Yearbook, 41, 24–37.

Leighton, F.A. (2002). Health risk assessment of the translocation of wild animals. Scientific and Technical Review of the Office International des Epizooties, 21, 187–195.

Maceda-Veiga, A., Figuerola, J., Martínez-Silvestri, A., Viscor, G., Ferrari, N., & Pacheco, M. (2015). Inside the red-box: applications of haematology in wildlife monitoring and ecosystem health assessment. Science of the Total Environment, 514, 322–332.

MacLeod, C.J., Paterson, A.M., Tompkins, D.M., & Duncan, R. P. (2010) Parasites lost – do invaders miss the boat or drown on arrival? Ecology Letters, 13, 516–527.

Manning, A.D., Wood, J.T., Cunningham, R.B., McIntyre, S., Shorthouse, D.J., Gordon, I.J., & Lindenmayer, D.B. (2011). Integrating research and restoration: The establishment of a long-term woodland experiment in south-eastern Australia. Australian Zoology, 35, 633–648.

Mathews, F., Moro, D., Strachan, R., Gelling, M., & Buller, N. (2006). Health surveillance in wildlife reintroductions. Biological Conservation, 131, 338–347.

Nichols, J. D. & Armstrong, D. P. (2012). Monitoring for reintroductions. In Reintroduction biology integrating science and management. (eds Ewen, J. G., Armstrong, D. P., Parker, K. A. & Seddon, P. J.) pp. 223–255. Wiley-Blackwell, Oxford, UK.

Pacioni, C., Robertson, I.D., Maxwell, M., van Weenen, J., & Wayne, A.F. (2013). Hematologic characteristics of the woylie (*Bettongia penicillata ogilbyi*). Journal of Wildlife Diseases, 49, 816–830.

Portas, T.J., Crowley, A., & Hufschmid, J. (2009). Ectoparasitic dermatitis in free-ranging swamp wallabies (*Wallabia bicolor*) in New South Wales. Australian Veterinary Journal, 87, 160–162.

Portas, T., Fletcher, D., Spratt, D., Reiss, A., Holz, P., Stalder, K., Devlin, J., Taylor, D., Dobroszczyk, D., & Manning, A.D. (2014). Health evaluation of free-ranging eastern

bettongs (*Bettongia gaimardi*) during translocation for re-introduction. Journal of Wildlife Diseases, 50, 210–223.

Robert, K.A., & Schwanz, L.E. (2013). Monitoring the health status of free-ranging tammar wallabies using hematology, serum, biochemistry, and parasite loads. Journal of Wildlife Management, 77, 1232–1243.

Roizman, B., & Pellett, P.E. (2001). The Family Herpesviridae: A Brief Introduction. In Fields Virology 4th Edition (eds Knipe, D.M., & Howley, P.M.), pp. 2381–2397. Lippincott Williams & Wilkins, Philadelphia, USA.

Schultz, D.J., Rich, B.G., Rohrig, W., McCarthy, P.J., Mathews, B, Schultz, T., Corrigan, T. & Taggart, D.A. (2011) Investigations into the health of brush-tailed rock-wallabies (*Petrogale penicillata)* before and after reintroduction. Australian Mammalogy, 33, 235–244.

Serrano, E., Gonzalez, F.J., Grandos, J.E., Morco, G., Fandos, P., Soriguer, R.C., & Perez, J.M. (2008). The use of total serum proteins and triglycerides for monitoring body condition in the Iberian wild goat (*Capra pyrenica*). Journal of Zoo and Wildlife Medicine, 39, 646–649.

Shield, J. (1971). A seasonal change in the blood cell volume of the Rottnest Island quokka, *Setonix brachyurus*. Journal of Zoology, 165, 111–119.

Shorthouse, D.J., Iglesias, D., Jeffress, S., Lane, S., Mills, P., Woodbridge, G., McIntyre, S., & Manning, A.D. (2012). The ‘‘making of’’ Mulligans Flat–Goorooyaroo experimental restoration project. Ecological Management & Restoration, 13, 112–125.

Spencer, P.B.S., Speare, R. (1992). Haematology of wild allied rock-wallabies, *Petrogale assimilis* Ramsay, 1877 (Marsupialia: Macropodidae), in North Queensland. Australian Journal of Zoology, 40, 355–364.

Stalder, K., Vaz, P. K., Gilkerson, J. R., Baker, R., Whiteley, P, Ficorilli, N., Tatarczuch, L., Portas, T., Skogvold, K., Anderson, G., & Devlin, J. M. (2015). Prevalence and clinical significance of herpesvirus infection in populations of Australian marsupials. *PLoS ONE*, 7: e0133807.

Stirrat, S. (2003). Body condition and blood chemistry of agile wallabies (*Macropus agilis*) in the wet-dry tropics. Wildlife Research, 30, 59–67.

Stockham, S.L., & Scott, M.A. (2008). Fundamentals of Veterinary Clinical Pathology. Second Edition. Blackwell Publishing, Ames, USA.

Travis, D.A., Hungerford, L., Engel, G.A., & Jones-Engel, L. (2006). Disease risk analysis: a tool for primate planning and decision making. American Journal of Primatology, 68, 855–867.

Van Der Peel, W.H.M., Kramps, J.A., Quak, J., Brand, A., & Van Oirschot, J.T. (1995). Persistence of bovine herpesvirus (BHVl) specific antibodies in cattle, after intranasal vaccination with a live virus vaccine. Veterinary Record, 137, 347–348.

Vaz, P.K., Motha, J., McCowan, C., Ficorilli, N., Whitely, P.L., Wilks, C.R., Hartley, C.A., Gilkerson, J.R., Browning, G.F., & Devlin, J.M. (2013). Isolation and characterization of a novel herpesvirus from a free-ranging eastern grey kangaroo (*Macropus giganteus*). Journal of Wildlife Diseases, 49, 143–151.

Vaughan, R.J., Warren, K.S., Mills, J.S., Palmer, C., Fenwick, S., Monaghan, C.L., & Friend, A.J. (2009). Hematological and serum biochemical reference values and cohort analysis in the Gilbert's potoroo (*Potorous gilbertii*). Journal of Zoo and Wildlife Medicine, 40, 276–288.

von Keéler, S. (1971). A revision of the Australasian Boopiidae (Insecta: Phthiraptera), with notes on the Trimenoponidae. Australian Journal of Zoology Supplement Series, 6, 1–126.

Webster, K.N., Hill, N. J., Burnett, L., & Deane, E.M. (2014). Ectoparasite infestation patterns, haematology and serum biochemistry of urban-dwelling common brushtail possums. Wildlife Biology, 20, 206–216.

Whitley, R.J. (2001) Herpes Simplex Viruses. In Fields Virology 4th Edition (eds Knipe, D.M., & Howley, P.M.), pp. 2461–2509. Lippincott Williams & Wilkins, Philadelphia, USA.

Work, T.M., Klavitter, J.L., Reynolds, M.H., & Blehert, D. (2010). Avian botulism: a case study in translocated endangered Laysan Ducks (*Anas laysanensis*) on Midway Atoll. Journal of Wildlife Diseases, 46, 499-506.