The ecology and host-parasite dynamics of a fauna translocation in Australia

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[The naturalist] looks upon every species of animal and plant now living as the individual letters which go to make up one of the volumes of our earth's history; and, as a few lost letters may make a sentence unintelligible, so the extinction of the numerous forms of life which the progress of cultivation invariably entails will necessarily render obscure this invaluable record of the past.

It is, therefore, an important object [to preserve them]. . . . If this is not done, future ages will certainly look back upon us as a people so immersed in the pursuit of wealth as to be blind to higher considerations.

Alfred Russel Wallace,
Journal of the Royal Geographical Society 1863

Declaration

I declare that this thesis is my own account of my research and contains as its main content work that has not been previously submitted for a degree at any other tertiary education institution.

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Abstract

Despite the frequency of fauna translocations as a technique to improve the conservation status of threatened species, new populations frequently fail to establish. Translocations often lack experimental manipulation to determine 'best practice' methods to improve success. One poorly understood element of translocation science is the impact of parasites and disease-causing pathogens on the animals moved and the ecosystem they are moved into. Of 58 published Australian translocations in the last 40 years, only 20 (35%) employed any level of parasite management, despite potential contribution of disease to initial fauna declines.

I closely investigated a translocation of boodies (*Bettongia lesueur*) from Barrow Island and Dryandra to Lorna Glen, and 'island dwarf' golden bandicoots (*Isoodon auratus*) from Barrow Island to Lorna Glen and Hermite Island. Bandicoots born into the new populations showed an increased skeletal size and body mass (males) and reproductive output in the number and average size of young (females). These changes occurred within 18 months of release, suggesting that responses were due to phenotypic plasticity, rather than selective pressure occurring over many generations. I conclude that the small size of bandicoots on Barrow Island is a response to resource limitation, rather than true island dwarfism.

I determined the impact on parasite load and survivorship of translocated animals by treating half the population with a topical antiparasitic. Despite frequent trapping (six-weekly) and very high recapture rate (64–99%), repeated dosage did not significantly impact ectoparasite or haemoparasite infection, or survival of the marsupials. I observed transmission of parasites between animals of different origin and to offspring, and a

decline in species diversity present in the translocated population due to the failure of some species to persist.

This thesis identified knowledge gaps in the translocation literature and addressed some key concepts of species ecology, population dynamics and parasitology via post-translocation monitoring.

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Thesis layout

This thesis has been presented as a “thesis by publication”, and includes published, submitted, and prepared but not yet submitted, manuscripts. The submission status of each chapter is outlined in Manuscripts in Preparation (page 208). Because each manuscript is intended to stand alone, there is some repetition in explanation of methods. Manuscripts within the thesis have been formatted uniformly for the thesis, rather than for each intended journal.

Chapter 1.

Introduction

The Hitchhiker's guide to Australia: a parasitological perspective on native and exotic fauna translocations

Abstract

No animal is an island; when an animal is moved to a new location, it may also carry its own parasite stowaways that have the potential to transfer to naïve wildlife at the new destination. The geographical isolation and recent colonisation of Australia by Europeans, along with the introduction of their associated exotic species makes Australia an interesting case study of how anthropogenically mediated movements of animals can lead to new host-parasite associations. These historical movements have included introductions of animals for agriculture, companionship, and accidental stowaways. More recently, faunal movements have also included a large number of deliberate translocations for conservation. While these movements have the potential to spread or introduce new parasite species into a naïve ecological community, we have a limited understanding of the extent of actual spread, and the potentially deleterious impacts on Australia's native fauna; many of which have undergone serious decline for other reasons. Negative effects of additional parasitic burden can impact both the animals moved, and the community they are moved into. This paper reviews introductions of exotic animals and their parasites into Australia, and movements of native animals within the Australia. In addition, we examined the disease management strategies implemented across 58 Australian translocations from the last 40 years. Fewer than 35% of all Australian fauna translocations reported some level of parasite or disease management (including external post mortems or visual health check on capture only), and of these, 25% included any kind of interventional measure (for example, quarantine procedures or swabbing for known pathogens). Although many identified disease as a factor in original species declines, few undertook any follow up monitoring to determine whether disease affected the outcome of the translocation. We emphasise the importance of parasite and disease risk assessments prior to translocating

fauna, and highlight the importance of research during follow-up monitoring post-translocation to quantify the impacts of parasites during faunal movements.

Introduction

The human-mediated movement of fauna is an important process by which wildlife parasites and other pathogens expand their geographic range. Relocation of animals from one place to another can occur for a variety of reasons, ranging from unintentional 'stowaways' to deliberate movements for agricultural purposes, biological control, and more recently, relocation for conservation management of wildlife species (Long, 2003; Fischer and Lindenmayer, 2000). With the movement of animals comes the potential for inadvertent transfer of microorganisms associated with these hosts.

The disease transmission risks associated with animal movements have been well recognised (Griffith et al., 1993), and several examples demonstrate the impacts of introduced pathogens on naïve species. For example, an infection with a microsporidian parasite (*Steinhausia* sp., probably introduced with exotic snails) caused the extinction of the land snail *Partula turgida* from the South Pacific islands (Cunningham and Daszak, 1998). Declines in amphibians throughout Central America, including the extinction of the Monteverde Golden Toad (*Bufo periglenes*) have been attributed to a concurrent wave of chytridiomycosis, caused by *Batrachochytrium dendrobatidis* (Cheng et al., 2011). The decline or extinction of a further 200 amphibians worldwide has been attributed to this highly transmissible fungus; the spread of which has been enhanced by the anthropogenic movement of infected frogs (Daszak et al., 2003; Skerratt et al., 2007).

Major concerns have been expressed regarding infectious disease transmission as a result of faunal movements such as viral or bacterial pathogens which can be highly contagious, virulent, and are frequently responsible for outbreaks in wildlife populations (Dobson and Foufopoulos, 2001). Less focus has been placed on eukaryotic parasites, such as protozoa, helminths and arthropods, because they are perceived to have less impact on

wildlife hosts (but see Tompkins and Poulin, 2006; Morgan et al., 2012, #10703). However, their effects on naïve hosts can be severe and have significant implications for conservation management activities (e.g. LoGiudice et al., 2003).

Baseline knowledge of parasites inhabiting Australian fauna species is generally poor, as is knowledge of the potential clinical impacts of parasites during stressful events (Teixeira et al., 2007). The importance of investigating and monitoring these effects of parasites during conservation activities has been highlighted in the literature (Armstrong and Seddon, 2007; Ewen et al., 2011; Kock et al., 2010). Furthermore, actual research that experimentally manages parasite risk during translocations is sparse (McGill et al., 2010), so managers have very little understanding of 'best practice' techniques. The first step in assessing the potential impact of parasites for a translocation is to survey both the source and destination populations.

Australia provides a unique perspective in understanding the importance and impact of faunal movements on parasites, and the consequences for conservation. The long geographical isolation of Australia has led to the coevolution of unique fauna and associated parasites (Carroll and Fox, 2008). However, in the last 250 years, there have been numerous deliberate and accidental introductions of animals, sometimes with the inadvertent introduction of their associated parasites. Environmental management in Australia now encourages the movement of native fauna within the continent as part of endangered species management, especially in maintaining genetic diversity (Ottewell et al., 2014). However, the consequences of such movements for parasite transmission and spread are rarely considered, nor quantified.

The aim of this review is to outline the extent of faunal movements into and within Australia and highlight the potential for spreading novel parasite infections, so as to inform

future conservation management of Australian wildlife. In this review, I examined the extent of faunal movements associated with conservation management over the past 40 years, and the parasite management measures undertaken for each. These translocations are discussed in the context of disease risks associated with faunal movements since European settlement of Australia (late 18th century); this ranges from the introduction of novel parasites carried by livestock into Australia (Jenkins and Macpherson, 2003), to the role of invasive species to act as potential vectors (Hartigan et al., 2011). By nature of the subject, it is difficult to obtain 'definitive proof' of the true impacts of introduced pathogens (Daszak and Cunningham, 1999), especially where there is limited quantitative or experimental data. However, a lack of modern day empirical evidence does not render retrospective analysis worthless. Any factor capable of causing severe fluctuations in populations may, in a worst-case scenario, contribute to local extinctions (MacPhee and Greenwood, 2013). Collation of these data is relevant for assessing the potential impacts of spreading parasite fauna to new locations for threatened species and their ecosystems.

Data collation and scope of review

I searched Google Scholar for translocations of wildlife in Australia using combinations of the following keywords: translocat*, relocat*, introduc*, releas*, wildlife and Australia. Additional studies were sourced from reference lists, book chapters, publicly available theses and reports, and unpublished data collected by the authors of this paper. References are displayed in *Table 1*.

Recent faunal movements in Australia

Faunal movements for conservation

Fauna translocations are a widespread conservation tool, for the purpose of expanding populations of threatened species (Fischer and Lindenmayer, 2000), mitigating the impacts of development (Maron et al., 2012) introduced predators or climate change (Seddon, 2010). In particular, there has been recent emphasis placed on the anthropogenic movement of animals to maintain and enhance genetic diversity of genetically isolated populations (Reynolds et al., 2013; Ottewell et al., 2014). Translocations normally involve the movement of species of conservation concern (Armstrong and Seddon, 2007) suffering a variety of threatening processes that sometimes include disease (Kock et al., 2010; Boyce et al., 2011).

The primary disease-related risks in translocations are the introduction of novel pathogens into ecosystems, and translocated hosts acquiring novel parasites or pathogens that circulate in the recipient host community. For example, Samuel et al. (1992) showed that elk are capable of hosting a pathogenic meningeal worm (*Parelaphostrongylus*), which could be translocated along with their elk hosts to regions inhabited by naïve elk. On the other hand, endangered Allegheny woodrats (*Neotoma magister*) became infected with the highly pathogenic racoon worm (*Baylisascaris*) when translocated into contaminated environments (LoGiudice et al., 2003). Trichomoniasis (caused by *Trichomonas gallinae,* probably introduced to Mauritius with exotic doves) has hampered recovery efforts of the endemic pink pigeon *Columba mayeri* in both the remnant and translocated populations (Swinnerton et al., 2005). In particular, the initial phase of a translocation, when animals are establishing home ranges, (such as during restocking events), is a time of higher

outbreak risk due to the increased contact between translocated and recipient individuals (Aiello et al., 2014).

While these and other case-studies (Larkin et al., 2003; Jule et al., 2008; Almberg et al., 2010; Larkin et al., 2011) provide *ad hoc* insights into the risks of parasites during translocations, we lack a broad understanding of how the act of translocation impacts on the transmission of parasites, and what the consequences are for both translocated hosts and the recipient ecosystem. Some parasites may be favoured through translocation and will become established, while the disruption of transmission cycles of other parasites may lead to local parasite extinction (MacLeod et al., 2010). Disruption of any aspect of the parasite's life cycle, including transmission mode, intermediate hosts or environmental requirements, may lead to non-establishment of the parasite (Clay, 2003). Invasive species may be experience a demographic release and become a pest when parasites that keep population numbers in check are lost (Clay, 2003; Torchin et al., 2003).

Endemic parasites can have negligible impacts upon host fitness (Davidar and Morton, 1993); however, several factors associated with translocations may alter this balanced association and exacerbate the negative impacts of these same parasites on their hosts. Stress, an inevitable component of translocations, may reduce the immunocompetence of translocated hosts, making them more vulnerable to the negative impacts of parasitism (Teixeira et al., 2007). Overlaid upon this, where parasite transmission occurs between the recipient host community and the immunocompromised hosts, translocations may result in a change in parasite species in the translocated hosts (Almberg et al., 2012) (some of which may be novel to the host), which may increase the incidence of polyparasitism (Bordes and Morand, 2011). Polyparasitism, where individuals have concurrent infections with different parasite species or intraspecific variants, can increase the pathogenicity of infection due to multiple immune challenges on the host

(Ulrich and Schmid-Hempel, 2012; Monteiro et al., 2010). Polyparasitism may also increase competitive interactions between parasite species or strains, promoting increased virulence, as shown in other host-parasite relationships (reviewed in Lymbery and Thompson, 2012). Although polyparasitism is a plausible consequence of the mixing of hosts and parasites during translocations, it has not previously been considered as a potential factor contributing towards translocation failures. Large gaps in our knowledge regarding parasites during translocations are likely to lead to ill-informed management practices.

Australian fauna translocations since 1970

I examined the literature concerning parasites and disease in the Australian fauna translocation literature during the last 40 years (*Table 1*) and identified 58 separate Australian translocations. This list of translocations builds on previous reviews (e.g. Sheean et al., 2011). The list comprises 37 species; 24 species of mammals (65%) seven birds, two reptiles, one frog, one fish and two invertebrate species (*Table 1*). Several species have been subject to multiple translocation efforts, such as the burrowing bettong (*Bettongia lesueur*), which appeared four times, and rufous hare-wallaby (*Lagorchestes hirsutus*), greater bilby (*Macrotis lagotis*) and numbat (*Myrmecobius fasciatus*) all of which appeared three times. It is likely that a large number of additional translocations have occurred, but published information is not available. The majority of animals came from captive colonies (41%), with 21% coming from islands, 10% from reserves, and the remainder from other source locations, such as adjacent land.

Table 1. Published translocations in Australia since 1970, and what health assessments or management strategies were undertaken when considering parasite and disease spread.

I used the terminology from Armstrong and Seddon (2007), who defined a 'translocation' as "any movement of living organisms from one area to another", and categorised three types of translocation:

Introduction: movement of an organism outside its historically known native range.

Reintroduction: intentional movement of an organism into part of its native range from which it has disappeared or become extirpated in historic times.

Re-stocking: movement of individuals to build-up an existing population". I further clarified re-stocking to differentiate those not purely for conservation purposes "human-animal conflict" – for example where an animal is moved within its current range to avoid clearing for development, and "experimental" where animals are moved within their current range for experimental changes in density.

NP, National Park; CP, Conservation Park; NR, Nature Reserve; Is., Island

ACT, Australian Capital Territory; NSW, New South Wales; NT, Northern Territory; QLD, Queensland; SA, South Australia; VIC, Victoria; WA. Western Australia

Reference	Date of relocation	Species relocated	Source location	Destination location	Pathogens reported in this reference	Disease management implemented	Type of translocation
Short et al. (1992)	1971-1981	Tammar wallaby *Macropus eugenii*	Garden Is., WA	Jandakot, WA	*Salmonella* infection	Many quarantined prior to release	Reintroduction
Short et al. (1992)	1972-1988	Quokka *Setonix brachyurus*	Rottnest Is., WA	Jandakot, WA	*Salmonella* infection, disease a factor in decline	Quarantined prior to release	Reintroduction
Short et al. (1992)	1972	Parma wallaby *Macropus parma*	Kawau Is., New Zealand	Pulbah Is., Lake Macquarie, NSW		None reported	Reintroduction
Short et al. (1992)	1974	Banded hare-wallaby *Lagostrophus fasciatus*	Dorre Is., WA	Dirk Hartog Is., WA		None reported	Reintroduction
Short et al. (1992)	1978	Parma wallaby *Macropus parma*	Captive-bred	Robertson, NSW		None reported	Reintroduction
Suckling and Macfarlane (1983)	1979	Sugar glider *Petaurus breviceps*	Captive-bred	Tower Hill State Game Reserve		None reported	Reintroduction
Danks (1997)	Late 1970s to early 1990s	Noisy scrub bird *Atrichornis clamosus*	Captive-bred, and Two People's Bay Nature Reserve, WA	7 sites up to 150km from Albany, WA		None reported	Reintroduction
Miller and Mullette (1985)	1980	Lord Howe Island woodhen *Tricholimnas sylvestris*	Captive breeding centre on Lord Howe Island, NSW	Lord Howe Is., NSW	Bird pox potentially caused initial decline	Captive colony maintained on island to reduce disease risk	Restocking
Short et al. (1992)	1980	Brush-tailed rock wallaby *Petrogale penicillata*	Captive-bred	Wombeyan Caves, NSW		None reported	Reintroduction
Delroy et al. (1986)	1980-1982	Brush-tailed bettong *Bettongia penicillata*	Captive-bred	Five small islands off SA coast		None reported	Reintroduction
Gibson et al. (1995)	1980-1992	Rufous hare-wallaby *Lagorchestes hirsutus*	Captive-bred	Sangster's Bore and Lander River, NT		Routine health checks of captive colony	Reintroduction

Reference	Date of relocation	Species relocated	Source location	Destination location	Pathogens reported in this reference	Disease management implemented	Type of translocation
Southgate (1994; 1995)	1983-1991	Greater bilby *Macrotis lagotis*	Captive-bred	West MacDonnell Ranges NP, and Warrtaka NP, NT		None reported	Reintroduction
Friend and Thomas (1995; Jones et al. (2003)	1985-2001	Numbat *Mymecobius fasciatus*	Captive-bred, wild caught from Dryandra, WA	Boyagin NR, Karroun Hill NR, Tutanning NR, Batalling CP, Dragon Rocks NR, Stirling Range NP, Karakamia Sanctuary, WA, Yookamurra Sanctuary, SA, Scotia Sanctuary, NSW		Post-mortems; one death attributed to an acanthocephalan	Reintroduction
Morris et al. (2003)	1987-2000	Western quoll *Dasyurus geoffroii*	Captive-bred	Julimar CP, Lake Magenta NR, Cape Arid NP, Mount Lindesay NP, Kalbarri NP, WA		None reported	Reintroduction
Ellis et al. (1990)	1988	Koala *Phascolarctos cinereus*	Captive-bred	Mutdapilly region, QLD	*Chlamydia* infection	None reported, but 50% of animals at destination were infected with *Chlamydia psittaci*	Restocking
Priddel and Wheeler (1994)	1988	Malleefowl, *Leipoa ocellata*	Captive-bred	Yalgogrin, NSW		Post-mortems to assess cause of death	Restocking
Backhouse et al. (1995; Serena (1994)	1989-1993	Eastern barred bandicoot *Perameles gunni*	Captive-bred	Gellibrand Hill Park, Hamilton Community Parklands and Mooramong, VIC	*Toxoplasma gondii*	None reported	Reintroduction
(Winnard and Coulson, 2008; Todd et al., 2002)	1989-2005	Eastern barred bandicoot *Perameles gunni*	Captive-bred	Various locations throughout south-western Victoria		None reported	Reintroductions
Priddel and Wheeler (1996)	1990	Malleefowl, *Leipoa ocellata*	Captive-bred	Yathong NR		External assessment of cause of death	Reintroduction
Brown et al. (1994)	1991-1993	Orange – bellied parrot	Captive-bred	Melaleuca, TAS	Parrot beak and feather disease	None reported	Restocking
Soderquist (1994; Olney et al. (1994)	1991-1993	Brush-tailed phascogale *Phascogale tapoatafa*	Captive-bred	Gippsland, VIC		None reported	Reintroduction

Reference	Date of relocation	Species relocated	Source location	Destination location	Pathogens reported in this reference	Disease management implemented	Type of translocation
Christensen and Burrows (1994)	1992	Golden bandicoot *Isoodon auratus*	Barrow Island	Gibson desert NR, WA		None reported	Reintroduction
Christensen and Burrows (1994)	1992	Burrowing bettong *Bettongia lesueur*	Barrow Island	Gibson desert NR, WA		None reported	Reintroduction
Short and Turner (2000)	1992	Burrowing bettong *Bettongia lesueur*	Dorre Is., WA	Heirisson Prong, WA		None reported	Reintroduction
Pietsch (1994)	1992-1993	Common Brushtail Possum *Trichosurus vulpecula*	Melbourne metropolitan area	Resovoir Closed Catchment in Dandenong Ranges, VIC		Health check on capture	Restocking (human-animal conflict)
Hancock and Hughes (1999; 2003)	1993	Common atyid shrimp *Paratya australiensis*	Branch Creek and Kilcoy West Creek, QLD	Within same creek		None reported	Experimental
Clarke and Schedvin (1997)	1994	Noisy miner *Manorina melanocephala*	Lurg district, NE Victoria	Goorambat district, VIC		None reported	Experimental
Priddel and Carlile (2001)	1995	Gould's petrel *Pterodroma leucoptera*	Cabbage Tree Island, NSW	Cabbage Tree Island, NSW		None reported	Experimental
Cooper and Walters (2002)	1996	Brown treecreeper *Climacteris picumnus*	New England Tablelands, NSW	New England Tablelands, NSW		None reported	Experimental
Ebner et al. (2007; 2009)	1996-2000	Trout cod *Maccullochella macquariensis*	Hatchery	Murrumbidgee River near Narrandera, NSW		None reported	Reintroduction
Pople et al. (2001)	1996-1998	Bridled nailtail wallaby *Onychogalea fraenata*	Wild-caught from Taunton (QLD) and Captive-bred	Idalia, QLD	Lumpy jaw	Health checked during trapping	Reintroduction
White and Pyke (2008)	1996-2001	Green and golden bell frog *Litoria aurea*	Captive-bred	Arncliffe, Botany, Collaroy, Marrickville, NSW	Chytrid fungus	Preventative measures to avoid spread of Chytrid fungus	Reintroductions
Anstee and Armstrong (2001)	1997	Pebble Mound Mouse *Pseudomys chapmani*	90km NW of Newman, WA	90km NW of Newman, WA		None reported	Restocking (human-animal conflict)

Reference	Date of relocation	Species relocated	Source location	Destination location	Pathogens reported in this reference	Disease management implemented	Type of translocation
Richards and Short (2003; 2006)	1997	Western barred bandicoot *Perameles bougainville*	Dorre Is., WA	Heirisson Prong, WA	Papilloma-like virus, *Toxoplasma gondii*, parasites	None reported	Reintroduction
Langford and Burbidge (2001)	1998	Rufous hare-wallaby *Lagorchestes hirsutus*	Tanami Desert, NT	Trimouille Is., WA		None reported	Introduction
Moseby and Bice (2004; 2011)	1998-1999	Greater stick-nest rat *Leporillus conditor*	Reevesby Island / Captive-bred	Arid Recovery Reserve, SA		None reported	Reintroduction
Moro (2003; 2006)	1998-2000	Dibbler *Parantechinus apicalis*	Captive-bred	Escape Island, WA	Parasites associated with density of *Mus musculus*	Quarantine, veterinary health check, faecal analysis before and after release	Introduction
Moseby et al. (2011)	1999-2000	Burrowing bettong *Bettongia lesueur*	Herisson Prong, WA / Bernier Island, WA	Arid Recovery Reserve, SA		None reported	Reintroduction
Moseby and O'Donnell (2003; 2011)	2000	Greater bilby *Macrotis lagotis*	Captive-bred	Arid Recovery Reserve, SA		None reported	Reintroduction
Hardman (2007)	2001	Rufous hare-wallaby *Larchestes hirsutus*	Captive-bred	Peron Peninsula, WA		Ivermectin injections, faecal samples monitored, checked for ectoparasites	Reintroduction
Hardman (2007)	2001	Banded hare-wallaby *Lagostrophus fasciatus*	Captive-bred	Peron Peninsula, WA		Ivermectin injections, faecal samples monitored, checked for ectoparasites	Reintroduction
Moseby et al. (2011)	2001	Western barred bandicoot *Perameles bougainville*	Bernier Island, WA	Arid Recovery Reserve, SA	Bandicoot papillomatosis carcinomatosis	None reported	Reintroduction
Priddel and Wheeler (2004)	2001	Brush-tailed bettongs *Bettongia penicillata*	St Peter Is. and Venus Bay CP, SA and Dryandra Woodland, WA	Yathong NR, NSW		Health check on capture, external assessment of cause of death	Reintroduction
Cardoso et al. (2009)	2003	Northern quoll *Dasyurus hallucatus*	Various; wild Northern Territory mainland	Astell Is. and Pobassoo Is., NT		None reported	Introduction
Ebner et al. (2007)	2004	Trout cod *Maccullochella macquariensis*	Hatchery	Murrumbidgee River and Cotter River, ACT		None reported	Reintroduction

Reference	Date of relocation	Species relocated	Source location	Destination location	Pathogens reported in this reference	Disease management implemented	Type of translocation
Mjadwesch and Nally (2008)	2004	Purple copper butterfly *Paralucia spinifera*	Footprint area of roadworks, Lisdale NSW	Outside footprint area, Lisdale, NSW		None reported	Restocking (human-animal conflict)
Moseby et al. (2011)	2004, 2007	Greater bilby *Macrotis lagotis*	Arid Recovery Reserve, SA	Adjacent unfenced reserve, SA		None reported	Reintroduction
Bester and Rusten (2009)	2005	Numbat *Myrmecobius fasciatus*	Scotia, NSW	Arid Recovery Reserve, SA		Dewormed with Ivermectin injection	Reintroduction
Moseby et al. (2011)	2005	Numbat *Mymecobius fasciatus*	Scotia Sanctuary, NSW	Arid Recovery Reserve, SA		None reported	Reintroduction
Daly et al. (2008)	2005-2007	Green and golden bell frog *Litoria aurea*	Captive-bred	Pambula, NSW	Chytrid fungus	Hygiene control protocols using anti-fungicide; frogs swabbed	Reintroduction
Clarke et al. (2013; 2011)	2005-2008	Western ringtail possum *Pseudocheirus occidentalis*	Wild and captive (rescued) from Bunbury area, WA,	Leschenault Peninsula CP and Yalgorup NP	*Toxoplasma gondii, Leptospirosis, Salmonella* and *Chlamydia*	Released and sympatric possums tested for Toxoplasma, leptospirosis, salmonella and chlamydia	Restocking (human-animal conflict)
Moseby et al. (2011)	2007	Woma python *Aspidites ramsayi*	Captive-bred	Arid Recovery Reserve, SA		None reported	Reintroduction
Christie et al. (2011)	2008	Napoleon's skink *Egernia napoleonis*	Near Dwellingup, WA	Nearby restoration sites at Huntly minesite, Dwellingup, WA		None reported	Restocking (human-animal conflict)
Moseby et al. (2011)	2008	Burrowing bettong *Bettongia lesueur*	Arid Recovery Reserve, SA	Adjacent unfenced reserve, SA		None reported	Reintroduction
Schultz et al. (2011)	2008-2010	Brush-tailed rock-wallaby *Petrogale penicillata*	Captive-bred, soft-released into Dunkeld prior to wild release	Moora Moora Creek, Grampians NP, VIC	Lumpy jaw, many parasites	Veterinary examinations before release. Ivomec injections, ectoparasite and blood sample checks. Disease status of sympatric macropods from adjacent areas.	Reintroduction
(Bennett et al., 2012; Bennett et al., 2013)	2009	Brown treecreeper *Climacteris picumnis*	Murrumbidgee region, NSW	Mulligan's Flat NR and Goorooyarroo NR, ACT		None reported	Reintroduction

Reference	Date of relocation	Species relocated	Source location	Destination location	Pathogens reported in this reference	Disease management implemented	Type of translocation
Lott et al. (2012)	2009-2010	Brush-tailed rock-wallaby *Petrogale penicillata*	Captive-bred	Warrumbungles NP, NSW		Faecal samples of wild and captive individuals before and after translocation	Restocking
Muhic et al. (2012)	2011	Black-footed rock-wallaby *Petrogale lateralis*	Captive-bred	Anangu Pitjantjatjara Yankunytjatjara Lands, SA		Health checks	Restocking

Of the 58 translocations, only 19 (33%) considered parasite or disease management during their investigation (*Table 1*), with some reporting an extensive examinations of parasite diversity and loads in the original (10.5%) and recipient communities (3.5%) (e.g. Clarke et al., 2013; Lott et al., 2012). Intervention (e.g. quarantine, screening for specific pathogens or worming) occurred in 25% of these translocations, with the remaining 8% undertaking monitoring only (e.g. visual health check on capture or visual post-mortems). Disease risk management efforts reported by researchers included: (a) planning actions, such as avoiding transmissible diseases by maintaining a captive colony of Lord Howe Island woodhen (*Tricholimnas sylvestris*) on Lord Howe Island, rather than at the supporting zoo (Miller and Mullette, 1985); (b) precautionary plans, such as preventing spread of Chytrid fungus between source and release populations of green and golden bell frogs *Litoria aurea* (White and Pyke, 2008); and (c) enforcing a quarantine period, as reported for captive-bred dibblers (*Parantechinus apicalis*) before release at their new relocation site (Moro, 2003; Short et al., 1992). Some health care presumably went unreported in these papers; for example animals that were captive-bred in zoo facilities probably underwent routine veterinary health checks.

Twelve (20.7%) of the 58 translocations cited disease as a contributing factor of original declines, such as *Chlamydia psittaci* infection in koalas (Ellis et al., 1990), and bandicoot papillomatosis carcinomatosis (*Papillomaviridae* / *Polyomaviridae*) for western barred bandicoots *Perameles bougainville* (Bennett, 2007; Moseby et al., 2011). Two studies (3.5%) recognised disease as a contributing factor for the failure of establishment of green and golden bell frogs (*Litoria aurea*), where chytrid fungus was a serious concern (White and Pyke, 2008; Daly et al., 2008). Another two studies used post-mortems to

assess the cause of death for relocated individuals (Friend and Thomas, 1995; Priddel and Wheeler, 1994); however disease was not identified as a contributing factor. A lack of empirical science during translocations has been recognised in the past (Armstrong and Seddon, 2007), with population and ecosystem level effects of parasites being highlighted as a key aspect of reintroduction biology. Eight studies (10.5%) incorporated parasites in their empirical analysis of translocation outcomes, for example Schultz et al. (2011). However, due to the huge variation in monitoring protocols and detail in reporting, it has proven difficult to assess whether parasites or other infectious agents were important factors impacting the success of these translocations, or whether there were any temporal trends of improvement.

Another confounding factor is that disease is rarely the sole influence for a translocation failure, with predators having the most substantial effect on translocation success in Australia (Sheean et al., 2011; Moseby et al., 2011). Woylie (*Bettongia penicillata*) translocations in Western Australia have been reported to fail primarily due to cat and fox predation (Clayton et al., 2014). However, diseases have been suggested to predispose individuals to predation (Wayne et al., 2013b; Thompson et al., 2014b). Therefore, although the end cause of translocation failure may be predation, the impact of disease may sometimes be masked by predation.

Early fauna introductions

Deliberate introductions linked to agricultural and pastoral enterprise

History helps provide context for understanding the potential impacts of moving parasites with other fauna. The predominant form of faunal movement in a historical context was the importation of livestock into Australia. This era (1788-c.1860) represented the highest potential for parasite introduction because there were repeated importations of large numbers of animals from a variety of sources and quarantine issues were not a major consideration for early settlers.

Animals introduced to Australia for agricultural purposes during the last 250 years brought with them an abundance of associated parasitic organisms (Henderson, 2009), which have had varying effects upon their agricultural animal and the native wildlife of Australia. Since their introduction into Australia, some parasite species such as *Babesia bovis* (the causative agent of babesiosis in cattle) and their ixodid tick vectors have remained an ongoing issue for farmers, while *B. bovis* does not appear to have transferred to native Australian fauna (Bock et al., 2004). Others, such as bush ticks (*Haemaphysalis longicornis*) have transferred from agricultural animals to Australian wildlife, but appear to have negligible effects on their new hosts (Besier and Wroth, 1985).

Some exotic parasites have been transferred to native Australian wildlife, and have established transmission cycles involving their new naïve hosts. An example of this scenario is *Echinococcus granulosus*, which was introduced to Australia at the time of European settlement with the arrival of sheep (*Ovis aries*) (Jenkins and Macpherson, 2003). In becoming naturalised to the Australian ecosystem, *Echinococcus* adopted macropodid

marsupials as new intermediate hosts in its life cycle (Jenkins and Macpherson, 2003). During the two-stage life cycle of this parasite eggs are ingested by an intermediate herbivorous host (which now includes the herbivorous macropodid wildlife of Australia), followed by development of larval hydatid cysts in their lungs (Barnes et al., 2007). These cysts reduce effective lung capacity and predispose the animal to predation, allowing the parasite to infect its definitive canid host (Jenkins and Macpherson, 2003). In Australia, this cycle has been transferred to herbivorous macropodid wildlife via accidental ingestion of *Echinococcus* eggs, passed in the faeces of canids (dingoes *Canis dingo* and feral dogs *C. familiaris*) after they themselves have become infected by ingesting hydatid cysts in infected sheep (Jenkins, 2006). The canid stage of the life cycle has also been found at low prevalence in Australian foxes (Saunders et al., 1995). Thus, a complete life cycle is now present in Australian wildlife, maintaining the parasite on a separate continent.

Echinococcus is now present in a number of macropod species throughout Australia, as well as wombats (*Vombatus urisnus*) and feral pigs (*Sus scrofa*) (Jenkins and Macpherson, 2003). This parasite has been reported to negatively impact Australian wildlife species: pulmonary hydatidosis caused by *Echinococcus* has caused death in captive macropods such as Queensland rock wallabies (*Petrogale persephone* and *P. mareeba*) and nailtail wallabies (*Onychogalea fraenata*) (Johnson et al., 1998) and impaired respiratory function of free-ranging Eastern grey kangaroos (*Macropus giganteus*) and common wallaroos (*Macropus robustus*) (Barnes et al., 2007). For *M. giganteus,* the effective lung capacity for infected individuals was reduced to approximately 55% of normal capacity in males, and 70–80% of normal capacity in females (Barnes et al., 2007). In addition, the distribution and abundance of potential macropod hosts across the

continent provides a significant reservoir of infection for other species (see Jenkins and Macpherson, 2003). Many of Australia's endangered macropods are found in small, isolated, remnant populations (Burbidge et al., 2008) that possess a higher risk of contamination due to nearby reservoir hosts located in surrounding farmland. In particular, there are concerns about the potential negative impact of hydatidosis on the conservation of severely restricted macropod species through infection and consequential increased predation risk (Barnes et al., 2007; Thompson et al., 2010).

Introduction for biological control

One of Australia's most notable introduced species, the cane toad *Rhinella marina* (formerly *Bufo marinus*), has been scrutinised for its potential to carry and disperse parasites. The 101 individual cane toads that founded Australia's deliberately introduced population came from Hawaii, but were themselves descendants of successive translocations from Puerto Rico, Barbados and French Guianan populations (Easteal, 1981). This mixed 'origin' provides the potential to introduce parasites from a variety of countries. The exotic parasites that imported cane toads have been infected with include: four species of intestinal and gall bladder protozoans (Delvinquier and Freeland, 1988), gastric-encysting nematodes, myxosporean parasites (Hartigan et al., 2011; Hartigan et al., 2010), chytrid fungus (Lettoof et al., 2013) and the South American nematode lungworm *Rhabdias pseudosphaerocephala* (Dubey and Shine, 2008). Although *R. pseudosphaerocephala* is capable of infecting Australian native frogs under laboratory conditions (Pizzatto et al., 2010), this nematode does not appear to have negative impacts on their growth, survival or locomotory performance (Pizzatto and Shine, 2011a; Pizzatto

et al., 2010). Despite decades of sympatry, *R. pseudosphaerocephala* has not transferred to native frog species in the wild (Pizzatto et al., 2012), perhaps because it does not complete its life cycle in the native frog species so far examined (Pizzatto et al., 2010). No blood protozoans have been identified from the cane toad (Delvinquier and Freeland, 1988).

The demonstrated dispersal capacity of the cane toad across Australia (Easteal et al., 1985) means that this species of frog has a great potential to facilitate the spread of other disease to the native species of Australia, particularly frogs. Cane toads are capable of hosting an array of parasites, transporting them to new location and providing the opportunity for spill-over into naïve fauna at these new areas. The cane toad has been implicated in the spread of two Australian *Myxidium* species (Hartigan et al., 2011), as well as the Brazilian frog parasite *Myxidium immersum* (Hartigan et al., 2010). Toads appear to be acting as a source for these parasites and effectively distributing them to the threatened native frog population of Australia. Museum material examined by Hartigan et al. (2010) revealed that *M. immersum* first became evident in native frogs after the invasion of the cane toad, and is now found in more than 20 endemic Australian frog species.

Cane toads have also been observed to harbour the endoparasitic pentastomid (*Raillietiella frenata*) and are capable of shedding fully embryonated eggs in their faeces (Kelehear et al., 2013), with cannabalism among competing cane toads appearing to be a method of parasite transmission between individual toads within a population (Pizzatto and Shine, 2011b). This parasite was transferred to the cane toad from its original host, the invasive Asian house gecko *Hemidactylus frenatus* (Ali et al., 1981), which has also become established in Australia. When considering the rapid spread of cane toads across northern Australia in large invasion fronts (Kelehear et al., 2013), this new host provides a

substantial host source for *Raillietiella frenata* and may facilitate an effective transport-system for this parasite to other potentially susceptible Australian fauna. As demonstrated with exotic parasite species, cane toads may similarly be influencing the infection dynamics of native Australian parasites by harbouring and transporting them across the country; however, further research is required to confirm this.

Introduction of companion animals and pest species

The protozoan parasite *Toxoplasma gondii* has become well-established in Australian wildlife (Canfield et al., 1990; Parameswaran, 2008; Pan et al., 2012). It affects a wide range of intermediate and paratenic hosts, with the domestic cat *Felis catus* recognised as its only definitive host. Australian native animals were probably first exposed to this coccidian parasite when cats were released in settlements in the early 1800s (Abbott, 2002; Abbott, 2008; Glen and Dickman, 2014). Cats were also deliberately released *en masse* at multiple locations to control rabbit populations (Dickman, 1996) and they spread rapidly throughout the continent. Since its arrival, *Toxoplasma* appears to have diversified genetically in its Australian hosts, compared to relatively consistent genotypes across North America, South America and Europe (Parameswaran et al., 2009). A high prevalence of *Toxoplasma* infection has been found in a wide range of wild populations without overt clinical symptoms (Pan et al., 2012). Toxoplasma has also been detected in marine mammals such as sea otters (*Enhydra lutris nereis*; Shapiro et al., 2012) and dolphins (Bowater et al., 2003; Roe et al., 2013) as a result of contaminated run-off, with negative population effects. Declines of several terrestrial native species have been correlated with elevated prevalence of *T. gondii* (reviewed by Thompson et al., 2010;

Thompson, 2013), with most evidence for the pathogenic impacts of *Toxoplasma* on Australian wildlife collected from captive animals (Hartley and Dubey, 1991). Despite an apparent lack of deleterious impacts in wild populations, infection with parasites such as *Toxoplasma* could predispose animals to mortality by other means, such as predation or roadkill. Hollings et al. (2013) found seroprevalence of *T. gondii* in Tasmanian mammals was elevated in areas of high cat density, and more prevalent in animals killed on roads than in culled animals. Therefore, feral cats may have an indirect impact on native populations through disease, in addition to direct predation effects.

While this is a notable and relatively well-described example of novel parasitic infection impacting naïve Australian mammals, other, less-documented examples suggest that these impacts are potentially wide-ranging. The release and colonisation of domestic goldfish into freshwater streams has enabled a parasitic copepod *Lernaea cyprinacea* to infect several native fish species, with serious pathogenic effects (Marina et al., 2008). Also, one of the major infectious diseases impacting wombats (*Vombatus ursinus*) is sarcoptic mange caused by the mite *Sarcoptes scabiei,* which is thought to have been introduced with European dogs in the 1800s (Skerratt et al., 1998).

Historical faunal declines associated with parasites

Although introduced parasites continue to persist in Australian wildlife, they probably had most impact during the early stages of European settlement. At each locality of European settlement, Australia experienced significant faunal declines between 1875 and 1925 following the arrival of European settlers and their associated introduced

animals (Burbidge and McKenzie, 1989). Abbott (2006) hypothesised that the drastic declines of Western Australian mammals at this time were exacerbated by the introduction of novel infectious agents. This is supported primarily by anecdotal records from trappers, farmers and local Aboriginal people who noticed these declines, and mention animals dead, dying, weak or "mangey and lousey" during this time frame (Abbott, 2006). On the opposite side of the continent, in south-eastern Australia, formerly abundant quolls (*Dasyurus viverrinus*) were observed suffering from tick infestations and "died off in the hundreds" during the mid to late 1800s, and have not recovered in numbers since (Peacock and Abbott, 2014).

The case of the Christmas Island rats (*Rattus macleari* and *R. nativitatis*) provides evidence of how this sequence of events may have occurred. Black rats *(Rattus rattus),* inadvertently introduced to Christmas Island in 1899 on board British ships, are thought to have ultimately caused the extinction of the island's native rat species *R. macleari* and *R. nativatus* (MacPhee and Greenwood, 2013). The arrival of *R. rattus* also introduced fleas infected with *Trypanosoma lewisi*, which were transferred to the native rodents (Pickering and Norris, 1996; Wyatt et al., 2008). Naturalists working on the island observed significant declines in *R. macleari* and *R. nativitatis*, which displayed clinical signs of trypanosomaisis (Durham, 1908). When autopsied, the Maclear's rats were found to be heavily infected with trypanosomes, which were morphologically similar to *T. lewisi* (Durham, 1908). Much later, *Trypanosoma lewisi* infection was confirmed by genetic sequencing of museum specimens of both native rats and ship rats collected from the island during this time (Wyatt et al., 2008). The two native rodents became extinct within a

period of eight years from the introduction of *R. rattus*, probably in part due to competition and predation, and most likely exacerbated by *T. lewisi* infection (Wyatt et al., 2008).

Trypanosoma lewisi was introduced to mainland Australia in the same way, via the introduced black rat *R. rattus*, brown rat *R. norvegicus*, (Mackerras, 1959) and house mouse *Mus musculus* (Johnston, 1909), and could have played a role in wildlife declines experienced between 1875 and 1925. *Trypanosoma lewisi* and *T. lewisi*-like strains have been identified in several native Australian species; the bush rat *Rattus fuscipes* in southern Queensland (Mackerras, 1958; Mackerras, 1959), water rat *Hydromys chrysogaster* in northern Queensland (Mackerras, 1959), the Shark Bay mouse *Pseudomys fieldi* the bush rat *Rattus fuscipes* and the ash-grey mouse *P. albocinereu*s in south-western Western Australia (Averis et al., 2009). Clinical signs of trypanosomaisis align with the anecdotal reports of sick and dying animals during Western Australia's faunal crash, for example Wansbrough (1919) "I have found *[Trichosurus vulpecula]* in hollow limbs, so weak and wasted as to be unable to get away… in most cases mangey and lousy". Urgent research is required to establish whether *T. lewisi* and *T. lewisi*-like strains are continuing to have similar effect upon the declining native mammal species of Australia.

Although clinical evidence is difficult to pinpoint in all of these historical cases, reports of disease-related population crashes and/or extinctions have accumulated in the last 20 years (MacPhee and Greenwood, 2013; Wyatt et al., 2008; Pickering and Norris, 1996; Laurance et al., 1996; McCallum et al., 2007) providing increasing support for the role of parasitic infections and disease during historical declines of Australian fauna. The faunal declines on mainland Australia at the time of European settlement were likely attributable to a sequence of threatening processes, with feral predators following exotic

diseases and habitat degradation (Johnson, 2006; Abbott, 2008). However, it is unlikely that the full impact of exotic parasites during these early stages of settlement will ever be entirely understood.

Management implications

The recommendations for managing disease risk during translocations have been frequently discussed, and many guidelines and recommendations are proposed (see Griffith et al., 1993; Mathews et al., 2006; Kock et al., 2010; Ewen et al., 2011; Sainsbury and Vaughan-Higgins, 2012; Harrington et al., 2013). The common theme of these recommendations is that a fundamental understanding is lacking for: (1) how parasites are spread during translocations; (2) what is the impact of novel parasite introductions on the translocated hosts and non-target species; and (3) what is the impact of current management strategies and their efficacy for translocation success. The lack of consideration given to disease in the studies reviewed here is surprising given that disease management planning is usually required for translocation proposals undertaken by Australian conservation agencies. Fauna translocations reported in the published literature probably represent a small proportion of those that have actually occurred in the last 40 years, as reporting of translocations has been recognisably poor (Sheean et al., 2011). However, to date, there is insufficient evidence about the role of disease in post-translocation failure to support recommendations in the literature.

Disease transmission is, of course, one of many factors that require consideration during a translocation; however, parasites and their spread by relocated animals have been

unjustifiably neglected, with the limited cases reported in the literature summarised in *Table 1*. Although more immediate, the visible and tangible threats, such as predation risk (Moseby et al., 2011) generally take precedence when it comes to risk mitigation during fauna translocations, it is evident from this review that the long-term impacts of accidental disease transmission are occasional, but can be severe, for both the target species and the destination ecosystem. For example, captive breeding of orange-bellied parrots (*Neophema chrysogaster*) in Tasmania, Australia, was hampered by mortality resulting from psittacine beak and feather disease *Circoviridae* (Ritchie et al., 1989). Although released animals were thought to be free of the disease, in 1991 psittacine beak and feather disease was discovered in the endangered wild population of orange-bellied parrots, already numbering fewer than 200 individuals (Brown et al., 1994). In Queensland, Australia, captive-rehabilitated koalas (*Phascolarctos cinereus*) were released into a wild population where 50% were infected with *Chlamydia psittaci* (Ellis et al., 1990), a pathogen that impacts the success of koala translocations (Lee and Martin, 1988) and has since contributed to the local extinction of koala populations in Victoria and South Australia (Brown et al., 1987; Melzer et al., 2000).

A precautionary approach has generally been advocated, by removing all parasites from translocated hosts to enhance the health of the animal during and after translocation (Ewen et al., 2011), and to avoid the introduction of novel organisms into the destination ecosystem (e.g. Work et al. 2010; but reviewed in Ewen et al. 2011). The rationale behind removing all parasites is to assist with the establishment and survival of the translocated animal, and to avoid the negative effects parasites may have on survivorship and reproduction (Watson, 2013). However, this strategy does not eliminate the risk of those

parasites circulating in the recipient host community spilling back into the translocated animals (Thompson, 2013); (Hartigan et al., 2011). In fact, the absence of parasites from captive-bred animals may inadvertently enhance their susceptibility to parasitic infections during translocations, as they may lack acquired resistance to endemic parasites, as was the case for captive-bred painted dogs reintroduced among wild populations in Africa, with all captive-bred dogs becoming chronically infected with hookworm (Ash, 2011).

The potential impact of parasites on translocation success should be considered alongside the ecological and evolutionary importance as a part of a sustainable population. A growing number of studies acknowledge the ecological and evolutionary importance of parasites in a healthy and sustainable population (Hudson et al., 2006; Hatcher et al., 2012), as well as the large contribution parasites make to global biodiversity (Dobson et al., 2008; Dunn et al., 2009). Of particular consequence for fauna translocations is the growing literature acknowledging the important role parasites play in promoting host immunity during and after translocations (Pizzi, 2009; McGill et al., 2010). Translocations involving captive-bred animals demonstrate that immunological naïvety can be associated with reduced survival following relocation (Jule et al., 2008; Faria et al., 2010; Boyce et al., 2011; Ewen et al., 2011). A growing number of studies suggest the potential value of retaining parasites during translocations, both for enhancing host immunity (Boyce et al., 2011) and re-establishing evolutionary and ecological processes (Almberg et al., 2012), as well as conserving the elements of biodiversity, and ecosystem-services that the parasites provide (Marcogliese, 2004; Gomez et al., 2012).

Disease effects are difficult to quantify because of their cryptic nature, and the fact that they may exacerbate other threatening processes, like predation. With the growing

trend of translocating threatened fauna for conservation purposes, these already vulnerable populations should have careful risk assessments and management in place. This is to ensure that, if disease could be contributing factor during translocation, that this risk is mitigated during this process and that it has minimal impact at the new location. Overall, it should be noted that the knowledge of parasites affecting Australian wildlife is generally poor, especially in determing what is 'normal', and what the survival impacts of parasite burdens could be during stressful situations, such as translocations. As noted by Armstrong and Seddon (2007), considerations about ecosystem impacts of relocated species and their parasites, as well as introduction of novel parasites into new ecosystems are important considerations for conservation managers. However, this is largely hampered by a lack of research quantifying the parasite transfered during translocations, and the consequent impacts on target and non-target hosts. Longitudinal monitoring and health surveillance is therefore recommended to determine the functional impacts of these concerns (Massei et al., 2010), especially where the benefits of such surveillance outweigh the costs to do so. Furthermore, the importance of cataloguing and reporting details of translocations in the pubished literature for purposes of conservation, genetics, and disease management should also be emphasized.

Conclusions and objectives of this thesis

No animal is an island; when an animal is moved to a new location, it may also carry its own parasite 'stowaways' that have the potential to transfer to naïve wildlife at the new destination. The geographical isolation of Australia and recent colonisation by Europeans,

along with the introduction of their associated exotic species makes this continent a compelling case study of how the anthropogenically-mediated movement of animals (agriculture, companionship, and accidental stowaways) can lead to new and novel host-parasite associations. .

More recently, faunal movements have also included a large number of deliberate translocations for conservation. Wildlife translocations have the potential to be impacted by disease in the following ways; (1) reduced survivorship of translocated animals following exposure to new parasites at the relocation site, (2) exacerbation of normally benign parasitic infections due to stressors associated with translocations and (3) the introduction of parasites (by the translocated animals) into the new ecosystem, and therefore changing to the host-parasite dynamics of species existing at the release site (Woodford and Rossiter, 1993; Cunningham, 1996).

Despite these apparent risks, the above analysis of the published literature revealed that fewer than 35% of all Australian fauna translocations reported any level of parasite or disease management (including post mortems or visual health check on capture only), and of these, only 25% included any kind of interventional measure (for example, quarantine procedures or testing for known pathogens). Although many translocations identified disease as a factor in original species declines, few undertook any follow-up monitoring to determine whether disease affected the outcome of the translocation. The importance of parasite and disease risk assessments prior to translocating fauna should be emphasized, in addition to research during post-translocation to quantify the possible impacts of parasites during faunal movements. It is apparent from this review that we currently have a limited understanding of host-parasite dynamics during translocations, and the

potentially deleterious impacts on Australia's native fauna; many of which have undergone serious decline for other reasons.

In this thesis, I aim to address some of these knowledge gaps by examining large numbers of founder animals of two species (boodie *Bettongia lesueur* and golden bandicoot *Isoodon auratus*) from two source populations (Barrow Island native animals and Dryandra captive breeding facility) as a part of a translocation. This work addresses some of these issues by longitudinally following translocated populations of these two mammal species after their release in a predator-proof enclosure at Lorna Glen, Western Australia. I investigated a mammal translocation in close detail and examine the population demographics, disease characteristics and survivorship of the new population. Specifically, I aimed to:

- Experimentally manipulate the ectoparasite and haemoparasite loads of released animals and determine whether these had an impact on post-release survival.
- Determine the parasite species prevalences of the boodies from two source populations and examine how these changed after both populations were mixed.
- Model the population growth and survivorship over time of both species according to different traits.
- Analyse the change in body size and breeding characteristics of 'island dwarf' Barrow Island golden bandicoots following release into uninhabited niches at Lorna Glen and Hermite Island.

I hope to provide further scientific evidence regarding the impact of parasitic infections and diseases during and after translocation, and to investigate the population dynamics of a translocation by longitudinal monitoring.

Chapter 2.

General methods: Translocation study site and species

Lorna Glen

This study took place at Lorna Glen, a former sheep and cattle station of approximately 244 000ha in the Western Australian rangelands, 150km NE of Wiluna (*Figure 1*). The Department of Conservation and Land Management (CALM; now Department of Parks and Wildlife) purchased the station in 2000 for purposes of biodiversity conservation. The proposed Conservation Park extends across the boundary of two IBRA (Interim Biogeographic Regionalisation for Australia) regions; the northern half is within the Gascoyne region, the southern within the Murchison region. The vegetation communities on the station remained in relatively good condition at the time of purchase and the diversity of habitat made it an important addition to the Department's conservation estate.

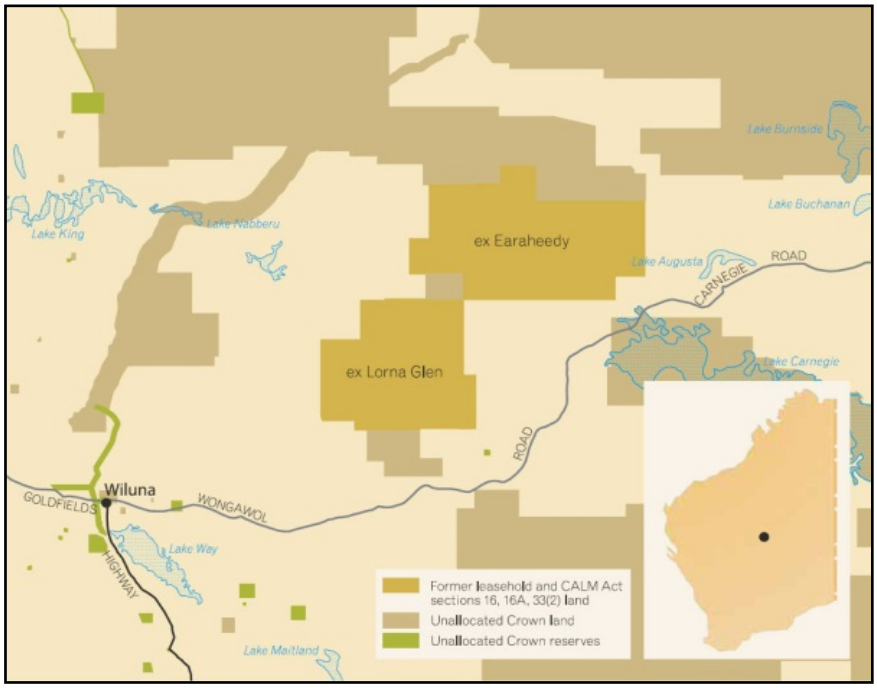

Figure 1. Location of two ex-pastoral leases managed by Department of Parks and Wildlife (Western Australia), showing Lorna Glen (Matuwa) and neighboring Earaheedy (Kurrara Kurrara) relative to the closest town, Wiluna.

Climate and rainfall

The climate is arid, with evaporation exceeding rainfall in all months. In general, the area receives summer rain (*Figure 2*) as a product of monsoonal systems of the north-west of Western Australia, however this rainfall is extremely variable and years of very low rainfall are not unusual (*Figure 3*). On average, 253 ± 11.6 mm falls per year, and monthly temperatures range from 5 to 37 degrees (Data: BOM climate data for Wiluna weather station 1899–2013).

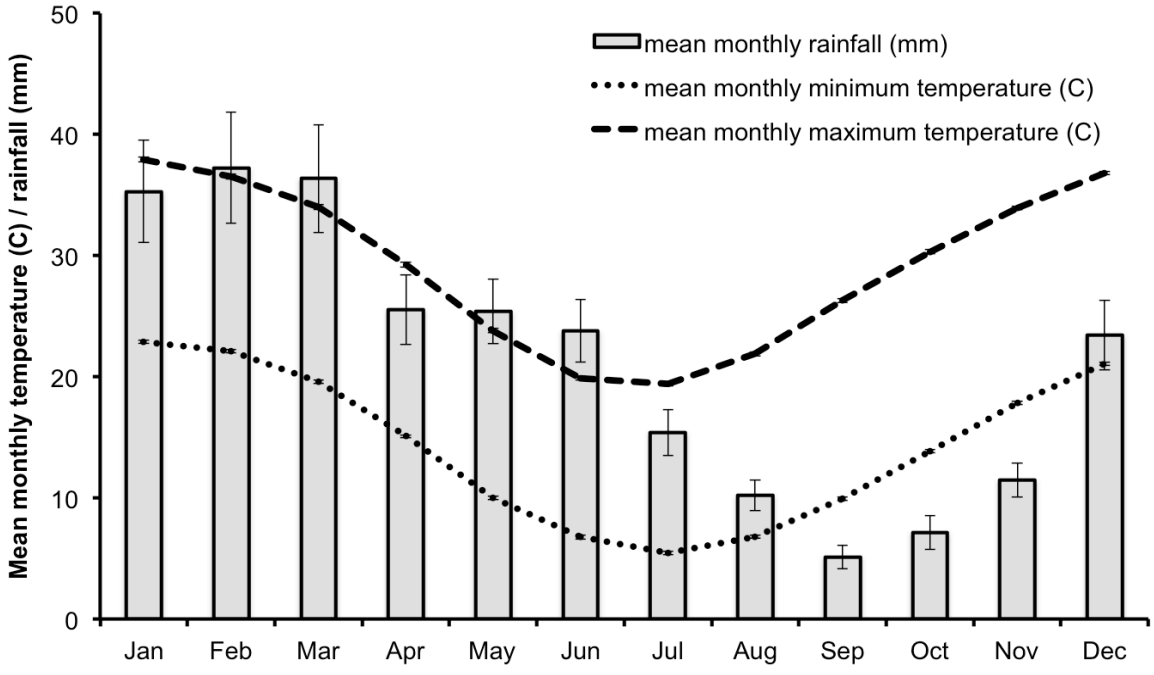

Figure 2. Mean monthly rainfall and temperatures for Wiluna weather station, 1899–2013. Data source; BoM (2013).

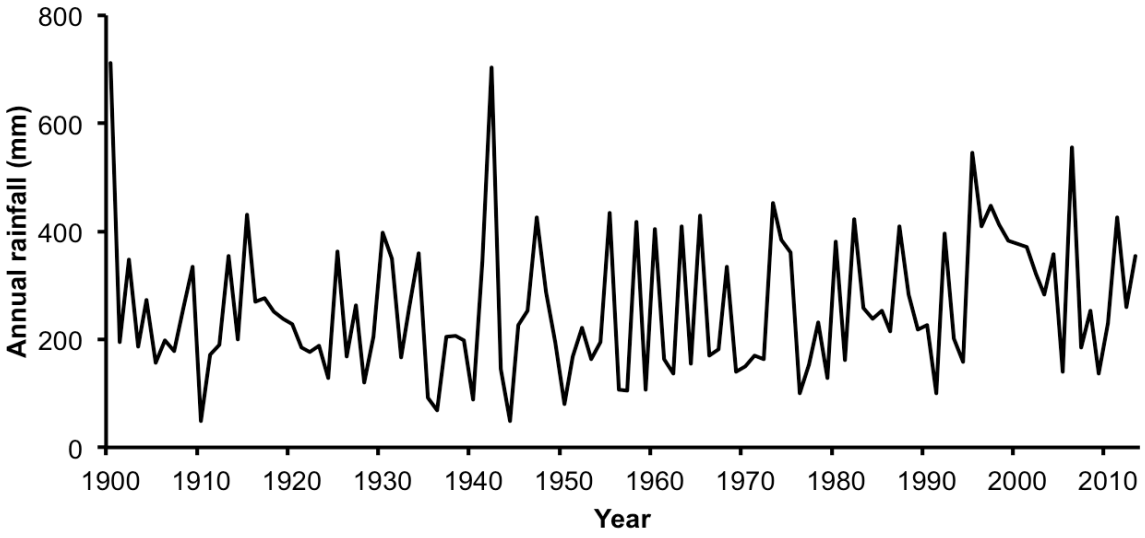

Figure 3. Annual rainfall for Wiluna weather station, 1900–2013. Source: BoM (2013)

Species diversity

The diverse habitat supports a remarkable array of flora and fauna, with 480 vascular plant species and 220 vertebrate species occurring on the property (Cowan, 2008). Throughout the southwestern portion of the property, the landscape is dominated by extensive *Triodia* sandplains with scattered dune systems and sparse Eucalypt overstory (*Eucalyptus gongylocarpa, E. kingsmilli;* Figure 4) (Mabbutt et al., 1963). The north-eastern section of the property features Mulga (*Acacia aneura* complex) claypans, low stony hills and rises and breakaway systems. Creek systems on the property are dominated by large stands of *E. camaldulensis (Figure 5)*.

Figure 4. Bullimore land system that dominates the southern half of Lorna Glen; spinifex (*T. basedowii*) understory and marble gum (*E. gongylocarpa*) overstory.

Figure 5. River gum (*E. camaldulensis*) stand at Lorna Glen.

The extant diversity of small vertebrates is one of the highest recorded in Australia, with records of at least 75 reptile, five frog, 133 bird, four bat, nine or ten dasyurid and four rodent species (Baynes, 2006; Coate, 2010; M. Cowan, pers. com., Feb 2014). Despite this small mammal diversity, only four of the native medium-to-large mammal species remain extant (echidna; *Tachyglossus aculeatus,* red kangaroo *Macropus rufus*, common wallaroo *Macropus robustus* and dingo *Canis dingo*).

Faunal declines in Australia

As is the case for the majority of Australia's arid and semi arid regions, the rangelands have undergone severe faunal declines and extinctions in the last 250 years. Australia has experienced the extinction of 29 native mammals since European settlement in 1788, (Burbidge and McKenzie, 1989; Morton, 1990; Johnson, 2006; McKenzie et al., 2007; Woinarski et al., 2015, #82078). Eight species currently exist only on islands or behind protective fences, and a further 36 species are now restricted to less than 25% of their original distributions (Burbidge et al., 2008). These declines were most extreme in the inland arid regions, having greatest impact on species with ground nesting strategies and those with body weights in the so-called Critical Weight Range of 35–5500 g (Burbidge et al., 2008). Threatening processes identified to be problematic for many species include the spread of introduced predators, especially cats (Christensen and Burrows, 1994; Short and Smith, 1994), changes in the landscape due to fire regimes (Allan and Southgate, 2002; Palmer et al., 2003), reduction in productivity by introduced grazing animals (Burbidge and McKenzie, 1989; Lunney, 2001; Woinarski and Fisher, 2003), and possibly disease

(Abbott, 2006). No one factor explains the decline or extinction of a species, and it is most likely that several threats are imposed simultaneously.

Management of serious declines such as this requires landscape-scale control of threatening processes and habitat restoration. Lorna Glen is a promising example of this concept and since the time of purchase Parks and Wildlife (in collaboration with the local Martu people) has undertaken a number of conservation initiatives to attempt to return the property to pre-European status. Destocking, removal of artificial watering points and construction of boundary fences has reduced grazing pressure and habitat degradation by feral herbivores such as camels (*Camelus dromedarius*), horses (*Equus caballus*), goats (*Capra hircus*) and cattle (*Bos taurus*) as well as stabilising the artificially high population of red kangaroos and common wallaroos. Rabbits (*Oryctolagus cuniculus*) are not generally controlled and live throughout the property. Regular patch burning has been reinstated in order to reduce the risk of homogenising wildfire, as well as to retain diversity in vegetation ages (Muller, 2006).

Feral cat control

A major component of the Lorna Glen Rangelands Restoration project has been feral cat (*Felis catus*) control (Algar and Burrows, 2004). Reduction of cat populations is seen as one of the most important factors for successful Australian mammal reintroductions (Moseby et al., 2011), and Lorna Glen is one of several WA sites undergoing intensive cat control since 2002 (Algar and Burrows, 2004). Toxic 1080 baits, the size and shape of a chipolata sausage (*Eradicat* ®; Department of Parks and Wildlife Bait Factory, Harvey,

Western Australia), were developed for this purpose. Baits are aerially dropped on an annual basis at the rate of 25-50/km^2 during the coldest time of the year. These bait drops probably also reduce populations of dingoes and the occasionally seen red fox (*Vulpes vulpes*). Other predator "by-kill" is not considered to be an issue; no dasyurids are extant at the site, varanids have very high 1080 tolerance (McIlroy et al., 1985) and Mulgara appear to experience a net population benefit from cat baiting, despite attempts at eating the chipolatas (Hamilton et al., 2013). Estimates of reductions in feral cat activity range between 0 and 80%, with effectiveness of cat control via baiting being highly dependent on ratio of cat abundance to small vertebrate prey abundance (Christensen et al., 2013). When small mammals are abundant relative to cat numbers, uptake of poison baits is low. This occurs in years following high rainfall events when small mammal abundance is high, as well as when prey abundance is normal but cat numbers are low. Research into the most effective methods of cat control is ongoing (see also Algar and Burrows, 2004; Algar et al., 2011; Campbell et al., 2011).

Mammal reintroductions

Reintroductions of the locally extinct mammal fauna are the last step in the restoration process, and represent an extension of the WA Parks and Wildlife *Western Shield* project (Wyre, 2004). Sub-fossil records combined with known species distributions were used to determine the pre-European fauna of the area (Baynes, 2006). Thirty-seven non-volant mammal species were identified, of which five are totally extinct, 13 are locally extinct, and 19 are extant or likely to be extant. Twelve of the locally extinct species were determined to be good candidates for the Rangelands Restoration project at Lorna Glen

(see *Table 2*). Several of these species have 'harvestable' populations due to being managed in captive breeding programs or persisting in good populations on islands.

In the presence of adequate cat control (up to 75% reductions; Christensen et al. (2013)), bilbies (*Macrotis lagotis*) and brushtail possums (*Trichosurus vulpecula*) have been successfully reintroduced to Lorna Glen with no further management interventions (Dunlop and Morris, 2009), and mulgara (*Dasycercus blythii*) populations have increased (Hamilton et al., 2013). Mala (*Lagorchestes hirsutus*), released at Lorna Glen in 2008, experienced heavy cat predation (55% of radiocollared individuals) and did not successfully establish (Miller et al., 2011). Mala and other 'cat-sensitive' species such as boodies and golden bandicoots (Christensen and Burrows, 1994; Langford and Burbidge, 2001) were deemed better protected within a fenced enclosure. An 1100ha predator-proof fence was constructed in 2009 (*Figure 6*) according to the decision-making processes described in Bode et al. (2012). The enclosure was declared cat-free after two sessions of four consecutive nights of perimeter track drags and leg-hold trapping for feral cats, using cat faeces and phonids as lures. The enclosure contains habitat from both the Gascoyne and Murchison IBRA regions, and thus supports a variety of animals with differing habitat requirements.

Figure 6. The 1100 ha predator proof enclosure constructed at Lorna Glen.

Table 2. Sub-fossil remains of species known or likely to occur at Lorna Glen, Western Australia. Items in bold indicate species to be reintroduced to Lorna Glen. Modified from Baynes (2006).

Common name	**Taxonomic name**	**Current status at Lorna Glen**	**Remains**
Echidna	*Tachyglossus aculeatus*	Extant	Remains found in breakaways
Kultarr	*Antechinomys laniger*	Extant	Present in owl pellet remains
Mulgara	*Dasycercus sp.*	Extant	Present in owl pellet remains – *D. cristicauda* or *D. blythi*?
Wongai ningaui	*Ningaui ridei*	Extant	Present in owl pellet remains
Woolley's pseudantechinus	*Pseudantechinus woolleyae*	Extant	Present in owl pellet remains
Fat-tailed dunnart	*Sminthopsis crassicaudata*	Extant	Not identified in owl pellets
Hairy-footed dunnart	*Sminthopsis hirtipes*	Extant	Not identified in owl pellets
Long-tailed dunnart	*Sminthopsis longicaudata*	Extant	Not identified in owl pellets
Stripe-faced dunnart	*Sminthopsis macroura*	Extant	Not identified in owl pellets
Ooldea dunnart	*Sminthopsis ooldea*	Extant	Owl pellet remains, confused with *S. dolichura?*
Euro	*Macropus robustus*	Extant	Many remains in caves, live animals seen around breakaways.
Red kangaroo	*Macropus rufus*	Extant	Seen around LG
Spinifex hopping-mouse	*Notomys alexis*	Extant	Present in owl pellet remains
Desert mouse	*Pseudomys desertor*	Extant	Present in owl pellet remains
Sandy inland mouse	*Pseudomys hermannsburgensis*	Extant	Present in owl pellet remains, mummified body found in cave.
Dingo	*Canis dingo*	Extant	Remains found in cave
Little Long-tailed dunnart	*Sminthopsis dolichura*	Possibly extant	Probable record from Millrose, confused with *S. ooldea?*
Lesser hairy-footed dunnart	*Sminthopsis youngsoni*	Possibly extant	Not identified on owl pellets

Common name	Taxonomic name	Current status at Lorna Glen	Remains
Bolam's mouse	*Pseudomys bolami*	Possibly extant	Present in owl pellet remains? Confused with *P. hermannsburgensis*?
Chuditch (western quoll)	***Dasyurus geoffroii***	**Locally extinct**	**No remains found but likely to have occurred at LG**
Red-tailed phascogale	***Phascogale calura***	**Locally extinct**	**Present in owl pellet remains on Millrose Station**
Sandhill dunnart	*Sminthopsis psammophila*	Locally extinct	Not identified in owl pellets, but probably occurred in sand dunes at LG
Numbat	***Myrmecobius fasciatus***	**Locally extinct**	**Not identified in owl pellets but unlikely to be – diurnal.**
Golden bandicoot	***Isoodon auratus***	**Locally extinct**	**Present in owl pellet remains, taxonomic issues with *I. obesulus*.**
Western barred bandicoot	***Perameles bouganville***	**Locally extinct**	**Not identified in owl pellet remains, but likely sand dune fauna**
Greater bilby	***Macrotis lagotis***	**Locally extinct**	**Not identified in owl pellets but likely to have occurred at LG.**
Boodie (burrowing bettong)	***Bettongia lesueur***	**Locally extinct**	**Not identified in owl pellets but many old warrens on LG**
Mala (rufous hare-wallaby)	***Lagorchestes hirsutus***	**Locally extinct**	**Not identified in owl pellets but likely sand dune fauna**
Black-footed rock-wallaby	***Petrogale lateralis***	**Locally extinct**	**Present in old scats found in caves**
Common brushtail possum	***Trichosurus vulpecula***	**Locally extinct**	**Present in owl pellet remains on LG and Millrose, old scats found in caves.**
Shark Bay mouse	***Pseudomys fieldii***	**Locally extinct**	**Present in owl pellet remains from LG and Millrose**
Pale field-rat	***Rattus tunneyi***	**Locally extinct**	**Present in owl pellet remains**
Pig-footed bandicoot	*Chaeropus ecaudatus*	Totally extinct	Present in owl pellet remains
Crescent nailtail wallaby	*Onychogalea lunata*	Totally extinct	May have been recorded in old scats found in caves.
Lesser stick-nest rat	*Leporillus apicalus*	Totally extinct	Present in owl pellet remains, old nests found in breakaways.
Short-tailed hopping- mouse	*Notomys amplus*	Totally extinct	Present in owl pellet remains
Long-tailed hopping- mouse	*Notomys longicaudatus*	Totally extinct	Present in owl pellet remains

In 2010, boodies from Barrow Island and Dryandra and golden bandicoots from Barrow Island were reintroduced to Lorna Glen, within the fenced enclosure. Numbers of animals, dates moved and their origin are detailed in *Table 3*. These animals made up the study species for the following chapters.

Table 3. Translocations of boodies and golden bandicoots to the Lorna Glen predator-proof enclosure in 2010.

	Release Date	**Barrow Island origin**			**Dryandra origin**			**Release**
		F	M	*Total*	F	M	*Total*	**Total**
Boodies *(Bettongia lesueur)*	13/01/2010				5	15	*20*	20
	31/01/2010 - 24/02/2010	26	39	*65*				65
	10/08/2010 - 13/08/2010				44	36	*80*	80
	21/10/2010				7	2	*9*	9
	Total	**26**	**39**	***65***	**56**	**53**	***109***	**174**
Golden Bandicoots *(Isoodon auratus)*	**Release Date**	**Barrow Island origin**						**Release**
		F	M	*Total*				**Total**
	31/01/2010 - 24/02/2010	82	78	160				160
	Total	**82**	**78**	***160***				**160**

Study species

Boodies

The boodie (or burrowing bettong, *Bettongia lesueur*) is one of nine species of Potoroidae, the small to medium sized rat kangaroos within the superfamily Macropodoidae (see *Figure 7*). It is the only macropod that regularly constructs and inhabits warrens (Short and Turner 1993, Burbidge and Short 2008). It is a gregarious animal with several males and females occupying one burrow, suggesting that this is a polygynous species (Sander et al., 1997). Boodie warrens form conspicuous mounds on the surface and contain large numbers of individuals; 20-40 animals inhabit each warren on Barrow Island (see *Figure 8*) (Sander et al., 1997).

Figure 7. A boodie, or burrowing bettong (*Bettongia lesueur*)

Figure 8. A calcrete boodie warren on Barrow Island. Bandicoots live throughout the island, occupying the spinifex (*Triodia angusta*) grasslands visible here.

Figure 9. An occupied boodie warren within the enclosure at Lorna Glen.

Adult boodies have a body weight in the range 600 - 1500g (larger on Bernier and Dorre Islands, smaller on Barrow and Boodie Islands) (Burbidge and Short, 2013). Boodies appear to be opportunistic breeders; on Bernier and Dorre Islands they breed predominantly during the wetter months when more resources are available (Short and Turner, 1993), whereas captive animals with access to artificial food are capable of breeding all year round (Thomas et al., 2003). Females have an oestrus cycle of 23 days and a gestation period of 21 days, with young remaining in the pouch for 115 days (Burbidge, 2008). Females reach sexual maturity at approximately 7-8 months of age; males are probably sexually mature prior to this (Short and Turner, 1999). Young male boodies disperse significantly further than females (4600m *cf*. 1100m, respectively) between the ages of 170 and 250 days, which coincides with the time they are weaned to being sexually mature (Parsons et al., 2002). Individual boodies have been recorded at over four years of age on Dorre Island (wild population) and over six years of age in captivity (Thomas et al., 2003).

Boodies are nocturnal, emerging from their burrows after sunset to feed. On the mainland boodies dig for tubers, bulbs, seeds, nuts and the green parts of plants, while on Barrow Island their diet includes native figs, seeds, roots, termites, carrion and fungi (Burbidge, 2008). Robley et al. (2002) found that reintroduced populations of boodies on Heirisson Prong have a broad diet that is seasonally variable, feeding on fungi, fruits, seeds, forbs, arthropods, stems, shrubs and carrion. Home ranges of boodies have been estimated at approximately 100ha, with males ranging slightly further than females (Robley et al., 2002; Short and Turner, 1999).

Boodies were once one of Australia's most geographically widespread animals, extending throughout most of Western Australia and South Australia and into central Northern Territory, eastern New South Wales and Victoria (*Figure 10*). The species is now extinct on the mainland (unfenced), and free-ranging populations are restricted to Barrow, Boodie, Dorre and Bernier, and Faure Islands off the Western Australian coast (Short and Turner, 1993). It is listed as Vulnerable under the Commonwealth Environment Protection and Biodiversity Conservation Act 1999 (EPBC Act) and Schedule 1 'Fauna that is likely to become extinct or is rare' under provisions of Section 14 of the Western Australian Wildlife Conservation Act 1950 (Richards, 2012).

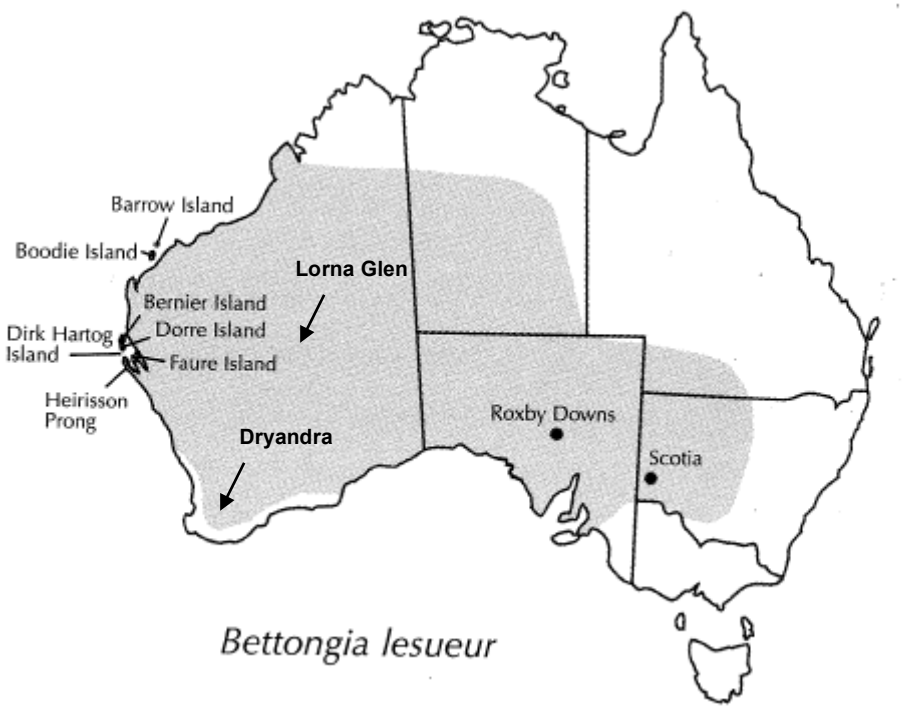

Figure 10. Current (labelled) and historic (shaded) boodie (*Bettongia lesueur*) distribution throughout Australia. For this reintroduction, animals were sourced from Dryandra and Barrow Island, and released at Lorna Glen (Adapted from Burbidge and Short, 2008).

Reintroductions are one of the conservation strategies listed in the national recovery plan for this species (Richards, 2012). Some translocations of this species have already occurred; to Heirisson Prong, Gibson Desert Nature Reserve, Faure and Boodie Islands in Western Australia, and Yookamurra Sanctuary and Roxby Downs in South Australia, with varying degrees of success (Richards, 2012). Prior to this, natural populations of boodies were confined to three islands: Bernier and Dorre Islands in Shark Bay and Barrow Island off the Pilbara coast (Short and Turner, 1993). In 1993, boodies from Barrow Island were successfully reintroduced onto Boodie Island after the population there was accidentally extinguished during a rat eradication campaign in 1985 (Morris, 2002). In 2002, the Australian Wildlife Conservancy successfully translocated them from Heirisson Prong to Faure Island (Page et al., 2012). Boodies have been reintroduced successfully into fenced mainland areas at Heirisson Prong in Shark Bay (Short et al., 1994), Scotia Sanctuary in western NSW and the Arid Recovery Project near Roxby Downs and Yookamurra Sanctuary, South Australia (Richards, 2012).

There have been two unsuccessful releases on the mainland into unfenced areas. Christensen and Burrows (1994) attempted to re-establish the boodie in an unfenced area of the Gibson Desert Nature Reserve, however this translocation failed due to heavy predation by feral cats. An unsuccessful translocation into the Dryandra Woodland also took place in 2003, where approximately half of the 70 released animals succumbed to predation by foxes or feral cats (N. Thomas, pers.com., 2009). Other causes of death included predation by native pythons, raptors and chuditch, unspecified disease causes and roadkill. In the case of this translocation, there were concerns about the animals being

translocated to an area where boodie warrens no longer remained and the animals spent an extended amount of time above ground, making them vulnerable to predation (N. Thomas, pers.com., 2009).

Golden bandicoots

Golden Bandicoots (*Isoodon auratus*) are a medium sized, mostly nocturnal, burrowing marsupial (*Figure 11*). The smallest of the short-nosed bandicoots, Golden Bandicoots weigh between 250 and 670g, putting them in the Critical Weight Range category (Burbidge and McKenzie, 1989) of mammals at greater risk of decline or extinction. Bandicoots are omnivorous, digging for arthropods and tubers. They are capable of breeding year-round, carrying up to 3 young at a time (Southgate et al., 1996).

Figure 11. A golden bandicoot (*Isoodon auratus*)

The taxonomy of the short nosed bandicoots *Isoodon* has been repeatedly revised in the last 60 years as a result of disputes over the specific taxonomic position of *I. auratus.* (Tate, 1948; Lyne and Mort, 1981; Pope et al., 2001; Westerman et al., 2011). Following several moves to group *I. auratus* with the southern brown bandicoot *I. obesulus* (Tate, 1948; Lyne and Mort, 1981; Pope *et al.*, 2001), Westerman et al. (2011) redefined *I. auratus* as a separate species on the basis of nuclear and mitochondrial DNA sequences. They recommend that the *I. auratus* (and subsequently the two subspecies *I. a. barrowensis* and *I. a. auratus*) retain their species status, separate from *I. obesulus*. This study adheres to the most recent classification (Westerman et al., 2011), focusing on *I. a. barrowensis*, translocated from Barrow Island.

Golden Bandicoots were previously widespread throughout a variety of arid and semi-arid habitats of central and northern Australia. They occurred throughout the majority of the Northern Territory, approximately half of Western Australia as well as parts of SA, NSW and QLD (McKenzie *et al.,* 2008). Their current distribution is restricted to Barrow and Middle islands (WA Pilbara), Augustus and Unwin islands (WA Kimberley) Marchinbar Island (NT Arnhem Land) and high rainfall areas of the north-western Kimberley between Yampi Peninsula and Mitchell Plateau (Palmer et al., 2003). The Kimberley mainland populations of Golden Bandicoots are recorded in rocky sandstone habitats and vine thickets in medium to high rainfall areas.

In 1912, “recently dead remains” of Golden Bandicoots were found on Hermite Island, in the Montebellos group (Montague, 1914). Montague (1914) concluded that they had become extinct only recently and attributed this extinction to the recent introduction of feral cats. They have not been recorded on any of the Montebello Islands in subsequent

surveys prior to the translocation (Burbidge et al., 2000). Pre-European skeletal remains of Golden Bandicoots were found at two locations on Lorna Glen and Earaheedy stations (Baynes, 2006). This provides a historical range extension of approximately 200km to the south west of that described by (McKenzie et al., 2008) (see *Figure 12*).

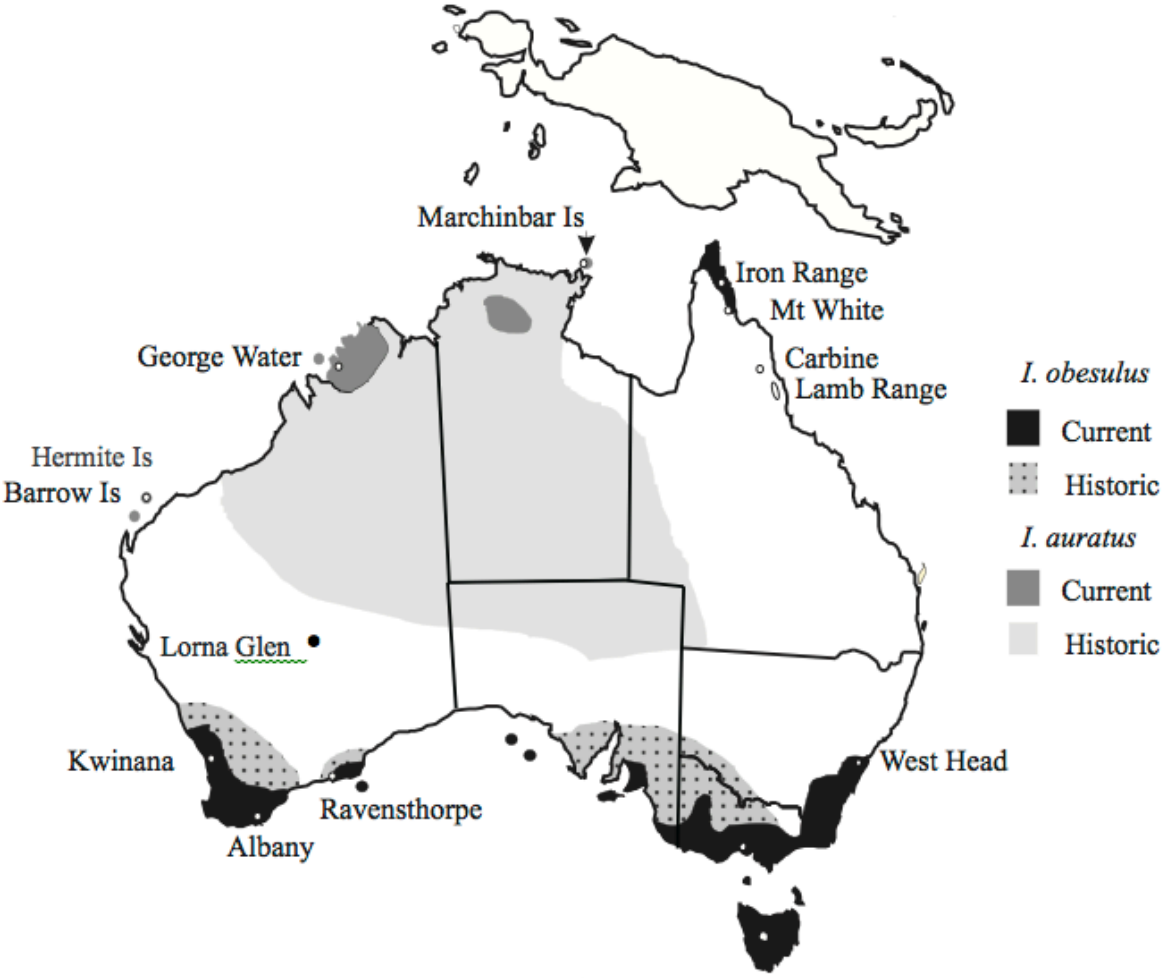

Figure 12. Current and historic distribution of *Isoodon auratus* (Golden Bandicoot) and *I. obesulus* (Southern Brown Bandicoot), adapted from Pope et al. (2001). Fossil evidence at Lorna Glen (Baynes, 2006) provides an extension to the former range of *I. auratus* to the south-west.

Population estimates of the species are uncertain, as there is limited regular monitoring for the species on the mainland. A population of 1400 were estimated to exist on Marchinbar Island in 1994, and this group is currently being considered as a source for translocation to other NT island sites (Palmer et al., 2003). The most well studied population is that on Barrow Island, where annual trapping has been carried out since 1989. It is the most common mammal on Barrow Island, with conservative population estimates at 20 000 (McKenzie et al., 2008).

Translocations

Golden bandicoots were sourced from Barrow Island, and boodies originated from both Barrow Island and the Dryandra captive colony (see *Figure 10* and *Figure 12*). On Barrow Island, animals were removed from the sites to be cleared or disturbed as part of the Gorgon Gas Development (Bamford et al., 2005). Reintroductions to Lorna Glen were part of the Rangelands Restoration fauna reconstruction adaptive management program (Burrows, 2006), and the Gorgon Gas Development Threatened Species Translocation and Reintroduction Program (Morris and Smith, 2010). Trapping of animals on Barrow Island for translocation to Lorna Glen took place over January and February 2010, and at Dryandra in January and August 2010. Animals were transported from their capture site by light aircraft to Lorna Glen and released into spinifex (bandicoots) or into one of the seven boodie warrens (boodies) in the enclosure within 24 hours of capture (*Figure 13*). Travel time from Barrow Island or Dryandra to Lorna Glen was 5-8 hours. Two or three animals were transported in each pet pack, inside individual breathable black cotton bags. Upon

arrival at Lorna Glen, they were checked for general health, injuries, weighed and measured. All individuals were implanted with a unique passive implanted transponder (PIT); Allflex® 12mm FD-X transponder (Allflex Australia, QLD, Australia) subcutaneously between the scapulae and sealed with *Vetbond*™ Tissue Adhesive (3M Australia, NSW, Australia) prior to release.

Figure 13. Local Martu women and children assist with the release of golden bandicoots *Isoodon auratus* at Lorna Glen.

In total, 160 golden bandicoots and 65 boodies were transferred from Barrow Island and released at Lorna Glen. During the same time, 160 golden bandicoots and 111 spectacled hare-wallabies were translocated from Barrow Is to the nearby Montebello Islands (Morris and Smith, 2010). In January and August 2010, an additional 109 boodies

were removed from the Dryandra captive facility and released at Lorna Glen. Animals were examined for ectoparasites and blood samples were taken from as many boodies as possible prior to release at Lorna Glen. This made a total of 160 golden bandicoots and 174 boodies (*Table 3*) at the Lorna Glen predator-proof enclosure as the founder population for the following studies.

Chapter 3.

Sensitivity testing of trypanosome detection by PCR from whole blood samples, using manual and automated DNA extraction methods

Abstract

Automated extraction of DNA for testing of laboratory samples is an attractive alternative to labour-intensive manual methods when higher throughput is required. However, it is important to maintain the maximum detection sensitivity possible to reduce the occurrence of type II errors (false negatives; failure to detect the target when it is present), especially in the biomedical field, where PCR is used for diagnosis. We used blood infected with known concentrations of *Trypanosoma copemani* to test the impact of analysis techniques on trypanosome detection sensitivity by PCR. We compared combinations of a manual and an automated DNA extraction method and two different PCR primer sets to investigate the impact of each on detection levels. Both extraction techniques and specificity of primer sets had a significant impact on detection sensitivity. Samples extracted using the same DNA extraction technique performed substantially differently for each of the separate primer sets. Type I errors (false positives; detection of the target when it is not present), produced by contaminants, were avoided with both extraction methods. This study highlights the importance of testing laboratory techniques with known samples to optimise accuracy of test results.

Introduction

Accurate diagnosis of the haemoprotozoan parasite *Trypanosoma* is of great concern for physicians and veterinarians worldwide, especially in tropical areas of developing countries where particularly pathogenic species exist. Human infection with *Trypanosoma brucei* gives rise to the chronic and acute forms of human African trypanosomiasis (sleeping sickness) via infection with *T. b. gambiense* or *T. b. rhodesiense,* respectively, in sub-Saharan Africa and is fatal if left untreated (Kennedy, 2013). American trypanosomiasis (Chagas disease) is caused by *Trypanosoma cruzi*, and can be fatal in both its chronic and acute forms (Kirchhoff, 1993). There is increasing evidence that trypanosomes are not only problematic for humans and livestock, but may also have impacts on wildlife, especially in cases where other threatening processes are present (e.g. black rhino, *Diceros bicornis*, Clausen, 1981; dromedary camels, *Camelus dromedarius* Mihok et al., 1994; Australian marsupials, Thompson et al., 2009; Thompson et al., 2014b).

In Australia, exotic trypanosome species have been inadvertently introduced with feral species such as the European rabbit *Oryctolagus cuniculus* (Hamilton et al., 2005) and black rats *Rattus rattus* (Pickering and Norris, 1996). These introductions can have potentially devastating results to immunologically naïve native species; for example, the arrival of black rats carrying fleas infected with *Trypanosoma lewisi* is likely to have caused the extinction of the native Maclear's rat *Rattus macleari* and probably Bulldog rat *Rattus nativitatis* on Christmas Island (Wyatt et al., 2008; see also Thompson et al., 2014a). More recently, new endemic trypanosome species have been identified and linked to declines in populations of the endangered woylie, *Bettongia penicillata* (Wayne et al., 2013b;

Thompson et al., 2014b). Accurate assessment of the impacts of trypanosomes on humans, livestock and wildlife relies on sensitive and reliable testing techniques.

Detection of pathogens within biological samples using molecular techniques (most commonly by polymerase chain reaction; PCR) is an efficient and often more sensitive alternative to traditional means, such as blood smear microscopy. It is possible to remove some level of observer error by using molecular detection techniques, and studies have shown situations where molecular techniques detect a pathogen that are missed by more traditional means (Smith et al., 2008). It is estimated that 20-30% of *T. brucei* infections are undetected by traditional microscopy screening methods (Robays et al., 2004). However, molecular techniques are not perfect and require quantifiable sensitivity testing to detect the margin of errors present when analysing biological samples.

PCR is a relatively simple and effective method for detecting trypanosome infections in whole blood samples (Desquesnes and Davila, 2002), with techniques continually being refined. The process of detecting trypanosomes by molecular methods involves extracting DNA in a blood sample (including both host and parasite DNA), then using PCR to exponentially replicate a specific sequence of DNA that is unique to the target (Kirchhoff et al., 1996).

When assessing host response to parasitic infections, it is crucial that detection techniques are reliable and have the best sensitivity and specificity possible. Much effort is concentrated on modification of PCR primers and reaction conditions to optimize specific detection, however there are few studies dealing with the sensitivity and reliability of different extraction methods, particularly using large numbers of replicates of the same samples.

Since the number of trypanosomes can be particularly low in biological specimens (Eastern barred bandicoot, *Perameles gunnii*, Bettiol et al., 1998), the DNA extraction step is particularly important in harvesting the maximum amount of high quality DNA for optimal detection by PCR. Although this study is specific to detecting trypanosomes within whole blood samples, the concept of extracting pathogenic DNA from host samples is common in medical research; for example, detection of *Chlamydia* within tissue samples (Apfalter et al., 2001), bacterial DNA from human faecal samples (McOrist et al., 2002) and fungal pathogens from blood (Loffler et al., 1997).

The DNA extraction process has traditionally involved manual pipetting methodology, with each sample being processed independently. Conventional manual extractions are cheap and often require inexpensive consumables, but can be more time-consuming and may become impractical when processing large numbers of samples. An alternative is small batch robotic DNA extraction systems such as the Maxwell® 16 Instrument (Promega); able to process up to 16 samples simultaneously (Krnajski et al., 2007). Small batch robotic systems may also assist in maintaining consistency in results when compared to variations of manual extraction batches due to human error and experience involved with individual processing.

The goal of our study was to compare the performance and sensitivity of two different nested PCR assays with trypanosome DNA extracted from blood using manual and automated methods. The analysis included diagnostic sensitivity for biological specimens of known concentrations (presence of type II errors), a contamination study (presence of type I errors) and a comparison of extraction times and costs.

Methods

Trypanosome sample preparation

Trypanosoma copemani were collected from infected woylies *(Bettongia penicillata)* via whole blood (Thompson et al., 2013) and cultured in medium. *Trypanosoma copemani* cultures were maintained as per (Botero et al., 2013) until there were adequate numbers of trypanosomes for the current experiment. The concentration of trypanosomes per mL was calculated by counting individual amastigote and trypomastigote forms in 10 µL of media using a haemocytometer chamber. From this known concentration, we diluted the solution with clean media and carefully pipetted appropriate quantities into clean vials. Trypanosome-free horse blood (250 µL) was added to each vial of parasites and mixed thoroughly with a pipette. Samples were stored at -20 °C until thawed for extraction. This work was carried out under the Department of Environment and Conservation animal ethics permit DEC AEC 2010 / 01, and Murdoch University animal ethics permit W2337/10.

Experimental design

The following known concentrations used were; 256, 128, 32, 16, 4 and 0 trypanosomes per 0.3 mL sample. Each of these individual concentrations was replicated 15 times for each of the two different DNA extraction techniques. These values represent a spectrum of very low to moderate levels of parasitaemia in blood (ILRAD, 1983). It was necessary to include 15 replicates of zero parasites for both techniques to ensure that no false positives occurred due to contamination during the experimental protocol.

DNA extraction

We tested two extraction methods; a manual method (Wizard® Genomic DNA Purification Kit, Promega Corporation) and an automated extraction (Maxwell® 16 LEV Blood DNA Kit, Promega Corporation). Both of these kits were designed specifically for DNA extraction from whole blood. Samples were run according to the manufacturer's instructions for frozen blood samples; the Maxwell® 16 Forensic Instrument (Cat.# AS1000) (which is required for the Maxwell® 16 LEV Blood DNA kits) was run using version 4.71 firmware for forensic casework. These two extraction methods differ substantially in price per sample and time required for the extraction (see *Table 4*). DNA was eluted in 50 µL of DNA rehydration solution for both protocols and stored at -20°C prior to use.

Table 4. Cost and time attributes of the two extraction techniques.

Type	*Automated*	*Manual*
Kit name	Maxwell® 16 LEV Blood DNA kit	Wizard® Genomic DNA Purification kit
Cost per sample	$7.50	$1.50
Additional consumables per sample	1 x 1000 µL Pipette tip	2 x 1.5mL Eppendorf tubes, 10 x 1000 µL and 3 x 200 µL pipette tips
Samples processed simultaneously	16	Limited by centrifuge size, usually 24
Total time for extraction	1 hour	2.5 hours
Handling time included	20 mins	2.5 hours

PCR primers and methods

All samples underwent one of two nested PCR protocols; one using *Trypanosoma* genus-specific primers and one using *T. copemani* species-specific primers. Details of the primer sets can be found in *Table 5*. PCR reaction conditions were as per McInnes et al. (2009), except for increasing the annealing temperature in the cycling phase to 56 °C (rather than 52 °C). We used 2 µL of DNA template solution for each reaction.

Table 5. PCR primers used to replicate sections of the Trypanosoma 18S rRNA gene, using nested protocols.

Reaction	Step	Name	Primer sequence	Source	Product size (bp)
T. copemani species-specific reaction	Primary reaction	S825F	5'-ACC GTT TCG GCT TTT GTT GG-3'	(Maslov et al., 1996)	959
		SLIR	5'ACA TTG TAG TGC GCG TGT C-3'	(McInnes et al., 2011)	
	Secondary reaction	WoF	5'- GTG TTG CTT TTT TGG TCT TCA CG-3'	(McInnes et al., 2011)	457
		WoR	5'-CAC AAA GGA GGA AAA AAG GGC-3'	(McInnes et al., 2011)	
Trypanosoma genus specific reaction	Primary reaction	SLF	5'-GCT TGT TTC AAG GAC TTA GC-3'	(McInnes et al., 2009)	1500
		S762R	5'-GAC TTT TGC TTC CTC TAA TG-3'	(Maslov et al., 1996)	
	Secondary reaction	S823F	5'-CGA ACA ACT GCC CTA TCA GC-3'	(Maslov et al., 1996)	904
		S662R	5'-GAC TAC AAT GGT CTC TAA TC-3'	(Maslov et al., 1996)	

There were 15 replicates of each trypanosome concentration, for each extraction technique, and for each primer pair (15 replicates x 7 concentrations x 2 extraction techniques x 2 primer sets = 420 samples in total). The DNA extractions were distributed among 96 well plates for PCR, with each plate containing extractions from both techniques and of varying trypanosome concentrations; plates were prepared this way to eliminate experimental variations of PCR reagents and protocols. Following PCR, each sample was confirmed as positive or negative according to presence of a fluorescent band following agarose gel electrophoresis. We also ran negative controls on each plate for the primary and secondary step of the nested PCR to check for contamination.

Data analysis

The experimental design was a nested three factorial for primer type (species or genus-specific primer) and extraction method (automated or manual) across different trypanosome concentrations. Data were determined to be quasibinomial (residual deviance/degrees of freedom > 1), so a general linear model was constructed of all three factors, and analysed by ANOVA in statistical program R, using package "stats" (Crawley, 2012).

Results

There was a significant relationship between detectability and trypanosome concentration ($F_{1, 26} = 30.13$, $P < 0.001$), where detection success increased with increasing

trypanosome concentration across all treatments. This caused significant positive interactions between concentration and primer ($F_{1, 23} = 7.203$, $P = 0.014$; *Figure 14* and *Figure 15*), as well as concentration and DNA extraction method ($F_{1, 22} = 14.60$, $P < 0.001$; *Figure 14* and *Figure 15*). These relationships differed significantly between the different primers used ($F_{1, 25} = 4.50$, $P = 0.046$) (where species-specific primers had greater success), and different DNA extraction method used ($F_{1, 24} = 8.45$, $P = 0.008$) (where manual extraction proved more successful than automated extraction). There was no interaction of extraction method and primer used ($F_{1, 21} = 56.84$, $P = 0.240$).

By correlating our percentage of successful detection with increasing trypanosome concentration for each of the four combinations of extraction method and primers (see *Table 5*), we were able to interpolate the levels of infection required to produce positive samples with different levels of confidence, as described in *Table 6*.

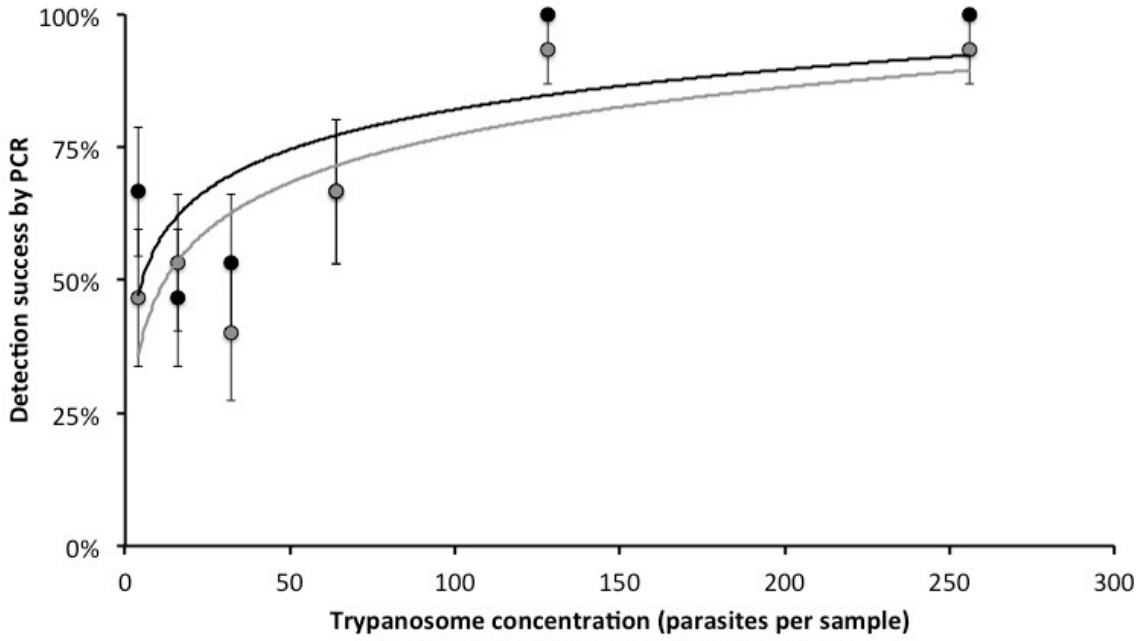

Figure 14. Species-specific primers: Sensitivity levels of *Trypanosoma copemani* detection from whole blood, using two different DNA extraction techniques and species-specific primers. Manual extraction: solid black circles, automated extraction: grey circles. Error bars indicate 95% confidence intervals.

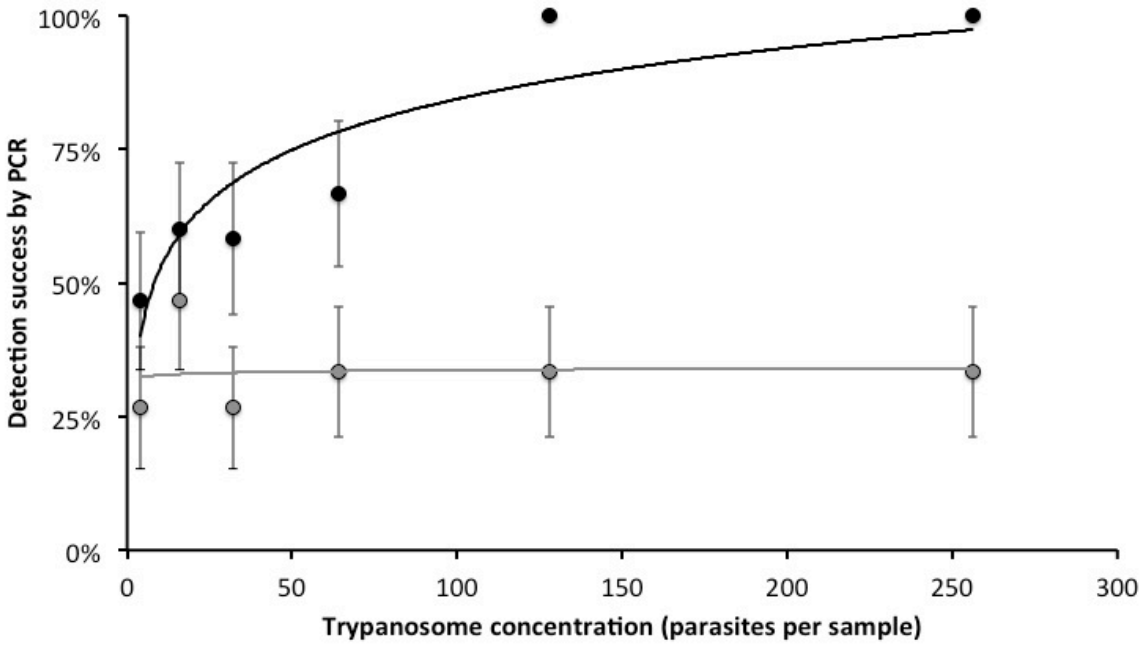

Figure 15. Genus-specific primers: Sensitivity levels of *Trypanosoma copemani* detection from whole blood, using two different DNA extraction techniques and genus-specific primers. Manual extraction: solid black circles, automated extraction: grey circles. Error bars indicate 95% confidence intervals.

From these data we conclude that the order of sensitivity for our protocols (listed here for 95% detection success; see *Table 6*) are;

1. Manual extraction with genus-specific primers; 221.7 trypanosomes per 0.3 mL sample (i.e. a concentration of 739 parasites per mL of blood),
2. Manual extraction with species-specific primers; 320.7 trypanosomes per 0.3 mL sample (i.e. a concentration of 1069 parasites per mL of blood),
3. Automated extraction with species-specific primers; 1324 trypanosomes per 0.3 mL sample (i.e. a concentration of 4413 parasites per ml of blood)

Our manual extraction method proved to be substantially better at detecting low concentrations of parasites, with a 50% chance of detecting ~20 parasites/mL. Neither method produced Type I errors (false positives). From this we can conclude that there was no DNA contamination present for either method.

Table 6. Number of trypanosomes required in a 0.3 mL blood sample for each method, for differing detection confidences.

	Species-specific primers		Genus-specific primers	
Detection sensitivity (%)	**Automated**	**Manual**	**Automated**	**Manual**
95	397.2	320.7	4.15E+68	221.7
80	124.2	81.0	2.20E+52	74.2
75	84.3	51.2	8.00E+46	51.5
60	26.3	12.9	4.15E+30	17.2
50	12.1	5.2	5.75E+19	8.3

Discussion

Our data demonstrate that the analytical sensitivity of trypanosome detection by PCR is significantly affected by both primer type and extraction method used. Many researchers focus on optimising molecular techniques used post extraction, but this study demonstrates the need to check and verify the efficacy of the DNA extraction technique as well. Furthermore, we highlight the importance of quantifying detection sensitivity with multiple replicates of known parasitic concentrations to accurately interpret results.

As expected, there was a significant relationship between trypanosome concentration and detection. Concentrations of 426 parasites per mL or higher were consistently detected. An exception to this was the combination of the manual DNA extraction protocol and the species-specific primers; which had 100% detection for parasite concentrations of 128 per sample or higher.

Overall, the detection sensitivity for our samples was improved by using manual DNA extraction. Regardless of the trypanosome concentration, only 30% of the samples containing trypanosomes presented as positive when extracted using the automated Maxwell® 16 system and screened with the genus-specific primers (*Figure 15*). Although it is likely that higher concentrations of cultured trypanosomes would eventually produce a consistently positive result, for the purposes of biological specimens with potentially low parasitaemia, low concentrations were more meaningful.

Although more time consuming, the manual method tested appears to outperform automated extraction method in trypanosome detection and cost-effectiveness. Other research has shown the Maxwell® 16 automated system to be an effective DNA extraction

method for situations of sampling from an environment that is rich in target DNA (Davis et al., 2012; Foley et al., 2011). In the case of pathological studies where the majority of DNA present is non-target (e.g. host) DNA, manual extraction methods were found to be more successful (Durnez et al., 2009; Affolabi et al., 2012). Perhaps, as appears to be the case with our samples, successful automated protocols are targeting shorter DNA segments.

We identified significantly different detection sensitivities between primer sets for the automated extraction process. Both primer sets targeted the same gene region of trypanosome DNA, with each extraction replicate of a given trypanosome concentration containing a similar numbers of target gene regions. A possible cause for the varying sensitivity of the different primer sets could be the difference in length of the target region of DNA. The target region for the genus-specific primer was 1.5 times the length of the species primer set. Longer amplicons are useful for target specificity – in this case, the 18S gene is common to a variety of taxa (Meyer et al., 2010) and a longer amplicon is useful to differentiate between them. However, as DNA within a sample deteriorates, long DNA fragments become less common and therefore less likely to amplify (Wiegand and Kleiber, 2001; McCarty and Atlas, 1993).

The addition of "host" blood to the replicated samples is important to the process, both for realism and for the creation of a physical DNA pellet required during the manual DNA extraction technique used here. If we used only trypanosomes in media, there would not be sufficient DNA in the solution to create this pellet and the target DNA would have been lost during the DNA extraction protocol. It also adds a more practical aspect to the experiment, as trypanosome detection is usually required from either whole blood or tissue samples, which contain host DNA in excess.

Several other studies have compared DNA yield from automated and manual extraction methods. Although less time consuming, automated methods do not tend to outperform manual extraction methods (Affolabi et al., 2012; Lindner et al., 2011; Durnez et al., 2009). However, Maxwell® appears to be one the most effective low-throughput robotic systems for extracting DNA from samples (Davis et al., 2012), especially when also considering material costs (see *Table 4*).

The varying DNA yield of the two DNA extraction techniques used here is possibly attributable to key differences in the purification process. Generally, manual extraction processes involves chemical and physical removal of cellular proteins and salts, leaving behind purified DNA, whereas automated systems use physical mean, such as paramagnetic particles to attract DNA and move it from well to well during the extraction process (Krnajski et al., 2007). We suspect that this difference may mean the automated system is either less efficient at picking up long, intact chains of DNA, or can become flooded by "host" DNA and therefore, is less likely to pick up relatively low concentrations of target parasitic DNA. Another possible cause could be the presence of some inhibitors from the automated DNA extraction kit that is less favourable for long chain replication.

The final aspect of interpreting detection sensitivity of biological samples is placing some context on whether our detection limits are relevant in a virulence setting. There are limited data, especially in a wildlife context, of what levels of parasitaemia correlate with the occurrence of overt symptoms and virulence of parasitic infections. Wild and domestic animals experimentally infected with *T. congolense* were observed to have parasite loads of up to 10 000 (oryx and waterbuck) and 1 000 000 (eland and cattle) parasites per mL, along with symptoms of anaemia (ILRAD, 1983). In human African trypanosomaisis

(caused by *T. b. gambiense)*, parasitaemia can occur between 100 and 10 000 parasites/mL of blood, with the former concentration being below the detection limits of most molecular techniques (Chappuis et al., 2005). Parasitaemia in the realm of thousands of parasites per mL is likely to be the case for wildlife trypanosomaisis as well; the host animal (*Bettongia penicillata*) that the parasites were collected from was apparently healthy with a blood parasite concentration of up to 2000 trypanosomes per mL (unpublished data), and lived for a further two years after blood sampling. From this information it seems that our best method (manual extraction followed by genus-specific PCR), which is capable of detecting 663 p/mL with 95% confidence, is a reasonable sensitivity for this biological context.

This study highlights the importance of testing laboratory techniques with multiple replicates of known samples to assess detection limits of pathological samples. Furthermore, each step of the laboratory process should be optimised for maximised sensitivity and reduction of type II errors.

Chapter 4.

Host-parasite dynamics of Trypanosomes and ectoparasites on a translocated population of Australian marsupials

Abstract

The host-parasite dynamics of a translocation of two species of Australian marsupials, their ectoparasites and trypanosomes was investigated from initial capture to 18 months post-release. Boodies (*Bettongia lesueur)* sourced from Barrow Island and Dryandra captive breeding enclosure, and golden bandicoots (*Isoodon auratus*) from Barrow Island were released into a large predator-proof enclosure at Lorna Glen, Western Australia. Individuals were randomly assigned to the "treated" and "untreated" groups; treated animals were dosed with the topical antiparasitic Selamectin at 6mg/kg at each six-weekly monitoring session in which they were captured. Ectoparasites were collected from all animals where present, and blood samples were taken from boodies in order to detect trypanosome prevalence in the populations. Boodies from Barrow Island were found to be harbouring both *Trypanosoma copemani* and *T. vegrandis* at the time of release, whereas only *T. copemani* was detected in animals of Dryandra origin. After six months, *T. vegrandis* was also detected in Dryanda boodies, and both trypanosome species were detected in offspring born at Lorna Glen, indicating successful transmission between individuals within the population. Of the original seven species of ectoparasite translocated with the mammals (four tick, one louse, one mite and one flea species), only three persisted to the following year. Ectoparasite prevalence was generally low for both mammal species, and was affected by time factors (time since release and season) but not treatment group. Likewise; treated animals did not show different levels of trypanosome prevalence. Survivorship analyses by program MARK did not differentiate treated and untreated animals, indicating that the antiparasitic treatment did not improve or reduce animal survivorship over the 18-month timeframe.

The circumstances of this translocation allowed for an unusually high level of regular animal recapture to enable regular antiparasitic treatment; even in this situation there appeared to be no management advantage to these species in doing so. It is possible that the treated and untreated groupings were confounded by sharing of the treatment, as the topically applied antiparasitic can be transferred from the fur of an animal during physical contact immediately after application. Translocation had a substantial impact on the parasite community of translocated hosts, and this impact persisted into the next generation of hosts born at the translocation site, creating a host-parasite community different to that of either source site. This study demonstrates the re-equilibration of host-parasite interactions following a substantial disturbance event, where some species are lost and others expand into new populations.

Introduction

Relocating animals has become an increasingly widespread and important method of restoring ecosystems and establishing self-sustaining populations, especially throughout the developed world (Bajomi et al., 2010). Species may be moved for conservation management (Abbott, 2000; Bajomi et al., 2010), to supplement recreational hunting (Linnel et al., 1997) or to resolve human-animal conflicts (Tribe and Brown, 2000; Massei et al., 2010). Despite the increased frequency of species translocations, the use of quantitative experimental manipulation to answer questions about best practice methods has been uncommon (Seddon et al., 2007). Armstrong and Seddon (2007) reviewed reintroduction literature and identified a number of important knowledge gaps. Among

these were questions regarding optimal pre- and post-release management, and the impact of parasites on the translocated population. To understand these questions, longitudinal monitoring should occur following the movement of a population (Massei et al., 2010).

Translocated wildlife provides an unusual scenario they may be affected by parasites in different ways to wild populations. Translocated animals usually undergo conservation actions because of their inherent rarity and/or importance. They may also be genetically bottlenecked, held in unnatural situations for undefined periods of time (for example in dense captive breeding programs), subject to stress of capture, handling and transport, and require time to acclimate to a particular habitat/climate which differs to that of their original habitat. Combinations of these factors, and the related stress upon the translocated animal may result in an increase in the impact of parasitism upon the host when compared to what may be expected under more natural conditions.

The significant physiological stress caused by translocations (Mathews et al., 2006; Teixeira et al., 2007), combined with stresses caused by nutritional challenges and establishment at a new site can contribute to susceptibility and severity of infection by parasites (White and Timms, 1994, #40343 Davey et al., 2006; Pedersen and Greives, 2008). This combination of factors may reduce survival rates and fitness substantially for a population that is required to grow and establish in a new environment. For example, the presence of the parasitic meningeal worm (*Parelaphotrongylus tenuis*) at the site of establishing elk (*Cervus elephas*) populations has been shown to hinder translocation efforts (Larkin et al., 2003). Translocated hosts may also spread parasites to other hosts in their new environment. Domestic reindeer (*Rangifer tarandus*) translocated to Greenland from Norway in 1952 transferred the warble fly (*Oedemagna tarandi*) and nostril fly

(*Cephenemyia trompe*) to the native subspecies of reindeer and caused large population declines through increased winter mortality (Kock et al., 2010, #11861).

Not all host-parasite interactions are negative, however; there may be indirect benefits to the individual animal, its conspecifics, or other species present at the establishing site (Robinson et al., 2010). For example, it has recently been demonstrated that the presence of some non-pathogenic species of *Taenia* tapeworms suppress transmission of the more pathogenic *T. solium* in pigs, dogs and humans in Laos (Conlan et al., 2012). Therefore, blanket eradication of parasites may negatively affect host immunity from other pathogens, as well as potentially impacting conservation of species diversity. From a species conservation perspective, an individual animal should be considered as an ecosystem in itself, hosting a variety of parasite fauna that may be unique to that species (Dobson et al., 2008). For this reason, cirl buntings (*Emberiza cirlus*) in the United Kingdom underwent health measures to reduce (but not completely remove) the coccidian parasite *Isospora normanlevini*, as they were recognised to be a natural part of the birds' endemic fauna (McGill et al., 2010). There is consensus in the literature that the role of parasites needs to be carefully considered in translocation planning and implementation (Gómez and Nichols, 2013; Jorgensen, 2014).

One common group of parasites identified as particularly detrimental to naïve hosts are trypanosomes, a ubiquitous group of potentially pathogenic single celled haemoparasites (Singla et al., 2003; MacPhee and Greenwood, 2013). Transmitted by various haematophagous ectoparasitic vectors, *Trypanosoma* species are associated with pathogenic illness in humans, including *T. cruzi* (Chaga's disease) and *T. brucei* (African sleeping sickness). Trypanosome infections also vary in virulence in non-human animals,

ranging from death to severe clinical disease to no measurable effect (Clausen, 1981; Mihok et al., 1994; Reid et al., 2001). They can also influence population abundances of immunologically-naive animals that have had no previous exposure to trypanosomes. For example, the murid-specific *T. lewisi* was introduced inadvertently to Christmas Island by a flea vector carried by *Rattus rattus*, and is thought to have been responsible for the extinction of the endemic rat species *R. macleari* and *R. nativitatis* (MacPhee and Greenwood, 2013).

Many Australian marsupials carry trypanosomes (Averis et al., 2009), as well as a variety of ectoparasites that are thought to act as vectors (Roberts, 1960; Roberts, 1963; Adams et al., 2004; Bennett, 2007; Thompson et al., 2010; Austen et al., 2011, #47352). Although sometimes innocuous, where balances between the pathogen and the host have been disrupted, trypanosomes can be pathogenic. They have been associated with the decline of a species of bettong; the woylie, *Bettongia pencillata*, (Smith et al., 2008; Averis et al., 2009; Thompson et al., 2013), although causal links have not been established. Therefore, understanding the transmission of trypanosomes in wildlife populations is important, particularly during inherently stressful situations such as translocations. I focused on ectoparasitic vectors of trypanosomes because ticks and fleas have been identified as common vectors of *T. copemani* and *T. vegrandis*, the two species in question (Austen et al., 2011; Thompson, 2014).

In this study, I undertook detailed monitoring of the ectoparasite infection dynamics of boodies (*Bettongia lesueur*) and golden bandicoots (*Isoodon auratus*) following their translocation to a predator-proof enclosure in central Western Australia. Boodies were translocated from two different source populations and were identified as being naturally

infected with different combinations of trypanosome species, enabling us to investigate potential cross-transmission and survival implications of trypanosome infections.

The specific hypotheses for this experiment were;

1) Ectoparasites present in the system should enable transmission of trypanosomes among hosts from different source populations.

2) A general antiparasitic treatment would disrupt the ectoparasite life cycle sufficiently to significantly reduce ectoparasite burdens in treated hosts, and reduce the prevalence of trypanosome infection.

3) These would have a flow-on effect to improve survivorship of the translocated animals receiving the antiparasitic treatment.

This study provides new insights into the host-parasite dynamics of a translocated population, and the management implications of moving parasites along with their mammalian hosts.

Methods

Study Species

Golden bandicoots (Marsupialia: Peramelidae) are small (200–650 g) marsupials that use their strong forepaws to dig up arthropods and tubers (McKenzie et al., 2008). Golden bandicoots are nominally solitary but have been observed to use burrows created by boodies (pers. obs.). Boodies (Marsupialia: Potoroidae) are the only species of regularly burrowing macropods, weighing between 600 and 2000 g (Burbidge, 2008). They create

extensive warrens in sand or under calcrete caps that are present throughout the semi-arid zones of Australia. Both species were historically abundant throughout the majority of the Australian continent, but have suffered extraordinary declines and are currently present in less than 2% of their former habitats (Short and Turner, 1993; Palmer et al., 2003; Burbidge and Short, 2013; McKenzie et al., 2013). In an effort to enhance the conservation status of these two marsupials, a large-scale translocation program was conducted (Miller et al., 2011), whereby large numbers of both were reintroduced to an area of their former habitat on mainland Australia.

Translocations and monitoring

In January 2010, 160 golden bandicoots (*Isoodon auratus*) and 67 boodies (*Bettongia lesueur*) from Barrow Island (20°46' S, 115°23'E) were released in a predator proof enclosure at Lorna Glen, Western Australia (26°12' S, 121°34' E), an ex-pastoral station within both species' former ranges. Lorna Glen has an extensive small mammal assemblage, including 12 species (eight dasyurids, four rodents,) that can move freely in and out of the enclosure due to being smaller than the wire gauge. The boodie population was later supplemented with an additional 109 individuals relocated from the Dryandra captive breeding enclosure (32°47'S, 116°58'E) in August 2010. During the course of this study, animals were born into the population and are referred to as of Lorna Glen origin.

For 18 months following the initial translocation in January 2010, animals were re-trapped on a six weekly basis for a total of 11 trapping sessions. Eighty-one wire cage traps baited with peanut butter and rolled oats were set within the enclosure for six nights (486

trap-nights per session). Animals were weighed, measured and checked for reproductive activity. Each animal was only processed on its first capture for each trapping session. All founder animals were released with a unique passive implanted transponder (PIT); Allflex® 12mm FD-X transponder (Allflex Australia, QLD, Australia) subcutaneously between the scapulae and sealed with *Vetbond*™ Tissue Adhesive (3M Australia, NSW, Australia). Newly captured individuals were implanted with a PIT on their first capture.

Each translocated individual animal was randomly assigned to 'treatment' or 'non-treatment' groups. Animals of Lorna Glen origin were assigned alternately as 'treatment' or 'non-treatment' as they were encountered. Those in the treatment group received a 6mg/kg dose of topical antiparasitic treatment (Revolution, 60 mg/mL selamectin, Pfizer) on release and subsequently on first capture within each follow-up trapping session. Selamectin is an Avermectin group compound used as a broad-spectrum endocide that is effective against fleas, ticks, intestinal hookworms and ascarids, and immature heartworms (Bishop et al., 2000). It has been shown to be effective against existing infestations of ectoparasites (Benchaoui et al., 2000) and safe for mammals, even at higher than recommended dosage (Krautmann et al., 2000; Novotny et al., 2000). As antiparasitic toxic effects have not been tested on marsupials, Selamectin was chosen in this study due to its low toxicity in other mammals, as well as for its non-invasive topical application. For the purposes of this study, Selamectin serves as a general antiparasitic that targets the likely vectors of the trypanosomes being studied in this experiment.

Sample collection

At each capture, I inspected animals for ectoparasites, with particular attention to areas around the tail, ears and genitals. If present, up to 20 specimens were collected and

stored in 70% ethanol until identified to species (where possible) using keys developed by (Roberts, 1963; Roberts, 1970) for ticks (Arachnida: Parasitiformes), Dunnet and Nardon (1974), for fleas (Insecta: Siphonaptera), Domrow and Lester (1985) for mites (Arachnida: Acari), and Calaby (1970; 1971) for lice (Insecta: Phthiraptera). Samples were also checked against specimens held at the WA museum. Ectoparasites burden was originally graded according to the level of infestation (0 = none seen; 1 = 1-20 present; 2 = 20+ present) however the rate of individuals having 20+ parasites was so low (boodies; 12/1035, bandicoots; 0/967 occasions) that I simplified this to presence or absence of ectoparasites for our analyses.

Whole blood was collected from the lateral tail vein of boodies (0.3 to 0.5mL of blood collected in EDTA tubes and stored at -20°C) to analyse for *Trypanosoma* infection using PCR (described in section 2.4). Blood smears were also made at time of blood collection for visual identification of trypanosomes. It was not possible to collect similar volumes of whole blood from conscious golden bandicoots to enable reliable detection of trypanosomes using PCR, therefore our analyses of trypanosome infection patterns were restricted to boodies only.

Sample analysis

Genomic DNA was extracted from whole blood samples using the Wizard® Genomic DNA Purification Kit (Promega, Madison USA) according to the manufacturer's instructions for a 300μL frozen whole blood sample. Each DNA sample was eluted into 50µL of DNA rehydration solution and stored at -20°C prior to further analysis by PCR.

A sample of 1μL of DNA extract was used as the template in a nested PCR using species-specific trypanosome oligonucleotides. For the *T. copemani* reaction, the primers used were: (Primary) S825F 5'-ACC GTT TCG GCT TTT GTT GG-3' (Maslov et al., 1996) and SLIR 5'ACA TTG TAG TGC GCG TGT C-3' (McInnes et al., 2009) (Secondary) WoF 5'- GTG TTG CTT TTT TGG TCT TCA CG-3' and WoR 5'-CAC AAA GGA GGA AAA AAG GGC-3' (McInnes et al., 2011). For the *T. vegrandis* reaction, the primers used were: (External) TVEF 5'-GGGGTCCTTTTATTTTATTTG -3' and TVER 5'- TAATTTATTGGCCAGACAAA -3' and (Internal) TVIF (5′- GACCAAAAACGTGCACGTG -3′) and TVIR (5′- AAATCGTCTCCGCTTTAAC AC-3′) (Botero et al., 2013). For reaction conditions, see Dunlop et al. (2014) and (Thompson et al., 2013). Details of the detection threshold of these methods for trypanosomes in whole blood samples can be found in Dunlop et al. (2014)

Amplified products were separated on a 1.5% agarose gel and run at 90 V for 45 min in Tris-acetate-EDTA buffer and visualized under UV light. Samples producing a band of approximately 400bp (*T. copemani*) and 350bp (*T. vegrandis*) were considered positive. Approximately 50% of positive results were sequenced to verify their species and clade.

Data analysis

We examined the factors influencing the ectoparasite dynamics post-translocation of boodies and golden bandicoots using a generalised linear mixed effects model (GLMM) with animal ID included as a random variable to account for multiple captures of the same individual. I used data for presence or absence of fleas, ticks, lice, mites for each host animal on its first capture per session, and for boodies, also included presence or absence

of *T. copemani* and *T. vegrandis.* For all models, presence or absence (1/0) was the dependent variable, with binomial error distribution. I discarded recaptures within the same trapping session. This gave me a dataset of 1035 boodie and 967 bandicoot capture events included, from 4043 total captures. Animals were therefore considered to be infected or not infected per trapping session.

For boodies, the independent variables for each model included animal origin (Barrow Island, Dryandra or Lorna Glen), flea treatment group (treated or untreated), presence of ectoparasites (each group coded as 0 or 1), season (summer, autumn, winter, spring), trapping session (where the release is session 1), sex, and presence of *T. copemani* or *T. vegrandis* (0 or 1). For bandicoots, I analysed animal origin (Barrow Island or Lorna Glen), flea treatment group (treated or untreated), presence of other ectoparasites (each group coded as 0 or 1), season (summer, autumn, winter, spring), trapping session (where the release is session 1) and sex. All analyses were conducted with R, using the LME4 and MULTCOMP packages. Only main effects were considered, and where variables had significant effects, differences in infection rates were compared among groups using Tukey's HSD.

In order to find out whether antiparasitic treatment or presence of trypanosomes was having an effect on survival, I analysed the capture-recapture data using MARK 2.1 (Cooch and White, 2013). The Cormack-Jolly-Seber (CJS) model (Lebreton *et al*., 1992) was used to estimate survival and recapture probability for different group characteristics over time. Survival probabilities refer to the chance of the animal surviving the interval from one trapping session to the next. The fully parameterised CJS model allows survival and

capture probabilities to differ between the groups for each time period. The model can then be simplified with different constraints to find the one most parsimonious to the data.

For boodies, I analysed the data twice, first with four groups; two antiparasitic treatment groups and two *T. copemani* infection groups, and secondly with two antiparasitic treatment groups and two *T. vegrandis* infection. For bandicoots, only the two antiparasitic treatment groups were used. Data from 11 sampling sessions were used in the analysis and there were 10 time intervals corresponding to the periods between each session. One of these intervals (December 2010 to Feb 2011) was three times the length of the others, as I did not trap in the peak of summer. The model accounts for this longer interval. In Chapter 6, I further examine survivorship, recapture probabilities and population growth parameters.

Results

Trapping data

The study encompassed 4010 captures of 222 individual boodies and 257 individual bandicoots, from 5346 trap nights across an 18-month period. Average capture rates were 37.3% and 38.4% for bandicoots and boodies, respectively (75.7% total). Each bandicoot was captured an average of 8.57 (± 0.65) occasions and each boodie was captured an average of 8.74 (± 0.67) occasions. Forty-eight boodies and 97 bandicoots born into the population were also captured during the study. Animals were only measured and sampled for parasites on the first capture of each trapping session, so excluding recaptures within

the same trapping period left 920 capture occasions for boodies and 967 capture occasions for bandicoots.

Ectoparasite prevalence and species richness

Although presence or absence of ectoparasites on both mammal species appears to be primarily affected by season, there was an overall decline in parasite species diversity over time (*Table 7*). Of the original seven species of ectoparasite translocated with the mammals (four tick, one louse, one mite and one flea species), only three persisted to the following year (*Figure 16*). Boodies were released with seven ectoparasite species, of which three persisted ($F_{1,9}$=2.67, P=0.009). The decline in species diversity from three species to one was not significant for bandicoots ($F_{1,9}$=2.67, P=0.14).

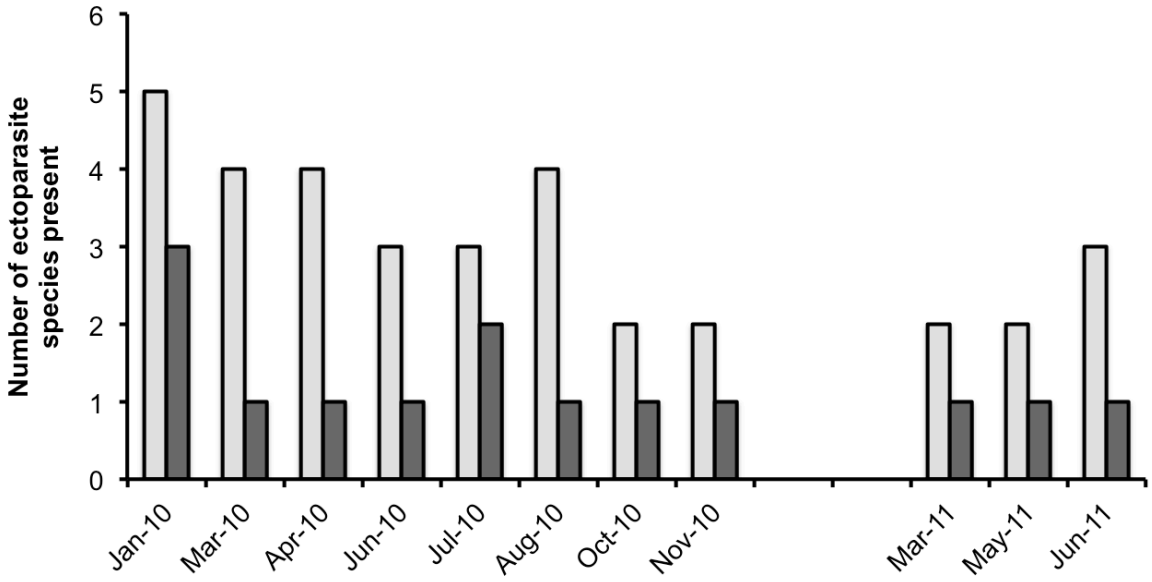

Figure 16. Number of ectoparasite species observed on the translocated populations of boodies (*Bettongia lesueur*: pale) and golden bandicoots (*Isoodon auratus*: dark). Releases occurred in

January 2010 (both mammal species) and August 2010 (boodies only). A significant decline in parasite species diversity was observed for boodies.

Table 7. Ectoparasites observed during the translocation and subsequent monitoring of two marsupial species, the golden bandicoot *Isoodon auratus* and the boodie *Bettongia lesueur*. Animals were first released in January and February 2010, with a follow up release of boodies in August 2010. Parasite origin was recorded when parasites were collected before release at the translocation site. Parasites were considered to have been transmitted to conspecifics if they were known only from one population origin, but were later found on animals of a different origin, or on offspring born at the translocation site. Animals were trapped approximately every 6 weeks for 18 months following release. Number of occurrences and overall prevalence refer to the times each parasite was observed from a potential 967 capture occasions for golden bandicoots and 1035 for boodies.

	Ectoparasite	Host species	Number of occurrences	Overall Prevalence (%)	Parasite Origin	Date last observed	Transmitted to conspecifics?
TICKS	*Amblyomma australiense*	*B. lesueur*	1	1.1	Dryandra	Feb 2010 (at release only)	No
	Amblyomma limbatum	*B. lesueur*	12	1.2	Barrow Island	Jul 2010	Yes
	Haemaphysalis humerosa	*I. obesulus*	76	9.7	Barrow Island	Persisted	Yes
		B. lesueur	89	0.3	Barrow Island	Persisted	Yes
	Haemaphysalis ratti	*B. lesueur*	3	3.3	Unknown	Persisted	Unknown
MITES	*Schoutedenichia emphyla*	*I. obesulus*	2	5.2	Barrow Island	Feb 2010 (at release only)	No
		B. lesueur	30	5.8	Dryandra	Aug 2010 (at release only)	No
FLEAS	*Echidnaphagus aranka*	*I. obesulus*	1	7.9	Unknown	Jul 2010	No
		B. lesueur	48	0.2	Barrow Island, Dryandra	Persisted	Yes
LICE	Boopid louse	*I. obesulus*	1	0.1	Barrow Island	Feb 2010 (at release only)	No
		B. lesueur	33	0.1	Barrow Island	Apr 2010	No

Boodies

Ectoparasites were detected in 20.2% of all boodie captures. A total of six species of ectoparasites were found on boodies over the course of the study, of which three persisted following translocation (*Figure 16*; *Table 7*). Boodies translocated from Barrow Island harboured three species of ticks (*Table 7*); primarily *H. humerosa,* but also *Amblyomma limbatum* and, on n=3 captures, *H. ratti*. An unknown Boopid species of louse was observed on released animals from Barrow Island, but was not observed beyond four months following release. Boodies from Dryandra harboured only one species of tick (*A. australiense*, on one individual at the time of release), and also carried the orange chigger mite *S. emphyla* around the pouch and genitals. This mite was present on 30 individuals at release but did not persist to the next trapping session six weeks later. Both populations of boodies harboured the flea *E. aranka*, which persisted in the population throughout this investigation.

Trypanosome prevalence in boodies

Boodies originating from Barrow Island were infected with the trypanosome *T. copemani* at release, with a prevalence of 12.5%. No Barrow Island boodies arrived at Lorna Glen infected with *T. vegrandis*. Boodies from the Dryandra captive enclosures possessed both *T. copemani* (11.4 ± 10.5% infected) and *T. vegrandis* (14.3 ± 13.2% infected). Two of these Dryandra individuals possessed a mixed infection of both *T. copemani* and *T. vegrandis*. Transmission of *T. vegrandis* from Dryandra boodies to Barrow Island boodies (n=18) was detected after June 2010, six months after the initial release (see

Figure 19). By 12 months post release both trypanosome species were present at overall prevalence of 16.7 (± 7.9)% and 2.4 (± 3.3)% for *T. copemani* and *T. vegrandis* respectively. *Trypanosoma vegrandis* was undetectable in the translocated boodies from Dryandra after 12 months, although *T. copemani* remained with a prevalence of 17.1 (± 12.5)% (*Figure 19*). Both species of trypanosome were transmitted to animals born at Lorna Glen; *T. copemani* was observed in nine individuals (26.1 ± 18.0%) and *T. vegrandis* was observed in one individual (4.3 ± 8.3%)(*Figure 19*).

Bandicoots

Ectoparasites were detected in 8.1% of all bandicoot captures. Bandicoots were host to a total of four species of ectoparasite over the course of the study, of which only one persisted at Lorna Glen following translocation (*Figure 16; Table 7*). The parasite most frequently observed on bandicoots was the tick *Haemaphysalis humerosa*. This tick was observed on translocated bandicoots as well as new individuals born into the population, indicating that the species had not only persisted, but was being transmitted to new individuals. The unknown Boopid species of louse and the orange chigger mite *Schoutedenichia emphyla* were observed at time of release only, on one and two individuals respectively (*Table 7*). The flea *Echidnophagus aranka* was observed once, on one individual several months after release (*Table 7*). Due to the extremely low prevalence, fleas, lice and mites were not included as factors in further statistical tests.

Factors associated with parasite prevalence

Ectoparasites

Considering all ectoparasites together, treatment of animals with antiparasitic had no significant effect on prevalence for either boodies ($P = 0.70$, two-tailed Fisher's exact test) or bandicoots ($P = 0.15$, two-tailed Fisher's exact test). Furthermore, treatment did not significantly affect the prevalence of any individual species of ectoparasite for boodies (*H. humerosa*: $F_{1,941}$=0.22, P=0.415; *E. aranka*: $F_{1,941} = 0.156$, $P = 0.61$) or bandicoots (*H. humerosa*: $F_{3,942}$=0.16, P=0.692).

For boodies, the only factors that significantly affected ectoparasite prevalence were time since release and season. Ectoparasites were significantly more likely to be present in summer ($F_{3,940} = 25.14$, $P < 0.001$), as well as positively correlated with time since release ($F_{3,940} = 0.24$, $P < 0.001$). When considering each ectoparasite species separately, season ($F_{3, 940} = 18.80$, $P < 0.001$) and animal origin ($F_{2, 940}$=13.20, P<0.001) were the only factor to have significant effect on tick prevalence (*Figure 17*) ($F_{3, 940} = 18.80$, $P < 0.001$). The prevalence of *H. humerosa* was greater in summer than in autumn, winter or spring (Tukey posthoc: $P < 0.001$, $P < 0.001$ and $P < 0.001$ respectively), and was lower on Dryandra born animals than on Lorna Glen born animals or those originating from Barrow Island (Tukey posthoc: $P < 0.001$ and $P < 0.001$ respectively). Prevalence also appeared to increase with time since release; however this may be confounded by the study period being 1.5 years rather than two full seasons. Season also was the only factor to have a significant effect on flea prevalence on boodies ($F_{3,940} = 3.69$, $P = 0.0013$), with the flea prevalence significantly lower in autumn than summer or winter (*Figure 17*) (Tukey posthoc: $P = 0.039$ and $P = 0.0067$ respectively). Other factors were not significantly related to flea prevalence.

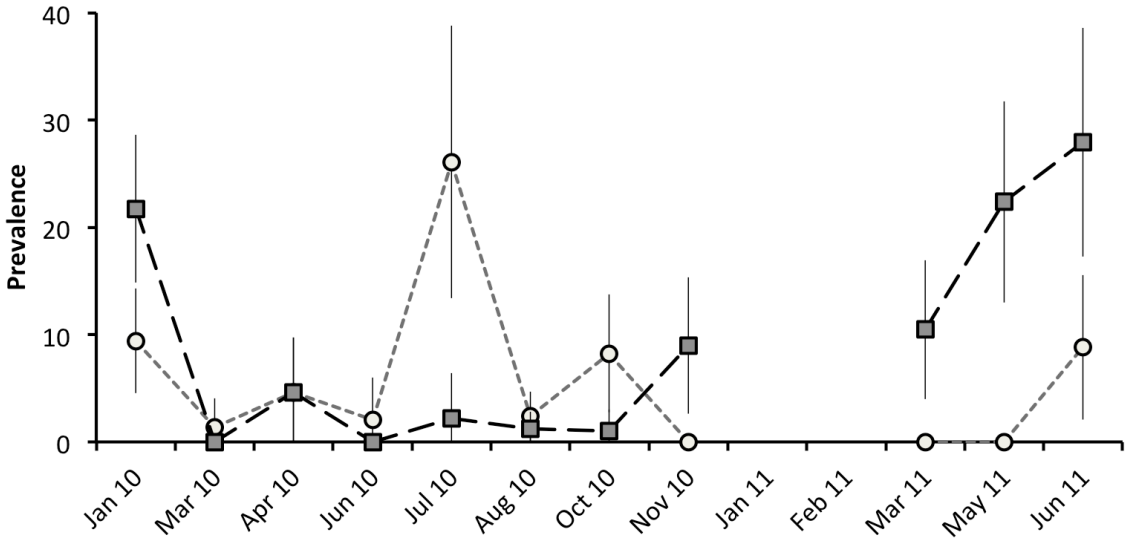

Figure 17. Prevalence of the two most common ectoparasite species on boodies (*Bettongia lesueur*), following translocation. Animals were sourced from Barrow Island, (20.76 °S, 115.40 °E) and Dryandra (32°47'S, 116°58'E) and released Lorna Glen, Western Australia (26° 13' S, 121° 33' E) in January and August 2010. Circles represent the flea *Echidnophagus aranka* and squares represent the tick *Haemaphysalis humerosa*, with 95% confidence intervals.

For bandicoots, season and time since release appeared to be the only factors influencing ectoparasite (tick) prevalence (*Figure 18*). Analysis of factors influencing the prevalence of other ectoparasites, including *A. limbatum* and the chigger mite *S. emphyla* was not possible due to the low prevalence and the rapid rate at which they disappeared from the boodie population. Only the tick *H. humerosa* occurred at a high enough prevalence for analysis. Season ($F_{3,942}$=14.82, P<0.0001) and time since release ($F_{3,967}$=51.03, P<0.0001) were the only factors significantly associated with tick prevalence

(*Figure 18*). Prevalence of *H*. *humerosa* (*Figure 16*) was substantially higher in summer than all other seasons (Tukey posthoc: $P < 0.001$), and decreased over time since release.

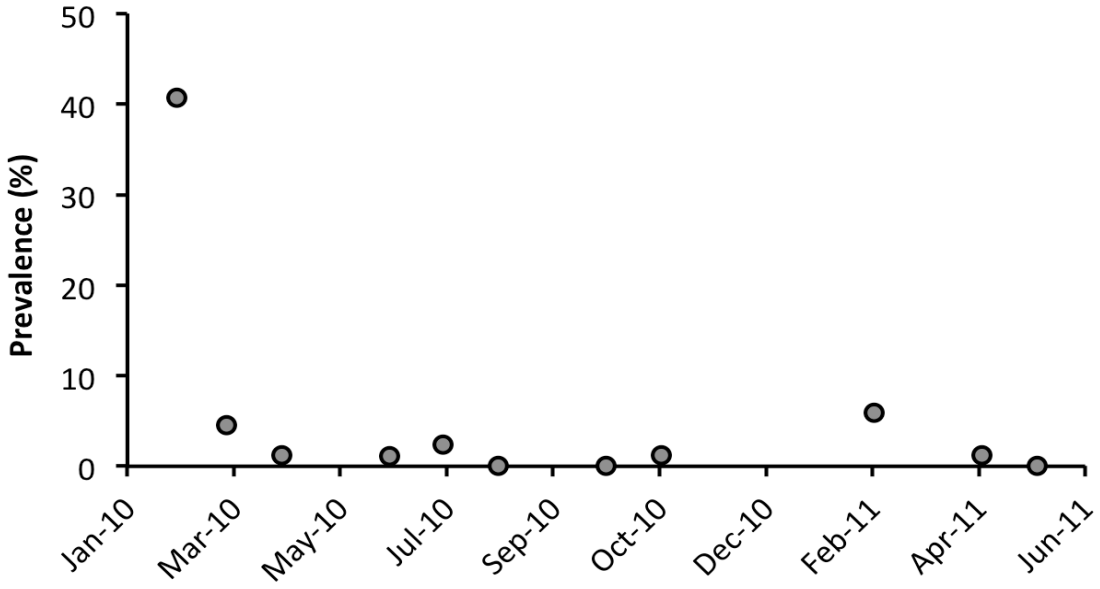

Figure 18. Prevalence of the tick *Haemaphysalis humerosa* on golden bandicoots (*Isoodon auratus*) following translocation from Barrow Island, Western Australia (20.76 °S, 115.40 °E) to Lorna Glen, Western Australia (26° 13' S, 121° 33' E) in January and February 2010. Occurrence of the tick species was significantly negatively correlated with time since release.

Trypanosomes

In total, 502 boodie blood samples from 920 captures (excludes animals re-trapped in the same session) were analysed by PCR for *T. copemani* and *T. vegrandis*. Prevalence was 11.6% for *T. copemani* and 8.6% for *T. vegrandis* respectively. As was the case for

ectoparasites, treatment of animals with an antiparasitic had no significant effect on prevalence for either *T. copemani* ($F_{1, 490}$ = 1.57; P = 1.18) or *T. vegrandis* ($F_{1, 496}$ = 1.63; P = 0.247). Season was the only factor significantly associated with prevalence of *T. copemani* from boodies, with the highest prevalence in autumn ($F_{3,471}$ = 11.2 P<0.0001). At release, *T. copemani* was detected in bodies from both Dryandra and Barrow Island, whereas *T. vegrandis* was only detected in animals originating from Dryandra. Samples from 12 months post-release revealed that transmission of *T. vegrandis* to Barrow Island and Lorna Glen born individuals had occurred (*Figure 19*).

There was a relationship between season and *T. vegrandis* prevalence, ($F_{3, 471}$ = 3.20, *P* = 0.0031) from boodies, with the highest prevalence in winter. No significant correlations with time since release, sex, animal origin, ectoparasite prevalence or antiparasitic treatment group were found.

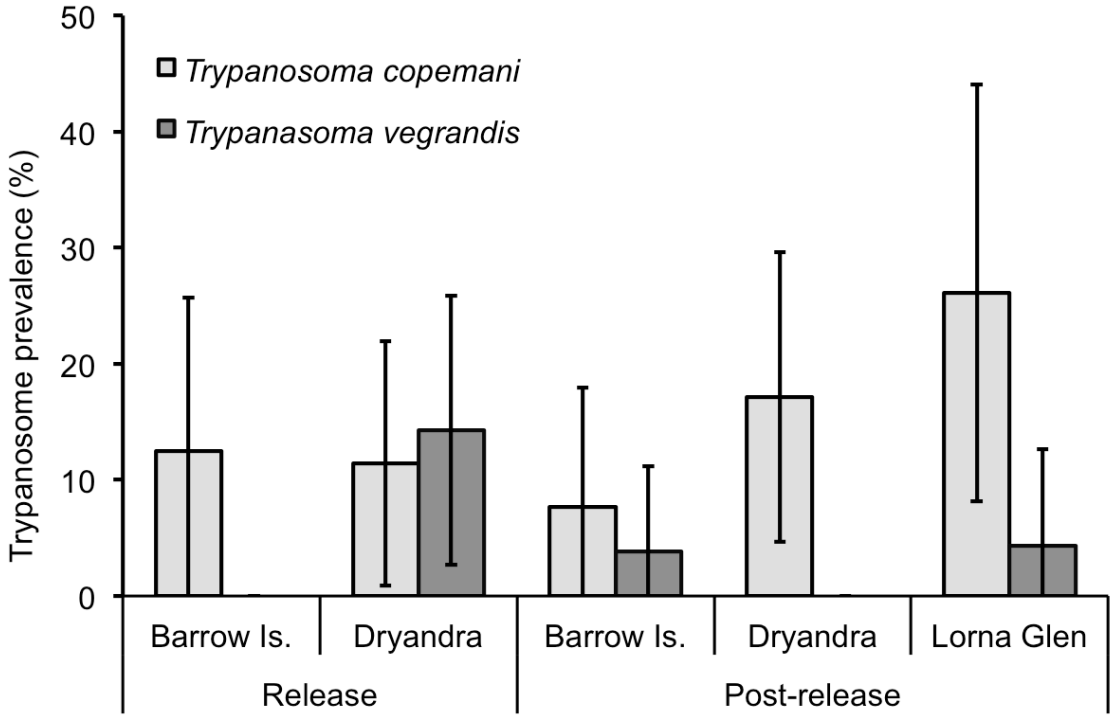

Figure 19. Infection prevalence of the trypanosomes *T. vegrandis* and *T. copemani* in the translocated boodie (*Bettongia lesueur*) population, at release and 12 months post-release. Error bars indicate 95% confidence intervals.

Post-release survival

In general, probability of surviving from one trapping session to the next (six-week interval) was high for both boodies and bandicoots (*Figure 20*). Apparent survival changed between trapping sessions, but on average was 87 (± 2.5)% for boodies and 86 (± 3.3)% for bandicoots. The best models of apparent survivorship did not support any separation of groups for boodies; that is, boodies of different origin, sex, antiparasitic treatment or trypanosome infection did not have different survivorship probabilities. For bandicoots,

survival was only influenced by sex, with females having slightly lower survivorship probabilities.

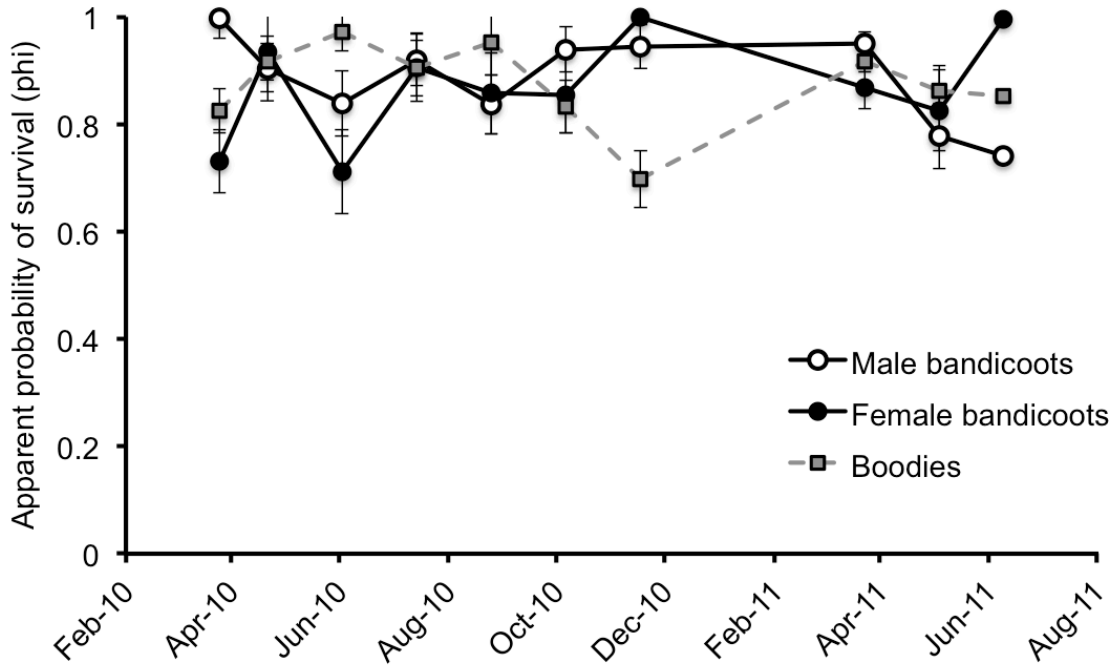

Figure 20. Apparent survivorship (phi) of translocated boodies (*Bettongia lesueur*) and golden bandicoots (*Isoodon auratus*) with 95% standard error bars. Overall survivorship was high, but differed from session to session. The best model grouped all boodies together, whereas male and female bandicoots had different survivorship estimates. Other population groupings (animal origin, antiparasitic treatment group and trypanosome occurrence) were not supported by the survivorship models.

Discussion

There is increasing recognition of the importance of parasites in ecosystem function, through their direct effects on host population dynamics and indirect mediation of

competitive and predatory interactions (Hudson et al., 2006; Lafferty et al., 2006). However, parasites are a poorly understood factor in reintroductions, both in the initial establishment and persistence of translocated animals, as well as subsequent effects on the ecosystem they are being released into (Woodford and Rossiter, 1993; Armstrong and Seddon, 2007; Sainsbury and Vaughan-Higgins, 2012). Parasitic infections may have both negative and positive effects on translocated animals and their environments (McGill et al., 2010; Almberg et al., 2012), but in general, a lack of follow up monitoring hinders our understanding of the long-term consequences of fauna translocations for host-parasite dynamics.

To the authors' knowledge, this is the first to experimentally manipulate and closely follow host-parasite interactions following the translocation of a marsupial species. Treating animals at six-weekly intervals with a topical antiparasitic had no effect on the prevalence of ectoparasites or trypanosomes, and made no improvement to the survival probability of the animals. The circumstances of this translocation allowed for an unusually high level of regular animal recapture to enable regular antiparasitic treatment; even in this situation there appeared to be no management advantage to these species in doing so. It is possible that the treated and untreated groups were confounded by sharing of the treatment, as the topically applied antiparasitic can be transferred from the fur of an animal during physical contact immediately after application (Gupta et al., 2005). This is more likely for the boodies, as they are gregarious animals that live communally and share burrows (Burbidge and Short, 2013). Bandicoots are, however, thought to be solitary except during the breeding season (McKenzie et al., 2013).

Presence of ectoparasites on animals appeared to mainly be driven by temporal factors (season and time since release) and I observed an overall reduction of ectoparasite species richness in the population over time. Four of the total seven species of ectoparasite present on released animals (in Jan and Aug 2010) did not persist beyond a few months at the release site (*Table 7*). This loss represents 57% of the parasite species potentially available for establishment, higher than that discovered by MacLeod et al. (2010) who reported 40% of chewing lice failed to establish in New Zealand.

As well as the antiparasitic treatment, the seasonality and life-cycle of the ectoparasites in this study may influence prevalence. Australian Ixodid ticks are three-host ticks, undergoing four different developmental stages during their life cycle; egg, larval, nymph and adult (Oliver, 1989; Nava et al., 2008). Each phase is more likely to be present in a host population at different times of the year. For example, the larval stage of the Australian paralysis tick, *I. holocyclus* is most abundant during summer and autumn, nymphs during autumn and winter and adult females during spring and early summer (Doube, 1979). Both the larval and nymphal stages require a blood meal before ecdysing to the next life stage, as does the adult female tick before laying her egg mass (Oliver, 1989). The abundance of adult Ixodid ticks may decline during hotter months, when they are sensitive to desiccation (Murdoch and Spratt, 2006). Unlike ticks, lice and fleas remain mobile throughout their life cycle and are capable of host switching, particularly when physical contact is made between two hosts. Their prevalence is therefore less likely to be impacted by season.

Following release at the new location, I observed the transmission of the ticks and fleas between animals of different origins and onto their offspring. I also observed the

transmission of *T. vegrandis* to the previously uninfected population of adult animals originating from Barrow Island, as well as to the new recruits born into the population.

Ectoparasite species diversity

The only two ectoparasites to persist 18 months after release were the tick *H. humerosa* and the flea *E. aranka* (present on boodies only). Conversely, the ticks *H. ratti*, *Amblyomma limbatum* and *A. australiense*, the Boopid louse, and the orange chigger mite *Schoutedenichia emphyla* did not persist on the population at Lorna Glen. Failure for parasites to establish following a new colonisation event is affected by the number of founder ectoparasites moved with the animals, presence of essential secondary hosts/life cycle requirements at the new location and suitable environmental conditions at the new site (MacLeod et al., 2010). Any one of these factors may have been unfavourable at Lorna Glen and may have been compounded by the anti-parasite treatment on half of the population, causing four of the ectoparasite species present in the founder populations not to establish. Perhaps *H. humerosa* and *E. aranka* were translocated with the hosts in higher numbers, are more resilient to changed environmental conditions or had suitable secondary hosts present at Lorna Glen.

Past studies on the ectoparasites of marsupials on Barrow Island have varied in their identification of *Haemaphysalis* species. The "bandicoot tick" *H. humerosa* was first described from specimens collected from Barrow Island bandicoots (Warburton and Nuttall, 1909) and also identified by Roberts (1963) as *H. humerosa* as an ectoparasite of the golden bandicoot *Isoodon barrowensis* (now *I. auratus*). Since the work of Roberts

(1963; 1970), *H. ratti* has also been identified on Barrow Island (Owen, 2007), as well as several sites on the mainland (Viggers and Spratt, 1995; Lorch et al., 2007; Owen, 2007). *Haemaphysalis humerosa* is very similar to *H. ratti,* differing in the length and shape of the scutum, and shape of coxae. Unlike Owen (2007), I identified only *H. humerosa* on golden bandicoots (present on 7.9% captures) and *H. ratti* on three boodies only. The similar appearance of these two tick species means it is possible that there is confusion in the literature as to which species are present for each host in various habitats. The Q-fever vector tick, *Amblyomma triggutattum*, although present on Barrow Island (McDiarmid et al., 2000; Callan et al., 2011), was not present on either of the translocated mammals.

Impact of translocation on trypanosome dynamics

This study detected transmission of the trypanosome *T. vegrandis* between hosts from different source populations, with the parasite infecting 18 naïve adults of Barrow Island origin, and at least one naïve individual of Lorna Glen origin (*Figure 19*). This transmission first occurred between founding adults, indicating that transmission is probably vector-driven, or direct (e.g. through sexual contact or mating contact; see Smith, 2008).

Although transmission of *T. vegrandis* between hosts from different source populations was first observed after six months, I hypothesise that vector transmission between individuals could have occurred soon after release, but *T. vegrandis* remained at an undetectably low level in the population until winter when parasitaemia appears to increase. The PCR used in our study detects an infection at a level of approximately 350

parasites per mL (Dunlop et al., 2014), thus, trypanosomes may remain undetectable during the initial phase of infection. Trypomastigote presence in the blood of the host varies throughout the period of infection; between the time of contracting the infection and the beginning of the acute phase of the disease, parasites may be concentrated in one area (e.g. lymph node) and not yet spread to the blood stream (Barrett et al., 2003; Botero et al., 2013). Wildlife can live with asymptomatic infections persisting at very low levels before or after the acute phase of infection (Noyes et al., 1999). Thus, recently infected individuals may exhibit a false negative result by PCR until trypomastigotes increase to a detectable level.

Lorna Glen-born recruits had a higher prevalence of *T. copemani* infection (relative to the number of individuals present) than adults from the two source sites (*Figure 19*). Although the transmission mode of these trypanosome species is largely unknown, it is suspected to be tick-borne (Austen et al., 2011; Thompson et al., 2014b). The difference in trypanosome prevalence according to animal origin could be a reflection of differences in immunity or differences in likelihood of exposure by age cohort. Recruits to the population may be immunologically naïve, having not yet developed an acquired immunity to *Trypanosoma* infection (Szép and Møller, 1999; Watson, 2013). They may also exhibit different behaviours that result in greater exposure to vectors, for example vertical transmission, refuge sharing with their mother or burrow sharing with other individuals (see also Davidar and Morton, 1993; Sol et al., 2003). A relationship between levels of infection and age-class has been reported for many parasites, where younger cohorts exhibit a higher infection rate, suggesting gradual acquired immunity in individuals (Woolhouse, 1998).

Translocation therefore had a substantial impact on the parasite community of translocated hosts, and this impact persisted into the next generation of hosts born at the translocation site, creating a host-parasite community different to that of either source site. This study demonstrates the re-equilibration of host-parasite interactions following a substantial disturbance event, where some species are lost and others expand into new populations.

Trypanosome vector correlation

None of the potential vectors (ectoparasites) were correlated with *T. vegrandis* or *T. copemani* infections when compared concurrently. However, there may have been a time lag between infection and detection of trypanosomes in the blood. Based on ectoparasite presence at source sites and persistence at Lorna Glen, some predictions about the potential vectors can be made. Prior studies have demonstrated ticks (*Ixodes australiensis*) as a vector for *T. copemani* in Gilbert's potoroo (*Potorous gilbertii*), quokka (*Setonix brachyurus*) and koala (*Phascolarctos cinereus*) (Austen et al., 2011; McInnes et al., 2011). In this study, the tick *A. limbatum* declined rapidly and was last seen in the host population five months after release at July 2010, suggesting it is not the only vector of trypanosomes in this population. I hypothesise that *H. humerosa,* also a known vector of *Coxiella burnetti* (Q-fever) in humans (Roberts, 1963; Owen, 2007) and *Theileria buffeli* (Stewart et al., 1987) in cattle, could also act as a vector of *T. copemani* at Lorna Glen due to its persistence. Thompson (2014) investigated potential vectors of *T. vegrandis* and *T. copemani* for woylies *(B. penicillata*) and found no evidence for flying insects (biting flies, mosquitoes and sand flies) being vectors of *T. vegrandis*, and only weak evidence for *T.*

copemani. The primary vectors of both trypanosomes appeared to be ticks (Thompson, 2014).

The Dryandra population of boodies (which harboured *T. vegrandis* at time of release) did not carry *H. humerosa* prior to their relocation to Lorna Glen. Therefore, a different vector (if a vector is involved) must have transmitted the *T. vegrandis* infection existing in this population. The tick *Amblyomma australiense*, present on Dryandra boodies (Burmej, 2012), could be responsible. This tick did not persist at the translocation site. This raises questions about how *T. vegrandis* is persisting in the environment at Lorna Glen; perhaps vectors are not essential and direct transmission is occurring (Smith, 2008), or the vector is a species not investigated here.

Interaction between ectoparasites, season and trypanosome infection

Ectoparasite prevalence appeared to be primarily driven by season for both boodies and bandicoots, as were trypanosome infections for boodies. The trypanosome infections were most prevalent in the population at different times; *T. copemani* in autumn, *T. vegrandis* in winter. There was also a seasonal effect for the tick *H. humerosa*, found to have been feeding on animals most frequently in summer. Seasonality likely affects host behaviour (e.g. huddling, den usage; Altizer et al. (2003)), and prevalence of feeding ticks according to their life cycle. For Ixodids, two of the four developmental stages (larval and nymphal) require a blood meal before ecdysing to the next stage, as does the adult female tick prior to laying eggs (Oliver, 1989). Australian Ixodid ticks tend to have seasonal life cycles (Doube, 1979; Nava et al., 2008), so developmental stages requiring a blood meal are

more likely to be present on hosts according to time of year. The semi-arid landscape our study is set in is characterised by cold, dry winters, and hot, wet summers. The observed cyclic prevalence of ticks appears to follow rainfall, with adult ticks in low abundance during dry times when they are most sensitive to desiccation (Murdoch and Spratt, 2006).

The origin of the animals also significantly influenced the infestation of the tick *H. humerosa*. Individuals most frequently infected were those originating from Lorna Glen (i.e. younger and potentially immunologically naive recruits) and Barrow Island (the original hosts of this tick species). Although transmission of *H. humerosa* onto animals originally from Dryandra occurred, they were significantly less likely to harbour ticks.

Management implications

As predicted in other translocation and disease literature, transmission of parasites and/or disease is an important consideration in translocations. The negative impact of parasites on their hosts can be rapid and unpredictable (Robinson et al., 2010) and have significant downstream ecological effects (Cameron et al., 2011).

This study demonstrates that 1) animals from different source populations had a different suite of ectoparasites and trypanosomes; 2) transmission of those parasites between individuals from different source populations occurred in the new location and 3) ongoing antiparasite treatment did not alter the prevalence of ectoparasites or trypanosome infection, or enhance the survival of translocated hosts. It appears that although ecto- and haemoparasites were transmitted between animals and some parasites

persisted following translocation, parasites did not negatively affect the establishment or survival of either mammal species.

Despite regular reapplication, antiparasitic treatment was not effective at reducing the prevalence of ectoparasites or trypanosomes in the treated group compared to the untreated group. This might be because of constant re-exposure via communal burrows or denning areas, and a time lag between sampling of six weeks meant that ectoparasites had recolonised in this time. Perhaps the effect of the antiparasitic was to reduce the overall ectoparasite population, but was not effective enough to eradicate ectoparasites on treated individuals. Or perhaps the treated and untreated animals were not practically different, due to sharing of the antiparasitic via physical contact, communal grooming or burrow sharing. Regardless, the presence of ectoparasites did not have a flow-on impact for trypanosome prevalence or survivorship. Based on this study, ongoing application of an antiparasitic for all or a proportion of the population is not an effective strategy for improving survivorship of translocated mammals. However, conservation practitioners may wish to use particular antiparasitics at the time of release to remove specific parasites identified to be problematic, or to reduce the overall loading on the translocated group in the initial phase of a translocation.

Chapter 5.

Environmental determination of body size in mammals: rethinking ‘island dwarfism’ in the golden bandicoot

Abstract

There are many cases of insular species exhibiting dramatic changes in body size compared with mainland counterparts. The pronounced morphological changes are considered to arise from divergent selection via resource limitation, the absence of competitors or reduced predation pressure. This chapter examines the response of populations of the Barrow Island 'dwarf' subspecies of golden bandicoot (*Isoodon auratus barrowensis*: Ramsay, 1887) following translocation to island and mainland sites. Skeletal and mass measurements, body condition and fecundity of the released animals and their offspring were compared to long-term monitoring data from the source population.

Upon establishment at new sites, translocated males increased in condition (i.e. body mass but not skeletal size). When comparing bandicoots born at the translocation sites compared to the founder population, males were significantly larger in skeletal size and mass, and females showed a significantly increased reproductive output in number, frequency and average mass of pouch young within 18 months of establishment.

This change in skeletal size, mass and fecundity took place in a single generation at both mainland and island translocation sites, suggesting that the response does not involve evolution by natural selection. This study demonstrates that the small size of golden bandicoots on Barrow Island is not a genotypic response to selective pressures, but rather a reversible phenotypic response to environmental conditions. We conclude that ecological processes relating to resource limitation drive "island dwarfism" in *I. auratus*, rather than generations of selective pressure.

Introduction

Since the times of Darwin and Wallace, biologists have studied island faunas due to the opportunity to examine evolutionary process. Islands are characterized by small areas with distinct boundaries and simplified biotas, and the development of endemic species or variations of species found on nearby landmasses. The 'Island Rule' predicts a critical body size between 250–700g (Lomolino, 1985; Lomolino, 2005), above which insular mammals dwarf, and below which trend toward gigantism (Foster, 1964; Van Valen, 1973; Lomolino, 1985). Classic examples of this trend include the dwarfing of insular elephants, hippopotamids and deer on islands in the Mediterranean Sea (Sondaar, 1991). Three-toed sloths (*Bradypus*) on geologically independent islands off Panama underwent gradual dwarfing according to time of isolation (Anderson and Handley Jr, 2002). Red deer (*Cervus elaphus*) on Jersey, Channel Islands, demonstrated gradual dwarfing to one-sixth their size in less than six thousand years (Lister, 1989). Although such confidence in the timeline of this process is uncommon, the consensus is that these effects are the result of selective evolution over many generations.

These taxa appear to follow the rule in predictable ways, however, many exceptions exist including small seed-eating insular rodents (*Peromyscus*) on islands off Mexico that display dwarfism instead of gigantism (Lawlor, 1982) and European badgers (*Meles meles*) becoming larger in the British Isles (Weckerly, 1998; Meiri et al., 2004). Different groups of vertebrates appear to respond to island isolation in different ways; for example, Meiri et al. (2004) found no reliable trend for island carnivores and insular varanid lizards in Australia may become dwarfed or gigantic, depending on prey availability (Case and Schwaner, 1993). In a meta-analysis Meiri et al. (2008) could find no consistent trend of dwarfism or

gigantism across a large dataset containing a variety of mammalian clades. They therefore attributed the rule to clade-specific responses to isolation rather than a consistent rule based on body size.

The mechanisms for these observed shifts in insular body size have also been explained by niche selection (Lawlor, 1982; Aubret et al., 2004), change in predation or competition pressures (Case and Schwaner, 1993; Raia and Meiri, 2006), specificity of diet (Lawlor, 1982), island size (Heaney, 1978) and genetic drift due to isolation (Barton and Mallet, 1996). There are many possible selective pressures acting on a species that could result in a change in size, and these are not mutually exclusive. Although large size is thought to be most efficient for food harvesting, the resources required to achieve this large size could be prohibitive on a resource-limited island (Case, 1978). Body size may be constrained by niche availability on the island; for example, a smaller adult body size may be favoured because of its ability to hide in small crevices (Case and Schwaner, 1993). Prey abundance and size has been demonstrated to have an effect on predators in reptiles (Keogh et al., 2005; Boback and Guyer, 2003) and mammals (Raia and Meiri, 2006). The strength of the island rule may also be dependent on the size of the island, and the period of isolation (Heaney, 1978). Of these explanations, those attributing changes to evolution rather than ecology have gained the most traction in the literature because most populations on islands have been separated from nearby landmasses for thousands of years (Lomolino, 1984; Anderson and Handley Jr, 2002). Although studies have examined parallel situations of dwarfing under different scenarios (Pafilis et al., 2009) as well as the experimental response of pressures manipulated in the laboratory (Aubret et al., 2004), it

is not known what happens to a population of island dwarf or giant upon being reintroduced to the mainland.

Our study characterises the response of populations of island 'dwarf' subspecies of golden bandicoot (*Isoodon auratus*) that have been translocated to two areas of former habitat; one on mainland Australia, and the other to another offshore island. Translocation of animals from the same source stock to both an island and a mainland site provides a unique opportunity to investigate whether dwarfism in *I. auratus* is genotypic (arising from many generations of selective pressure) or phenotypic (a consequence of environmental restrictions present on the source island).

Methods

Study species

Golden bandicoots are a medium-sized, mostly nocturnal, burrowing marsupial with stout forepaws for digging up arthropods and tubers (McKenzie et al., 2008). The smallest of the short-nosed bandicoots, golden bandicoots fall within the Critical Weight Range category (Burbidge and McKenzie, 1989) of mammals between 35 and 5500 g that are at a disproportionately high risk of decline or extinction. Although golden bandicoots were formerly widespread throughout Australia's arid zones prior to European arrival (McKenzie et al., 2008), they have suffered severe declines throughout the Australian continent (See *Figure 12, Figure 21*) and are currently listed as Vulnerable under the *Environment Protection and Biodiversity Conservation Act* 1999 (McKenzie et al., 2013). Threatening processes imposed on the species include the spread of introduced predators,

especially cats (Christensen and Burrows, 1994; Short and Smith, 1994), changes in the landscape due to fire regimes (Allan and Southgate, 2002; Palmer et al., 2003), changes in the landscape by introduced grazing animals (Burbidge and McKenzie, 1989; Lunney, 2001; Woinarski and Fisher, 2003), and possibly disease (Abbott, 2006).

The formerly widespread mainland subspecies *(I. auratus auratus*) weighs 300–800 g and is currently restricted to the North Kimberley, including Augustus and Unwin Islands, Marchinbar Island off the coast of Arnhem Land, and high rainfall areas of the north-western Kimberley between the Yampi Peninsula and the Mitchell Plateau (Friend et al., 1991; Palmer et al., 2003). The Barrow Island golden bandicoot (*I. a. barrowensis*) is a dwarf form (250–600 g) restricted to Barrow Island (20.76 °S, 115.40 °E) off the northwest coast of Western Australia, and nearby Middle Islands in the Western Australian Pilbara region (Palmer et al., 2003). Population estimates vary, but the largest and most stable population is present on Barrow Island, with estimates ranging between 20 000–80 000 individuals (McKenzie et al., 2008). Golden bandicoots have a similar reproductive capacity to other *Isoodon* species, giving birth to up to eight young but generally raising only one or two young to independence (Southgate et al., 1996). Independence occurs after 80 days, and animals are capable of reproduction shortly afterward; generation time is approximately 150 to 200 days (Gemmell, 1986; Southgate et al., 1996; Duffy and Rose, 2008).

The taxonomy of the short nosed bandicoots (*Isoodon* spp.) has been repeatedly revised over the last 60 years as a result of uncertainty surrounding the taxonomic status of *I. auratus* (Tate, 1948; Lyne and Mort, 1981; Pope et al., 2001; Westerman et al., 2011) and other morphologically distinct populations. This study adheres to the most recent

classification (Westerman et al., 2011), which maintains *I. auratus* as a distinct species. This study focuses on the subspecies *I. a. barrowensis*, endemic to Barrow Island.

Study sites and release protocol

Barrow Island golden bandicoots (*I. a. barrowensis*) were reintroduced to two areas of the species' former distribution from Barrow Island, Western Australia (BI: 20° 51' S, 115° 24' E) in February 2010. Barrow Island is a large rocky limestone island dominated by clumping grasses (*Triodia wiseana* and *T. augusta)*. Although the island separated from the WA mainland 8 000–10 000 years ago (Williamson, 1981), many of Western Australia's fauna that are extinct or greatly reduced on the mainland still persist on the island (Butler, 1970). Bandicoots from BI were translocated to Hermite Island (HI: 20° 29' S, 115° 31' E), and Lorna Glen (LG: 26° 13' S, 121° 33' E) on mainland Australia. Hermite Island is a cat and rat-free island (Algar et al., 2002) in the Montebellos group, approximately 30km north of BI. The majority of HI is dense *T. wiseana* and *T. augusta* on a rocky limestone substrate. The other recipient site for the translocations, LG, is a former pastoral lease, 160km NE of Wiluna, Western Australia, now managed for conservation and the site of a fauna reconstruction program. Releases occurred within an 1100 ha predator proof enclosure (described inBode et al., 2012) of sandy grassland (*T. basedowii*) and open *Acacia aneura* woodlands with a rocky clay substrate. Supplementary food (intermittent) and water (constant) was provided in the enclosure for the duration of the study.

Both of these translocations are considered to be reintroductions, based on historical distributions and the fossil record. The carcass of a golden bandicoot (probably *I.*

a. barrowensis) was found on HI in 1912 (Montague, 1914) but not by any surveys after this date. Pre-European skeletal remains of golden bandicoots (*I. a. auratus*) were found on LG and at the neighbouring Earaheedy station (Baynes, 2006).

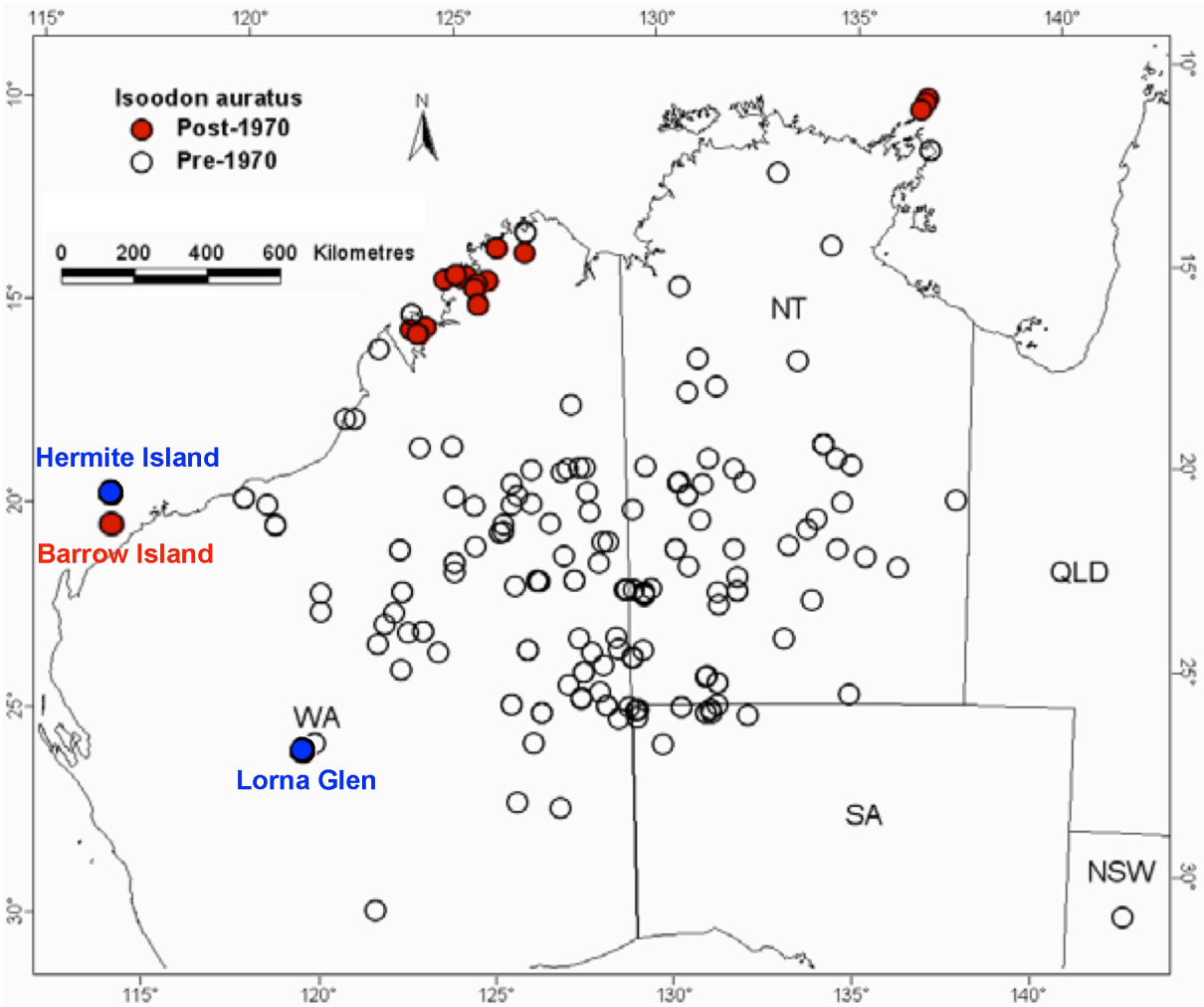

Figure 21. Previous (open) and current (closed) distribution of the golden bandicoot, *Isoodon auratus.* Translocation origin site is Barrow Island; establishment sites were Hermite Island and Lorna Glen. Map adapted from (Palmer et al., 2003).

In 2010, 160 golden bandicoots were captured and moved by helicopter and light aircraft to each of the two new sites (320 individuals in total). The animals were released at HI and LG within 24 hours of capture. All individuals were implanted with a unique passive implanted transponder (PIT); Allflex® 12mm FD-X transponder (Allflex Australia, QLD, Australia) subcutaneously between the scapulae and sealed with *Vetbond*™ Tissue Adhesive (3M Australia, NSW, Australia) prior to release. Baseline measurements of long pes (heel to base of claw on longest toe on hindfoot), head length (tip of nose to protrusion at back of skull), body mass, and reproductive age and condition were taken at time of capture. Long pes, rather than head length, was used as a measure of body size due to it being a less variable and more repeatable measure. At LG, I took all measurements. On BI, there were two observers and on HI there were four observers. The animals sourced from BI and translocated to HI and LG were representative of the BI source population in terms of size (male pes length; source: 41.8 ± SE 0.15 mm, founders: 41.2 ± SE 0.39 mm, $P = 0.48$. Female pes length; founders: source: 39.99 ± SE 0.13 mm, 39.9 ± SE 0.28 mm, $P=0.28$) and genetic variation (Ottewell et al., 2014).

Table 8. Summary and breakdown of golden bandicoot (*Isoodon auratus*) captures at the origin site (Barrow Island) and the two translocation sites (Hermite Island and Lorna Glen).

	Male		Female		Total	
	All captures	**Recaptures**	**All captures**	**Recaptures**	**All captures**	**Recaptures**
Barrow Island	601	151	566	133	1167	284
Hermite Island	427	180	301	160	728	340
Lorna Glen	636	450	421	257	1057	707
Total	1664	781	1288	550	2952	1331

Monitoring and trapping

Post-translocation monitoring at HI and LG was undertaken using cage traps baited with peanut butter and rolled oats. At LG, 81 Sheffield cage traps (20cm x 20cm x 56 cm) were set 200m apart in transects along roads within the 1100ha enclosure. At HI, 105 cage and Elliott traps (9cm x 10 x 33cm) were laid in 5 x 25 m grids (0.3 ha). The traps were opened for six consecutive nights at each location and repeated trapping efforts were undertaken every six weeks at LG and every 12 weeks at HI for a period of 18 months after the initial release. Trapping effort was different between sites due to the presence of other species (e.g. high numbers of *Bettongia lesueur* at LG) and logistics of monitoring (i.e. access to a remote island), however golden bandicoots readily enter both styles of trap. The data from BI was collated from annual long-term fauna monitoring between 1998 and 2008. Golden bandicoots were trapped in both Elliott traps and cage traps baited with peanut butter and rolled oats. Annual trapping consisted of five 1 ha grids of 25 traps opened for four consecutive nights during spring (Morris et al., 1999).

Each bandicoot trapped was weighed to the nearest 5 g using digital scales, and long pes was measured to the nearest 0.1 mm using vernier callipers. Females were assessed for reproductive activity and categorized as: Breeding (with pouch young), Lactating (pouch young not captured but still at heel), Non-breeding (no pouch young but pouch and teats developed), or Virginal (never bred). The number of pouch young and the crown-rump length (to nearest 5 mm) of these young were recorded. Animals were classified as "adult" according to their reproductive condition; an independent female with fully formed pouch or an independent male with scrotal width of 20mm or greater. New recruits were marked with an individual PIT at the time of first capture. An ear tissue sample was also taken for each new animal for genetic archiving, also allowing easy identification of new and recaptured animals. No animal was observed to have lost its PIT tags following initial tagging and ear notching.

Body Condition Index (BCI)

The most accurate measures of body condition are direct measures of body fat and water (Bakker and Main, 1980), but are lethal and therefore not practical here. Non-lethal alternatives estimate body condition using ratios of a morphological measure to body mass (Hayes and Shonkwiler, 2001). Pes length was used as the measure of skeletal size as it was the most reliable measurement during the 10 years of monitoring at BI. Body condition index (BCI) was calculated as the residuals of a linear regression between log of body mass and log of pes length (Viggers et al., 1998). Only males were included in this analysis due to the confounding influence of pouch young mass for females. Bandicoots that had high,

positive residuals were heavier than expected for their size and were interpreted as in good condition, and *vice versa*.

Statistical analyses

A linear mixed effects model was used to test the effects of translocation site upon morphometric measurements, where bandicoot identity was included as a random variable to account for multiple measurements of the same individual. For these comparisons, only individuals born on site were used; *i.e.*, founder individuals from BI have been excluded. Females were excluded from comparisons involving mass, as they were carrying pouch young up to 85% of the time, with young accounting for up to 60% of total body mass. Instead, linear mixed effects comparisons were made between the reproductive output (number of young and estimated pouch young biomass) of females at different sites, again using bandicoot ID as a random variable to control for repeated measurement effects.

To estimate the pouch young biomass produced by females in each population, we fitted a curve to pouch young of known crown-rump length (CR) and body mass. This enabled the generation of a predictive relationship between mass (g) and length (mm). These measurements came from a small number of pouch young that were ejected and dead in traps (n=6), and larger furred pouch young able to be weighed and measured independently of their mother (n=3). The predicted mass of a pouch young is equal to $0.00009(CR)^{2.885}$ (R^2=0.985). Estimated pouch young biomass was compared between populations by ANCOVA, again with bandicoot identity as a random variable to control for repeated measurement effects.

Subadults (independent but not reproductive) were included in the analyses as they show the same size-weight ratios as adults, and excluding animals on the basis of an arbitrary size would confound the analysis. This species is capable of reproducing after 150 days, so while subadults represent only 10-15% of the analysed population, they form the lower part of the size curves. Pouch young (still dependent on mother) were not included.

Results

A total of 2952 measurements of golden bandicoots were used to generate this dataset (see *Table 8*). Of these, 1331 were recaptures, i.e. animals that had been tagged and measured in a previous trapping session. Animals were only measured once per trapping session. The majority of captures (1167) were from BI long term monitoring, 728 from HI and 1057 from LG. Trapping at LG consisted of a high level of recaptures of the same individuals, necessitating individual identification as a random variable in our analysis to account for repeated measures.

Comparisons between recruited (HI, LG) and source (BI) bandicoots

Table 9 presents a summary of the following results for reproductive output, pes, body condition, and body mass.

Reproductive output

Female bandicoots at LG had a significant overall increase in reproductive output compared to the founders on BI. Females at LG were in a breeding state (with pouch young or lactating) on more than 70% of captures, whereas those on BI and HI were in a breeding state only 30% of the time.

Of the reproductively mature females (*i.e.*, excluding females with an undeveloped pouch), females carried a significantly different number of pouch young ($F_{2, 309} = 23.35$, $P < 0.0001$) between different sites. LG females carried significantly more individual pouch young than those from HI or BI (Tukey posthoc: $P < 0.0001$ and $P < 0.0001$ respectively). Females at LG carried up to five pouch young, whereas a maximum of three pouch young were observed on BI. Repeated measurement of individuals did not influence the dataset, because there was no effect of ID on pouch young number ($F_{1, 413} = 0.33$, $P = 0.57$).

Consequently, there was a significant difference in the average pouch young mass estimated for each site ($F_{2, 264} = 8.91$, $P < 0.0002$). Females from LG had significantly higher reproductive output (Tukey posthoc: $P < 0.001$) than those from BI, with no effect of ID on pouch young mass ($F_{1, 346} = 0.137$, $P = 0.712$). Comparisons with HI were not significant.

Size

Male bandicoots born at different sites had significantly different pes lengths ($F_{3, 542} = 13.23$, $P < 0.0001$); males born at the translocation sites LG and HI had significantly longer pes than those at BI (Tukey posthoc: $P < 0.00001$ and $P < 0.00001$ respectively) (*Figure 22*) with no effect of ID ($F_{1, 764} = 0.68$, $P = 0.41$).

Female bandicoots born at different sites had significantly different pes lengths ($F_{2, 377} = 7.39$, $P < 0.0007$); females born at the translocation sites LG and HI were significantly larger than those on BI (Tukey posthoc: $P = 0.0058$ and $P = 0.018$ respectively). There was no effect of ID on pes ($F_{1, 499} = 0.876$, $P = 0.46$).

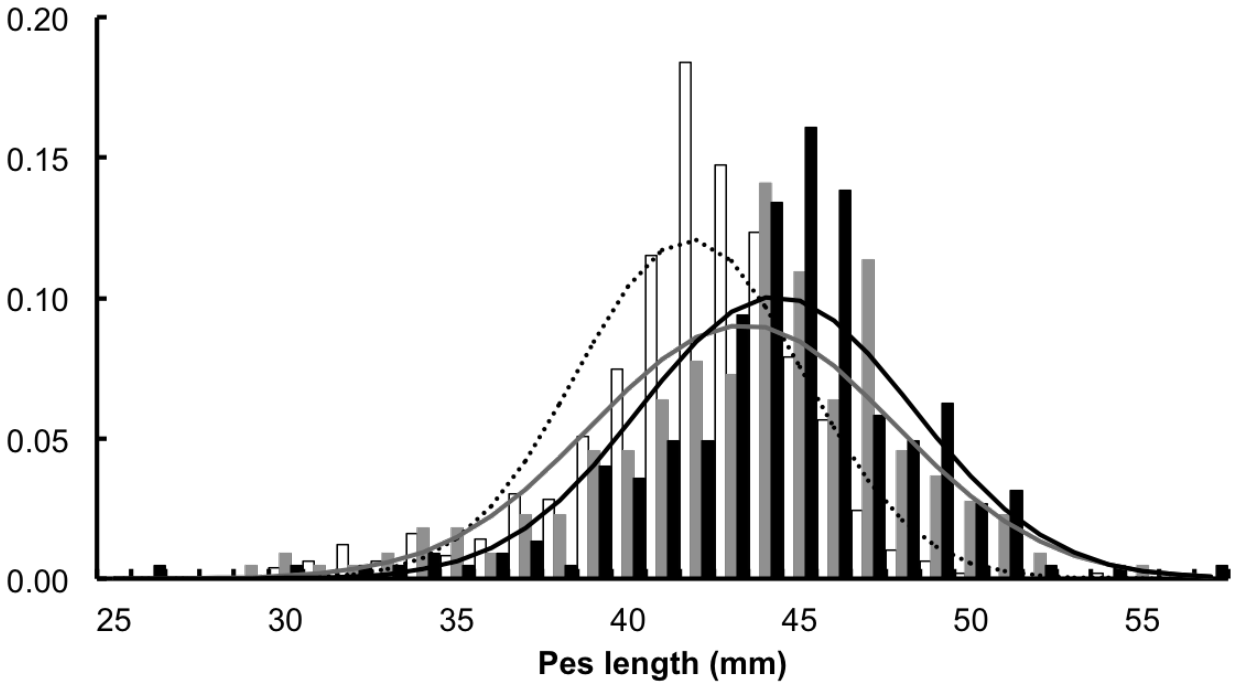

Figure 22. Frequency distributions of body size of male golden bandicoots (*Isoodon auratus*) at origin site (Barrow Island: white) and the two translocation sites (Hermite Island: grey, and Lorna Glen: black). Lines indicate the fitted normal distributions for Barrow Island (dashed line), Lorna Glen (black line), and Hermite Island (grey line). Male bandicoots born Lorna Glen (μ=44.3mm) and Hermite Island (μ=43.4mm) were significantly larger than those from Barrow Island (μ=41.8mm; Tukey posthoc: $P < 0.00001$ and $P < 0.00001$ respectively).

Mass

There was a significant difference in body mass of male bandicoots between sites ($F_{3, 587}$ = 13.180, P < 0.0001); those at LG and HI were significantly heavier than those on BI (Tukey posthoc: P < 0.0001 and P = 0.0158 respectively) and males at LG were significantly heavier than those on HI (Tukey posthoc: P = 0.0057) (*Figure 23*). There was no effect of ID ($F_{1, 853}$ = 0.079, P = 0.778). Of the 41 individual males over 600g, only four were from BI, 16 were from HI and 21 were from LG.

Golden bandicoot pouch young are physically attached to the female's teat from the time they enter the pouch until they are well developed. As a consequence, it is not possible to weigh females independently of their young. However, an adjusted female mass was estimated by subtracting the estimated pouch young mass (based on the size and number of pouch young) as described above from the female's total mass. The adjusted masses we calculated are most different from the actual measurements of female mass for LG and HI animals due to greater average numbers of PY carried and larger average PY (see above and *Table 9*). Females had significantly different adjusted masses at each of the three sites ($F_{2, 404}$ = 14.698, P < 0.001), with females at LG being significantly heavier than females at both HI and BI (Tukey post hoc: P <0.001 and P <0.001 respectively).

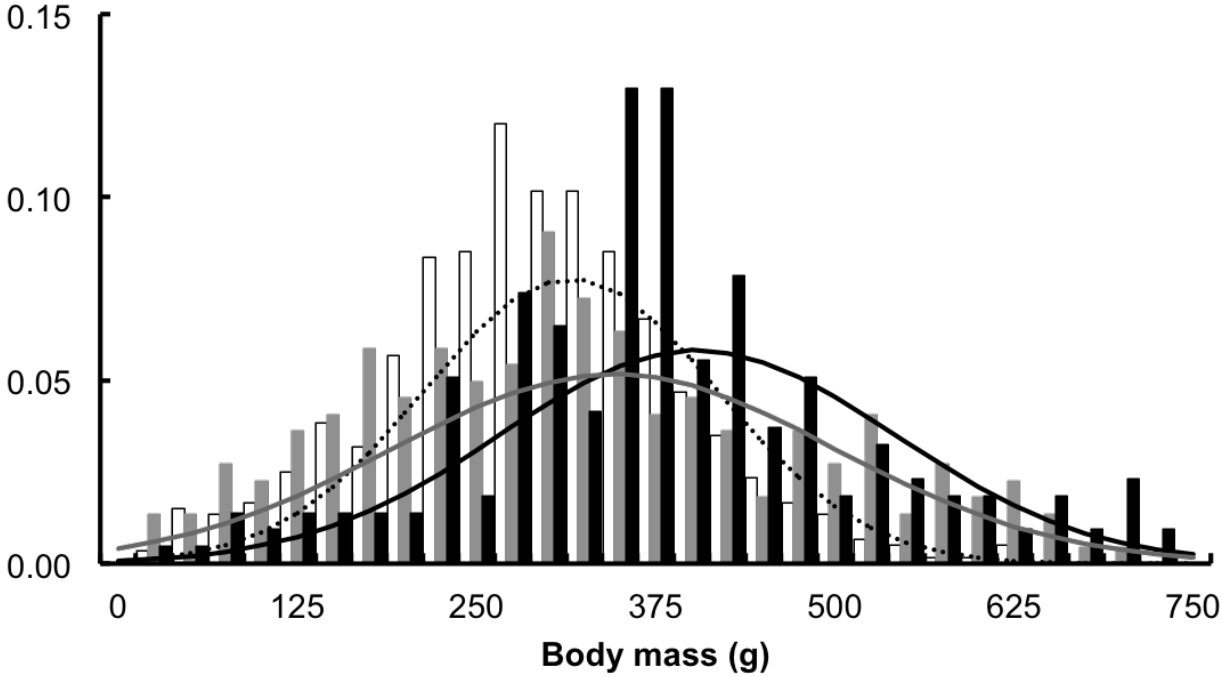

Figure 23. Frequency distributions of body mass of male golden bandicoots (*Isoodon auratus*) at origin site (Barrow Island: white) and the two translocation sites (Hermite Island: grey, and Lorna Glen: black). Lines indicate the fitted normal distributions for Barrow Island (dashed line), Lorna Glen (black line), and Hermite Island (grey line). Male bandicoots born at Lorna Glen (μ=404g) and Hermite Island (μ=345g) were significantly heavier than those from Barrow Island (μ= 315g; Tukey posthoc: P < 0.0001 and P = 0.0158 respectively).

Body condition index

Not only were male bandicoots at the three sites different in size and weight, they also showed a significant difference in body condition indices between locations ($F_{3, 536}$ = 8.381, P < 0.0001); with males from BI and LG had a significantly higher BCI than those born at HI (Tukey posthoc: P = 0.0024 and P < 0.0001 respectively). There was no effect of ID ($F_{1, 751}$ = 1.646, P = 0.2) (*Figure 24*).

Table 9. Differences in size, weight, condition and reproductive output of golden bandicoots (*Isoodon auratus barrowensis*) born at Barrow Island (source population), Hermite Island and Lorna Glen (translocated populations). Pairwise differences are shown using letters (a, b, c), where significantly different pairs do not share the same letter.

		Barrow Island		Hermite Island		Lorna Glen			
		mean ± se	**n**	**mean ± se**	**n**	**mean ± se**	**N**	**F** $_{DF1, DF2}$	**P**
Male	**Pes length (mm)**	**41.8** ± 0.2 *a*	495	**43.4** ± 0.3 *b*	221	**44.3** ± 0.3 *b*	224	19.87 $_{2, 535}$	<0.0001
	Body mass (g)	**315.8** ± 4.2 *a*	599	**344.8** ± 10.4 *b*	222	**404.1** ± 9.3 *b*	216	19.83 $_{2, 580}$	<0.0001
	Body Condition Index (BCI)	**-0.01** ± 0.01 *a*	493	**-0.10** ± 0.02 *b*	220	**0.02** ± 0.01 *a*	212	12.05 $_{2, 529}$	<0.0001
Female	**Pes length (mm)**	**40.0** ± 0.13 *a*	483	**40.9** ± 0.29 *b*	139	**41.9** ± 0.47 *b*	86	7.39 $_{2, 377}$	0.0007
	Adjusted body mass (g)	**242.8** ± 3.8 *a*	390	**265** ± 7.9 *b*	138	**325** ± 14.8 *c*	80	14.698 $_{2, 404}$	<0.0001
	Pouch young number	**0.73** ± 0.05 *a*	315	**0.81** ± 0.10 *a*	86	**1.56** ± 0.14 *b*	85	23.35 $_{2, 309}$	<0.0001
	Pouch young biomass (g)	**0.53** ± 0.29 *a*	377	**4.11** ± 1.22 *ab*	141	**9.48** ± 1.79 *b*	85	8.91 $_{2, 264}$	<0.0002

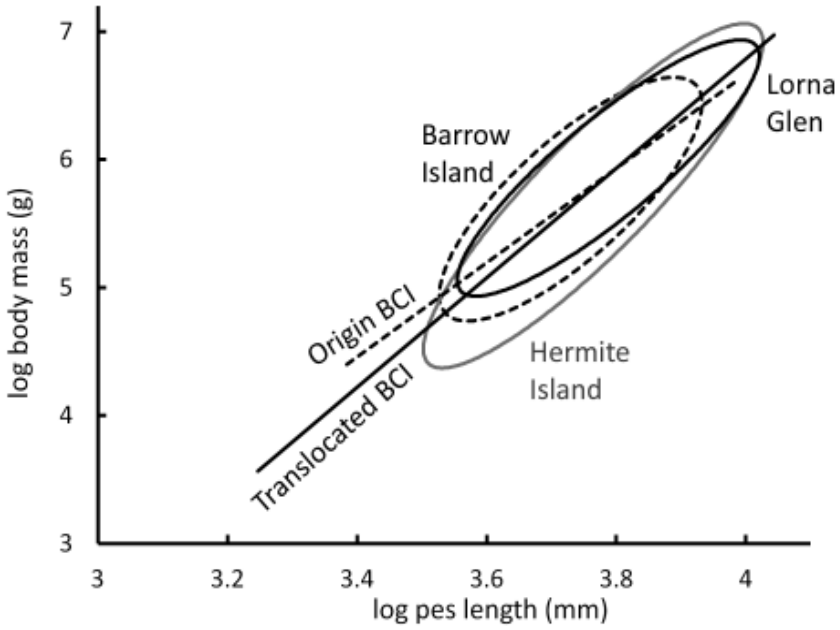

Figure 24. Body condition of male golden bandicoots at different sites. Log transformed pes length and body mass ellipses represent the 95% CI for the populations of male bandicoots born at Barrow Island (dashed lines), Hermite Island (grey line), Lorna Glen (solid line). The different trendlines represent statistically different body condition index (BCI) relationships for the three populations; the original Barrow Island population (dashed trendline), and the Lorna Glen and Hermite Island populations (solid trendline), which did not differ from each other. The animals at Lorna Glen and Hermite Island were larger and heavier than those at Barrow Island.

Effect of translocation on founder individuals

The founder population translocated from BI to HI and LG went through a period of intense weight gain following release at new sites. Average mass of males on HI was up to 1.8 times the average for BI males. Males released at LG stabilised at approximately 1.5 times the average mass of BI adults. Skeletal measurements showed that there was also a 10% increase in actual body size relative to the source population (*Figure 25*). This may be accounted for by

growth of some sub adults in the translocation group, suggested by the lower than expected body mass at time zero (see *Figure 25*).

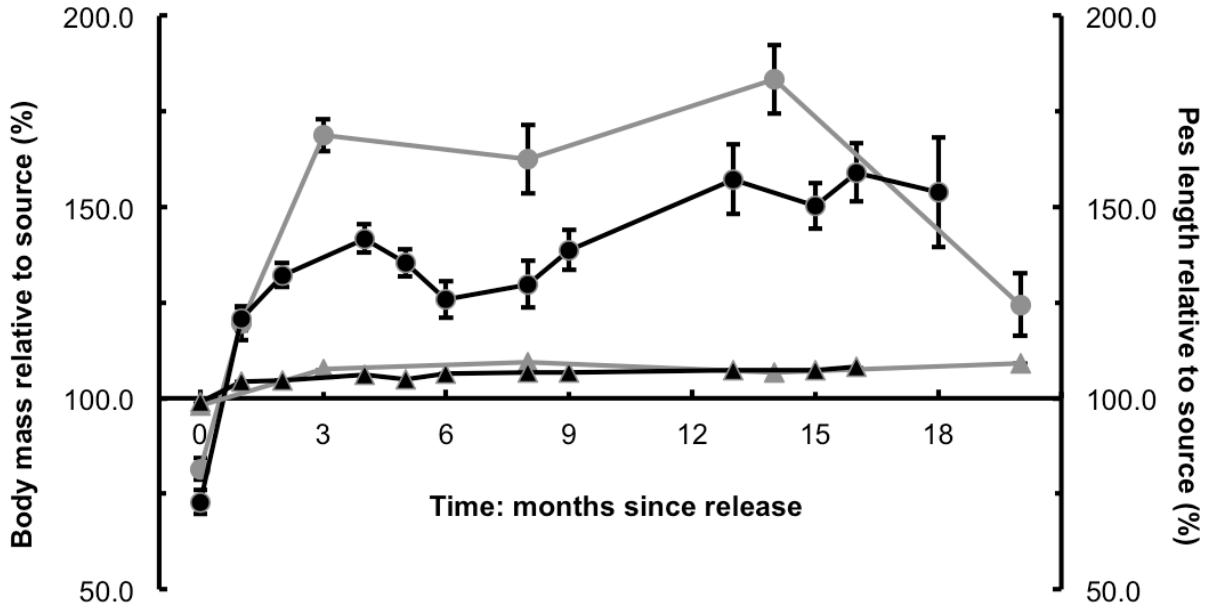

Figure 25. Change in average size (triangles) and mass (circles) of individual adult male golden bandicoots (*Isoodon auratus*) translocated from Barrow Island to Hermite Island (grey) and Lorna Glen (black). The X horizon (100%) represents the average mass of an adult male Barrow Island bandicoot (315.8 ± 4.2 g) and average pes length (41.8 ± 0.15 mm).

Discussion

This study demonstrates changes in the morphology and reproductive output of golden bandicoots when they are translocated to locations free of other bandicoots, at both island and mainland sites. The most startling aspect of this finding is that these changes occurred within 18 month of release, equivalent to the first one to four generations. Although the authors acknowledge that there are too few generations to definitively understand what natural selection may be contributing to the observed results, the immediate increase in size suggests a phenotypic rather than genotypic explanation.

Founder individuals and recruited individuals responded to colonisation of a new habitat in different ways.

Response of founder individuals

Translocated individuals (founders) showed a rapid increase in both size and mass soon after arrival at both new sites. The founder animals went from being below 100% to more than 150% times the mass of an average BI individual. The increase in skeletal measures indicates that some translocated individuals must not have been fully grown (despite being sexually mature), and thus grew slightly larger at the new sites. However, they still did not grow to be as large as those born on site; this indicates that resources from birth are important. The increase in mass, however, greatly outweighs the small increase in skeletal size, suggesting that the majority of translocated males increased in mass that is not associated with somatic growth (i.e. this increase in mass is predominantly an increase in BCI). This increase in BCI in translocated individuals is likely a response to resource availability (Jakob et al., 1996), and suggests that translocation has relaxed the ecological constraints of resource limitation that occurred on BI.

Response of recruited individuals

Newly recruited males at both translocation sites (HI and LG) were significantly heavier by mass and larger by skeletal measures than those at the source site (BI). Males showed a significant increase in body condition. On HI, despite being larger and heavier than the source population, newly recruited males had a slightly lowered body condition, which was significantly different to those in the source population and at LG. One

explanation for this is that the initial population expansion on the island reached unsustainable densities in some areas, and large individuals lost condition as resources depleted. The intermittent supplementary feeding at LG probably offset this effect. This fits the pattern of mass over time for founder individuals, where the final point (*Figure 25;* approx. 1.5 years post-release) shows a dramatic drop in body mass back towards the average.

Recruited females at LG showed a significantly increased reproductive output in both the frequency of breeding, as well as the number and average size of young present (see *Table 9*). This effect was greatest at LG, where some supplementary food was available. While HI females demonstrated an increase in average pouch young biomass from the source site, this data was not significantly different to either BI or LG. An increase in average pouch young biomass represents not only an increase in the number of young being carried, but also the proportion of young being carried to independence. Continuously breeding marsupials are known to abort or 'throw' their young in times of stress or resource hardship, and are thereby more likely to successfully rear young to independence in good seasons (Higginbottom and Johnson, 2000).

Relevance to the Island Rule

The Island Rule, as discussed by Van Valen (1973), Foster (1964) and Lomolino (1984, 2005), describes gradual, evolutionary morphological change due to new selection pressures on an island compared to the mainland. These differing pressures can include potential competition, predation, feeding ecology or habitat availability. Changes occur on an individual basis, over many generations (Lister, 1989).

Another mechanism under which morphological change can operate is phenotypic plasticity. These are opportunistic responses to changing environmental conditions and have been recorded to occur almost immediately. For example, Aubret et al. (2004) demonstrated morphological change within one generation of 'island dwarf' tiger snakes, which due to smaller prey size, have become smaller themselves. When larger prey is available young snakes will grow to have a larger jaw size, relative to their parents. This phenotypic variation is attributed to adaptive plasticity, enabling individuals to take advantage of resources when they are available.

The morphological and reproductive changes reported here occurred immediately following the translocation, within the first four generations of new recruits. The entire study took place over 18 months following release, which could be a maximum of four generations (assuming a generation to be 150 days; which is similar to *I. macrourus* and *I. obesulus* (Gemmell, 1986)). Resource limitation has an impact on marsupial fecundity (Ramsey et al., 2002), and a release of competitive effects will have an impact on the physical development of individuals from gestation to adulthood. Prolonged maternal under-nutrition has been shown to have a profound impact on the number of young, foetal growth rates and mature adult size in a variety of taxa (Erikstad et al., 1997; Swain and Jones, 2000; McMahon and Hindell, 2003; Skogland, 2010), including humans (Lummaa and Clutton-Brock, 2002). Here, the release from resource limitation appears to have allowed golden bandicoots to grow to a greater body size, presumably due to better nutrition from birth. Females with access to supplementary food were able to breed almost continuously and raise 2–3 young at a time. The implication of these findings for the Barrow Island population is that total population numbers are likely to fluctuate greatly according to temporal resource availability.

Krebs and Singleton (1993) emphasize that observer differences can provide confounding error. At LG, one observer took all measurements. On BI, there were two observers and on HI there were four observers. Although observer error may be a factor for accurate, repeatable skeletal measurements taken with callipers, it would be difficult for observer error to apply to body mass measurements when the same equipment was used between observers. A difference was detected for both skeletal measurements and body mass of animals at different sites and as such unlikely to be attributed to observer error. Furthermore, observer error in measurements would likely be reflected in standard errors between sites, which was not evident in these data.

We hypothesise that on Barrow Island, the combination of constant intraspecific and interspecific competition in a low productivity habitat (Bradshaw et al., 1994) means that adult animals are constrained to never grow to their largest potential size (see Millar and Hickling, 1990, Functional Ecology, 4, 5-12;). The number of golden bandicoots present on Barrow Island is thought to be between 20 000 and 80 000 (McKenzie et al., 2008); density of animals on the 202km^2 island could therefore be up to 400 individuals per km^2. Total population number as well as the condition of animals within the population is seasonally dependent. Bradshaw et al., (1994) demonstrated an increase in condition of golden bandicoots with the marked increase in availability of terrestrial invertebrates between the (then) driest year on record and following a cyclonic rain event the next year.

Barrow Island also supports several other species of mammals that operate in similar niches to golden bandicoots. Spectacled hare-wallaby (*Lagorchestes conspicillatus)*, boodie (*Bettongia lesueur)*, and two native rodent species are all present on the island and are potentially competing for similar resources. Translocation to an empty niche, whether island or mainland, represents a substantial relaxation in the ecological constraints of

resource availability present at Barrow Island. The high density of animals on Barrow Island seems to suggest that reproduction is favoured over body size.

The resource availability hypothesis, in which optimal body size of vertebrates on islands is tied directly to food availability (Keehn et al., 2013; Case, 1978), has been demonstrated in a variety of taxa to be one of the most important determinants of animal body size. Red kangaroo *(Macropus rufus)* populations respond to drought by shifting to a higher proportion of more efficient small males that are replaced by large males when resources return (Moss and Croft, 1999). A lack of overwinter resources correlate with reduced calf and adult doe size in wild reindeer (*Rangifer tarandus)* (Skogland, 1983). Individual Galapagos marine iguanas *(Amblyrhynchus cristatus)* are capable of switching between growth and shrinkage throughout their lifetimes, according to environmental conditions. These individuals, during severe El Niño events, individuals were observed to shrink up to 20% in body length, conferring an advantage during time of sparse resources (Wikelski and Thom, 2000). Reptiles on islands with differing prey availabilities show size variation, correlated with resource availability and competition (Keehn et al., 2013). Barrow Island golden bandicoots have a low field metabolic rate and diet comprising mostly small invertebrates such as termites (Bradshaw et al., 1994). The two main findings for this study (increased reproductive output for females and greater body size for males) are undoubtedly linked: in times of plentiful resources, males respond by putting on size and condition, females respond by producing more, larger young.

This study demonstrates a shift in the normal distribution of traits relating to size, mass and reproductive output upon change in the environmental conditions caused by translocation to empty niches. We predict that the small size of golden bandicoots on Barrow Island is not a genotypic response to selective pressures, but rather a phenotypic response to conditions on the island. When translocated to vacant ecological niches, they

have the capacity to produce offspring with increased body mass, size and reproductive output. This flexible phenotype means that the translocated populations at HI and LG may return to a smaller size as they reach carrying capacity. The *I. a. barrowensis* subspecies, although morphologically defined by its smaller size, is not a true 'island dwarf', but exists on Barrow Island under environmental constraints.

Chapter 6.

Getting close to the MARK: An empirical test using captive populations of boodies and golden bandicoots

Abstract

Information about the success or failure of translocations relies on quantification of the population of animals and the variables affecting their establishment. I compared estimates from two different population-modeling techniques, Known To Be Alive and Capture-Mark-Recapture, for a population of translocated boodies (*Bettongia lesueur*) and golden bandicoots (*Isoodon auratus*). Population estimates were calibrated using known numbers (founder individuals, and number of births). I also assessed the differing survival rates and capture probabilities for animals according to their sex and animal origin in order to determine the attributes of animals that are most appropriate for translocation.

Of 5,346 trap nights, I had 4010 capture occasions (75% capture success) of 479 target individual animals. No animals lost their unique identifying tags (passive implant transponders) during the study.

Both species showed high capture probability (p) of between 64 and 99%. Capture probabilities were not differentiated between the sexes or animal origins for either species, but were variable between trapping sessions. Including animal origin as factor did not improve the survival models for either species; captive-bred boodies were not differentiated from wild-caught animals. Furthermore, the lack of differentiation in survivorship of animals of Lorna Glen origin (i.e. born on-site) indicates that there was not a significant difference in survival for newly recruited animals compared to founder adults for either species. The most parsimonious model for bandicoot survivorship indicated that across the whole timeframe of this study, males had better survival than females, but did not differentiate between male and female boodies.

Effective founder populations were calculated from the apparent survival rates in the period immediately after release. For boodies, survival was 82.5% (70 of the 85 released individuals) and for bandicoots, survival was estimated to be almost 100% for males (77.7 of 78 released individuals) and 73% (59.9 of 82 released individuals). Population estimates for both species appeared to plateau, regardless of additional releases or high birth rates. This may indicate that at this time, the enclosure had a carrying capacity beyond which additional recruits were not supported.

Both methods of population analyses aligned very closely over time, probably due to the very high capture probabilities. The quantification of population characteristics for these two translocated species provide useful data for other models, e.g. for "harvest" VORTEX models, and for assisting with management decision making.

Introduction

Effective conservation management depends on accurate information about biological populations for decision-making processes (Sandercock, 2006; Wayne et al., 2013a; Frederiksen et al., 2014). Usually, managers seek information on total population size to assess the impacts of threatening processes, the success of breeding programs or to determine levels for harvest for translocations. For conservation monitoring, knowledge of population size and other demographic parameters is needed but often difficult to obtain for non-game species (Wayne et al., 2013a). Inherent issues with estimations of total population size exist; for example the inability to differentiate between births and immigration events. Because of this, some ecologists argue that other indices of success are more relevant to a population (Heppell et al., 2000; Sandercock, 2006). For example, survival to a reproductive age may be more important for a long-lived species with relatively low fecundity than absolute population numbers (for example the southern water skink, Eulamprus tympanum,Blomberg and Shine, 2001; or gopher tortoise Gopherus poluphemus,Tuberville et al., 2008). Depending on the species, these may include survivorship of first year animals, fecundity of breeding females or persistence of dominant males. In a translocation scenario, initial survivorship of founders is likely to be an important determinant as to whether or not the population establishes.

Population estimations are also affected by the methods available for the collection of detection data. Data from animals with high detectability and low heterogeneity produce the most accurate models. Usually these include live encounters (e.g. trapping), identifiable sightings (e.g., dead recoveries (e.g. fisheries or hunting) or age cohort distributions. Live encounter approaches have recently been enhanced by the introduction of DNA

'barcoding', in which animals are individually identified by genetic tagging via methods such as hair traps (Peakall et al., 2006) or scat collection (Wasser et al., 2004).

Few studies have the opportunity to calibrate the results of outputs from population models with known population sizes. Some species offer the opportunity for multiple survey techniques to validate population modelling, for example on Macquarie Island, where estimates of abundance of elephant seals (*Mirounga leonina*) surveyed at ground level were highly correlated with counts using satellite imagery (McMahon et al., 2014). Trap capture rates of woylies aligned closely with track counts, diggings and nest densities, allowing Wayne et al. (2013a; 2013b) to verify that declines modeled using MARK were real. Estimates are improved when ecologists have a better understanding of the population, for example islands with no migration (Bonnet et al., 2002), long-term studies with high detection rates (Bradshaw et al., 2007), or where established populations are augmented with known stock (Tuberville et al., 2008).

Aside from examples such as these, the majority of population estimation studies attempt to model the number of individuals present starting from a position of little or no baseline data. In this study, I used mark-recapture analysis to estimate the demographic parameters (survivorship of animals according to different attribute groups, capture probabilities and population numbers) of a known population of animals released into a large captive enclosure. I therefore had good knowledge of apparent survival at the start of the study, due to the known starting population and limited emigration, and of births into the population because all of the founders were tagged. The population was monitored regularly after release by trapping, and population estimates and survivorship were derived from recapture data. This study provided an unprecedented opportunity to estimate the accuracy of demographic parameters estimated from mark-recapture analysis.

Here, I aim to assess the survivorship of released animals according to different attribute groups, and to compare two modelling methods to estimate the size of the population. These data inform us of the effective founder populations, and enable better planning for future translocations by indicating the most appropriate animals to release.

Methods

Study Species

Boodies, also known as burrowing bettongs (*Bettongia lesueur*) are small rat kangaroos of the potoroid family that live in communal burrows (Sander et al., 1997). Golden bandicoots (*Isoodon auratus*) are the smallest of the short-nosed bandicoots and inhabit scrubby undergrowth (Southgate et al., 1996). Both species breed opportunistically in response to resource availability, rather than strictly by season, and are capable of reproducing within a few months of independence. They are both mostly nocturnal, omnivorous marsupials that were once distributed over approximately two-thirds of the Australian mainland, but have undergone severe declines following European settlement (Burbidge and Short, 2013; McKenzie et al., 2013). This study examines a population of both species that was reintroduced to the Western Australian mainland in order to improve their conservation status. Boodies and golden bandicoots are readily trappable and therefore make ideal candidates for a mark recapture study.

Study area

The reintroduction site Lorna Glen (26° 13’ S, 121° 33’ E) is a former sheep and cattle station in the rangelands of Western Australia. Animals were translocated from a wild population on Barrow Island (20° 51’ S, 115° 24’ E) or a captive population (boodies

only) at Dryandra (32°47′S 116°58′E) into the 1100 ha predator-proof enclosure at Lorna Glen (*Figure 26*). The wild populations on Barrow Island number approximately 40 000 – 50 000 bandicoots, and 3000 – 4500 boodies (R. Teale, pers. com., Feb 2015). Boodies from Dryandra consisted of a captive colony of 109 individuals in a 10 ha enclosure founded in 1998 by 20 Dorre Island individuals. Additional details about the translocation sites and procedure can be found in Chapter 2.

The boodies and bandicoots in the enclosure at Lorna Glen are intended to provide a breeding population of acclimatised animals that could be released from the enclosure at a later date. Adult animals could not escape from the enclosure, however, a small number of juvenile bandicoots could move in and out of the enclosure and a small number remained outside (C. Sims, pers.com., 2013).

Trapping

After the release, animals were trapped for six consecutive nights every six weeks for 18 months. I used 81 Sheffield cage traps (20cm x 20cm x 56 cm; Sheffield Wire Co, Welshpool, Western Australia) within the 1100 ha enclosure each night. I set 56 traps 200 m apart along track transects (*Figure 26*), plus clusters of five extra traps at each of five boodie warrens in order to adjust for trap saturation at these high density locations. The trap layout was biased toward the northern half of the enclosure in order to adequately cover the habitat containing boodie warrens. Due to high capture rates, it was not possible from a practical or welfare perspective to set traps at higher density or cover the southern boundary. In total, trapping consisted of 11 x 6 x 81 = 5,346 trap nights.

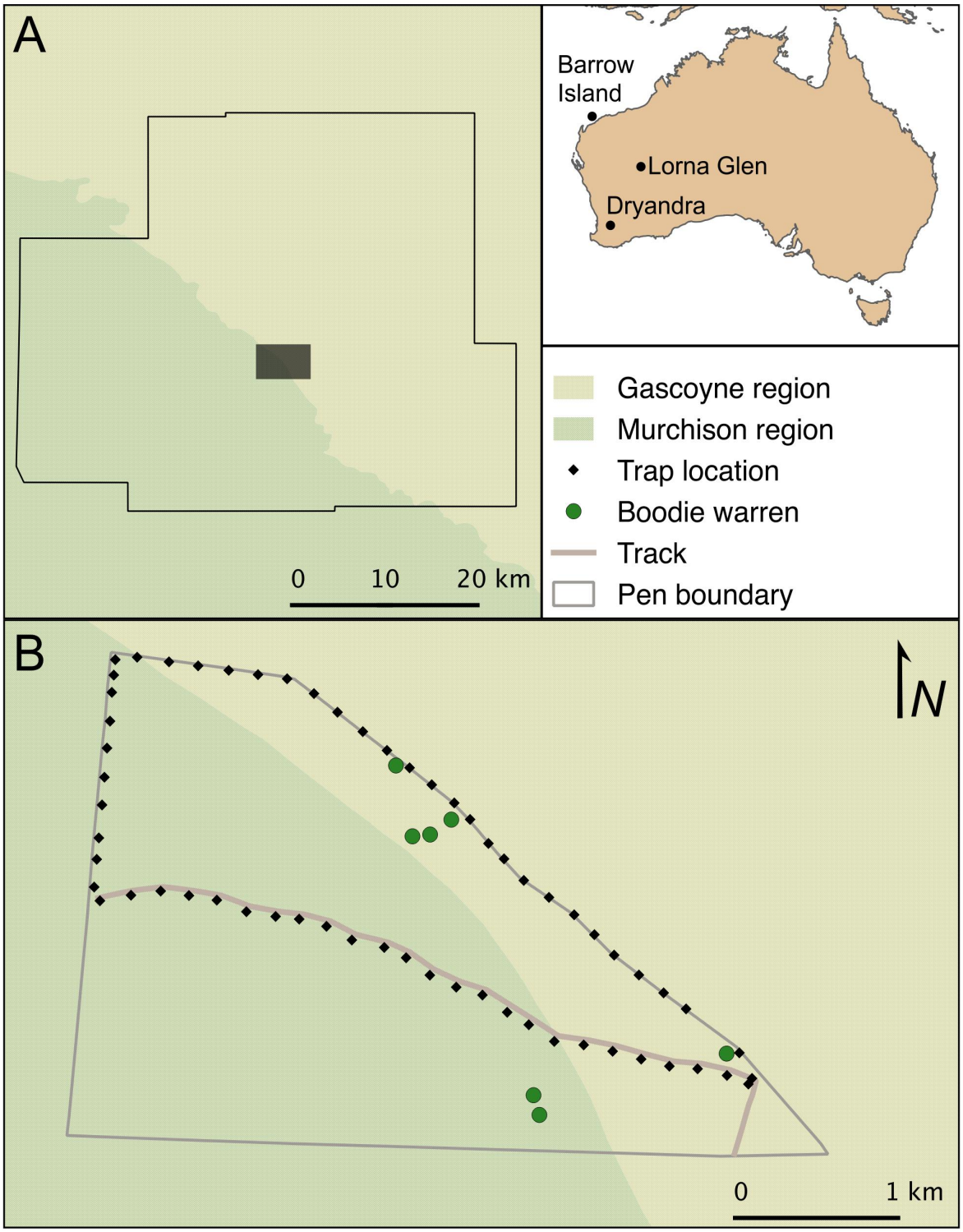

Figure 26. Map of study site showing A: Lorna Glen, B: the predator-proof enclosure at Lorna Glen, including location of tracks, trapping transects and boodie warrens. Inset shows the locations of Lorna Glen relative to the source sites Barrow Island and Dryandra, Western Australia.

Individual marks

All released animals were tagged with unique identifying passive implant transponders (PIT); Allflex® 12mm FD-X transponder (Allflex Australia, Queensland, Australia) implanted subcutaneously between the scapulae. Following implantation, I used *Vetbond*™ Tissue Adhesive (3M Australia, NSW, Australia) to hygienically seal the insertion wound and to prevent tag loss. New animals born into the population were PIT tagged on first capture when their weight was 120 g or greater. DNA samples in the form of 2mm ear notches were also taken from each new animal.

Data analysis

I estimated survivorship and capture probabilities of animals by their different groupings (sex, animal origin) using Cormack-Jolly-Seber (CJS) models in program MARK (Cooch and White, 2013). The fully parameterised CJS model allows survival and recapture probabilities to differ between groups, for each time interval. This means that each population group, capture probability, survivorship and time can operate as interactive factors. This study included 11 sampling sessions, with 10 time intervals between. One time interval was three times the length of the others due to no trapping in the peak of summer; this was accounted for in the model. Animals were divided into groups according to sex and animal origin (Barrow Island, Dryandra or Lorna Glen). Because all animals were released with PIT tags, newly marked animals must have been born on site.

Models were ranked by AICc value (Akaike's Information Criterion, adjusted for small-sample bias, White and Burnham, 1999), where lower AICc values indicate a better fitting model. If the **Δ**AICc (the difference between a candidate model and the best model) of a candidate model is less than 2.0 then it can be considered to have reasonable support.

The most parsimonious CJS models were used to inform the construction of JS open-population models (using POPAN model in program MARK) to calculate population estimates.

Models created using program MARK were compared to a more traditional method of population estimation, Known To Be Alive (KTBA), which calculate the minimum number of animals alive in a session based on their later recapture. This known value was compared to the POPAN estimations of births.

Results

Trapping

We released a total of 174 boodies, of which 109 originated from Dryandra and 65 originated from Barrow Island (see *Table 10*). The ex-Dryandra boodies were released in three stages, an initial 20 in Jan 2010, 80 in August 2010 and nine in October 2010. All 160 bandicoots were of Barrow Island origin and were released in Jan–Feb 2010. A further 48 boodies and 97 bandicoots were born into the population during the study. Juvenile animals large enough to be microchipped (heavier than 120 g) but still dependent on their mothers (e.g. young-at-heel) were excluded from the analysis until caught independently. A total of 16 individual boodies and 39 bandicoots were excluded in this way.

	Release Date	Barrow Island origin			Dryandra origin			Release
		F	M	*Total*	F	M	*Total*	**Total**
Boodies *(Bettongia lesueur)*	13/01/2010				5	15	*20*	20
	31/01/2010 - 24/02/2010	26	39	*65*				65
	10/08/2010 - 13/08/2010				44	36	*80*	80
	21/10/2010				7	2	*9*	9
	Total	**26**	**39**	***65***	**56**	**53**	***109***	**174**

	Release Date	Barrow Island origin			Release
		F	M	*Total*	**Total**
Golden Bandicoots *(Isoodon auratus)*	31/01/2010 - 24/02/2010	82	78	160	160
	Total	**82**	**78**	***160***	**160**

Table 10. Dates and number of boodies and bandicoots released at Lorna Glen in the predator proof enclosure during 2010.

Of 5,346 trap nights, I had 4010 capture occasions (75% capture success) of 479 target individual animals. This does not include the further 98 incidences of 'bycatch', consisting of one Stimson's python (*Antaresia stimsoni*), one yellow-spotted monitor (*Varanus panoptes*), one black-headed monitor (*V. tristis*), two little button-quails (*Turnix velox*), one crested bellbird (*Oreoica gutturalis*), 21 mulgara (*Dasycercus blythi*), 13 kultarr (*Antechinomys laniger*), one stripe-faced dunnart (*Sminthopsis macroura*), 26 common brushtail possums (*Trichosurus vulpecula*), 28 spinifex hopping-mice (*Notomys alexis*), one Shark Bay mouse (*Pseudomys fieldi*) and two European rabbits (*Oryctolagus cuniculus*). These animals were not permanently marked so represent capture occasions rather than number of individuals.

Mark-recapture analyses require there to be a plateau in the individual accumulation curves within a trapping session, indicating that the trapping effort is sufficient compared to the population size (Lebreton et al., 1992). In an attempt to offset high population density and incomplete spatial coverage of the enclosure, I trapped for six

consecutive nights. The individual accumulation curves for each species were asymptotic, indicating that trapping effort was adequate for surveying this population.

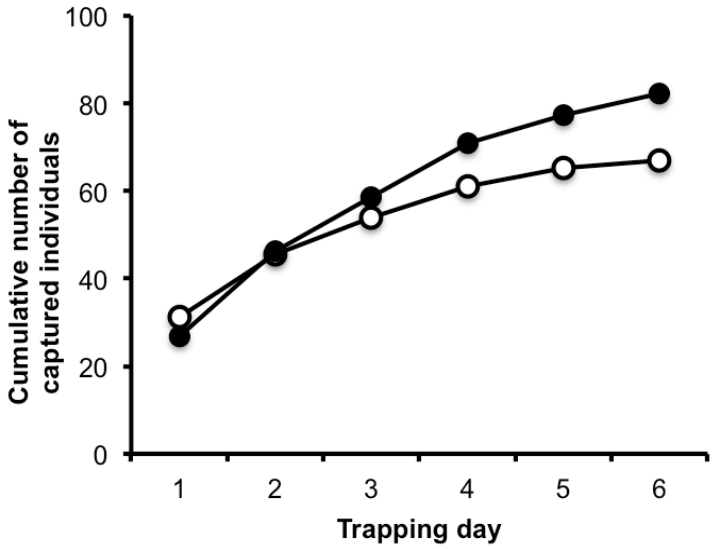

Figure 27. Individual accumulation curves for boodies (*Bettongia lesueur*: open circles) and golden bandicoots (*Isoodon auratus*: closed circles), within trapping sessions of six consecutive nights. Cumulative captures were averaged over the ten trapping sessions following release. Error bars demonstrating 95% CI are too small to see on this scale.

The ratio of boodie and bandicoot captures was statistically even; there were 1962 and 2048 captures respectively ($\chi 2,1 = 1844$, $P < 0.01$) of 222 and 257 individuals. Each individual was captured on average 8.3 ± 0.6 (boodie) and 7.7 ± 0.6 (golden bandicoot) occasions.

Two key assumptions of mark recapture modeling are that marks on individuals are permanent and uniquely identifiable, and that samples are instantaneous relative to the interval between trapping occasions (Arnason and Mills, 1981). Our combined method of permanent ear notch (for DNA sampling purposes) and unique tag implant at the time of first capture allowed me to determine that no animal with an ear notch had lost its PIT tag.

Our sampling of six nights every six weeks was a trade-off between being effectively instantaneous and having long enough sampling periods for sufficient detections.

Model choices

The best CJS models for survival and capture probability of boodies and bandicoots are shown below. For boodies (*Table 11*), the best survivorship model did not differentiate between the six groups (three animal origins x two sexes), providing a survivorship estimate for the whole population over time. Capture probabilities were different over time and by animal origin, probably due to the fact that Lorna Glen animals did not appear in the population for the first few trapping session. The following models were a substantially worse fit, as demonstrated by the delta AICc value >2.

For bandicoots, the best three models were very similar in goodness of fit, but treated the parameters differently (*Table 11*). For the best two models, survivorship was constant over time but differentiated between groups. The third model differentiated survival and capture probabilities across time. As the top three model outputs were very similar, I used the third best model for the following estimations in order to answer questions about how the population changed following translocation.

Table 11. Model rankings for Cormack-Jolly-Seber survivorship and capture probability analysis of boodies (*Bettongia lesueur*) and golden bandicoots (*Isoodon auratus*) following release at Lorna Glen, Western Australia. Group parameters included animal origin (from Barrow Island, Dryandra or Lorna Glen for boodies, or from Barrow Island or Lorna Glen for bandicoots) and sex. Lower AICc values indicate a better fitting model. If the **Δ**AICc of a candidate model is less than 2.0 then it can be considered to have reasonable support.

	Model	AICc	ΔAICc	AICc Weights	Model Likelihood	Number of Parameters	Deviance
Boodies	Phi(time) p(animal origin*time)	1263.7	0	0.87	1	36	419.7
	Phi(animal origin*time) p(time)	1267.5	3.78	0.13	0.1519	36	423.4
	Phi(time) p(time)	1278.8	15.09	0.00	0.0005	19	471.3
	Phi(animal origin*sex) p(time)	1282.9	19.21	0.00	0.0001	16	481.7
Bandicoots	Phi(animal origin*sex) p(t)	1582.8	0	0.41	1	14	524.3
	Phi(animal origin*sex) p(t*sex)	1583.8	1.03	0.24	0.5975	24	504.3
	Phi(time) p(time)	1584.1	1.34	0.21	0.5125	19	515.2
	Phi(sex*time) p(sex*time)	1586.1	3.26	0.08	0.1956	38	476.4

Survivorship

Apparent survivorship (Phi) per six-week interval was high for both boodies and bandicoots (See *Figure 28* and *Figure 29*). Adding groupings of animal origin (Dryandra, Barrow Island or Lorna Glen) or sex did not improve the boodie survival models, so all animals were considered together. The most parsimonious model for bandicoot survivorship indicated that across the whole timeframe of this study, males had better survival than females, and that animals originating from Barrow Island survived better than new recruits born on site at Lorna Glen (*Table 12*). For the remainder of the results below, I am considering the third-best model, which grouped survival of all animals together, differentiating survivorship and capture probabilities over time.

Table 12. Survivorship (Phi) estimates ± standard error for golden bandicoots (*Isoodon auratus*) in the 18-months following release at Lorna Glen, Western Australia. These estimates are derived from the best Cormack-Jolly-Seber model, which differentiated the survivorship of animals in different groups but not over time.

	Males, Barrow Island	Males, Lorna Glen	Females, Barrow Island	Females, Lorna Glen
Survivorship (Phi)	0.91 ± 0.01	0.88 ± 0.03	0.85 ± 0.06	0.78 ± 0.02

In the first interval following release, 82.5% of boodies survived to the first trapping session. Models did not distinguish boodies of different groups. Overall survivorship for all boodies across the 18-month study was 87.1 (± 2.5)%. When examining the model including time as a factor, female bandicoots had a lower apparent survival than males in the sessions following release (*Figure 29*). Almost all male bandicoots 99% (± 3.7) survived from release to the first trapping session 6 weeks later, whereas apparent survival for females was 73.1% (± 5.9). Overall survivorship across the 18-month study for male and female bandicoots were 88.4 (± 2.6)% and 86.8 (± 3.1)% respectively.

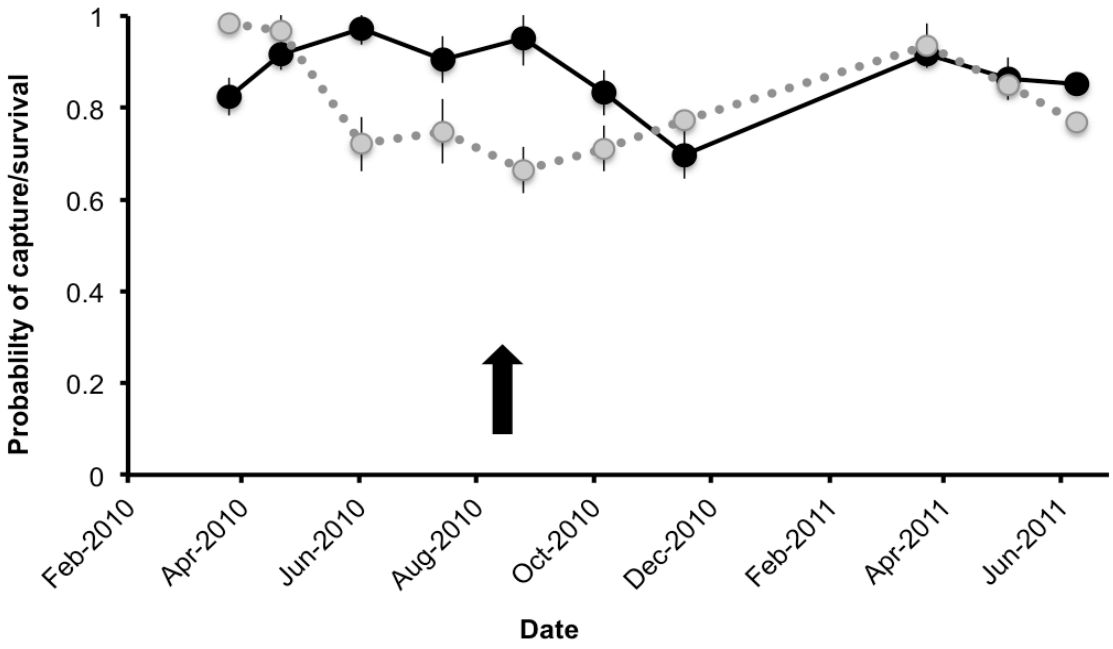

Figure 28. Apparent survival (phi: black) and capture probability (p-hat: grey) of boodies (*Bettongia lesueur*) at Lorna Glen, following initial release in January 2010. The arrow denotes the release of an additional 80 boodies in August 2010. Error bars denote standard error.

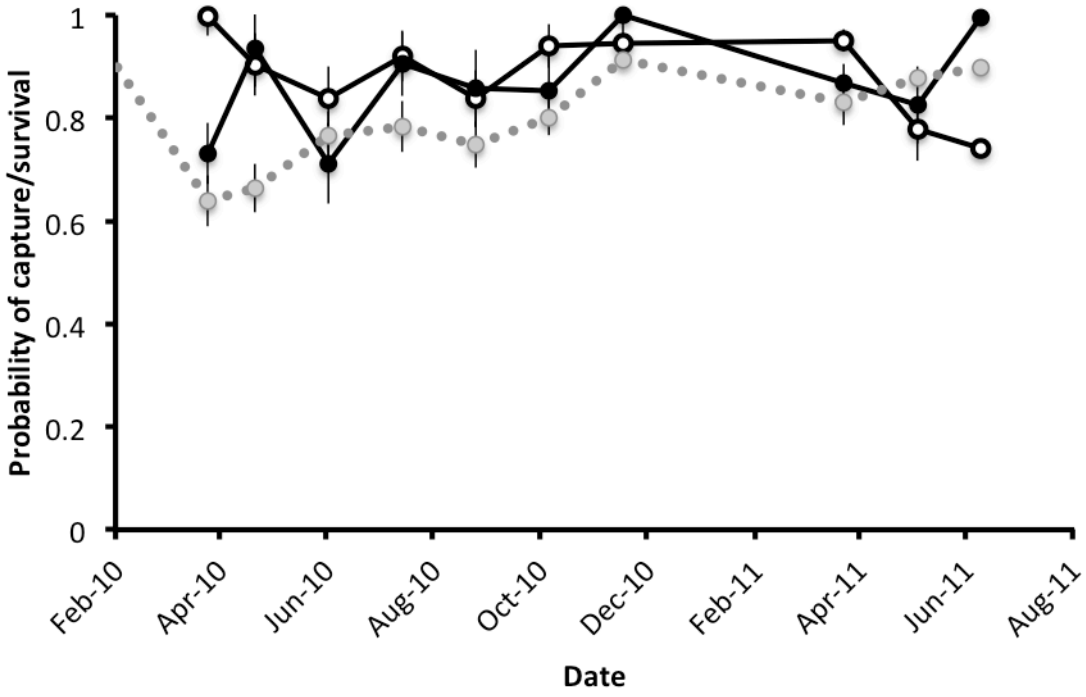

Figure 29. Apparent survival (phi) and capture probabilities (p-hat) of golden bandicoots (*Isoodon auratus*) at Lorna Glen, following release in January 2010. Capture probabilities are shown in grey, with survival probability in black (male: open symbols, female: closed symbols). Error bars denote standard error.

Capture probability

Both species showed high capture probability (p) of between 64 and 99% (see *Figure 28* and *Figure 29*). Capture probabilities were not differentiated between the sexes or animal origins for either species, but were variable between trapping sessions. For the first two trapping sessions, boodies had significantly higher capture probabilities than bandicoots (~ 95% cf 65%, respectively), indicating that boodies could have been excluding bandicoots from traps. For the remainder of trapping sessions, boodies and bandicoots had very similar capture probabilities.

Population estimates

Both methods of population analyses aligned very closely over time. As expected, boodies showed a sharp population increase when the additional 80 individuals were released in the enclosure, but declined again shortly afterwards (*Figure 30*). Survivorship immediately following the second release dropped to 60 and 70% (for males and females, respectively) at this time, consistent with the decrease in population numbers.

Bandicoot population estimates appeared to decline slightly over time, plateauing at approximately 30-35 female and 50-70 male individuals (*Figure 31*). Population estimates for females were consistently lower by both calculation methods.

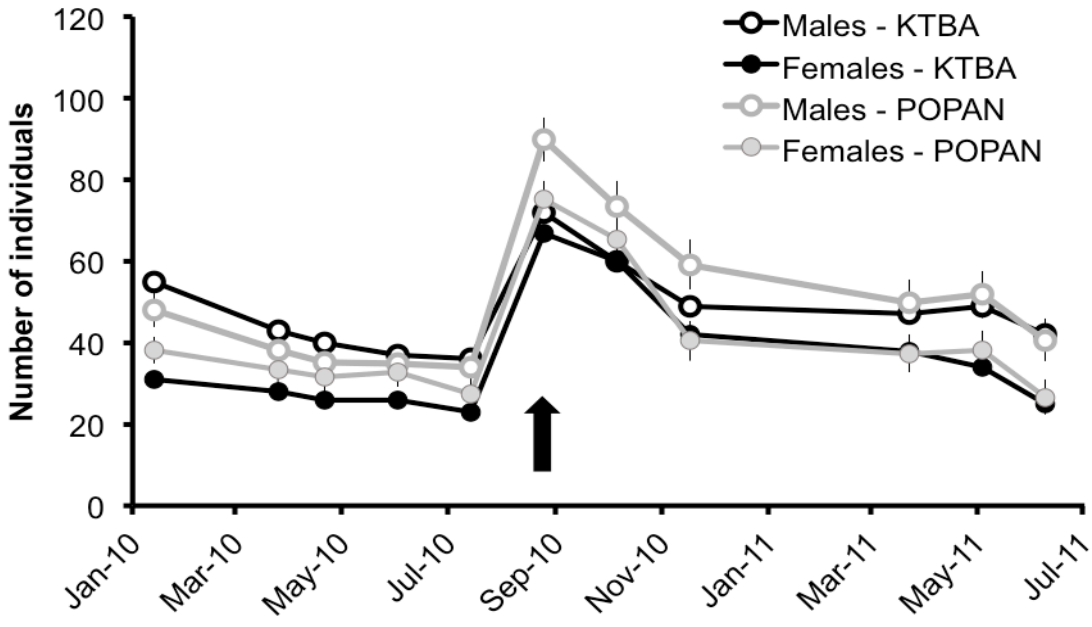

Figure 30. Population estimates for boodies (*Bettongia lesueur*) in the enclosure at Lorna Glen, as estimated by CJS – POPAN analysis and Known To Be Alive (KTBA) calculations. Animals were

initially released in January 2010, with an additional 80 boodies in August 2010 denoted by the arrow. Error bars signify standard error.

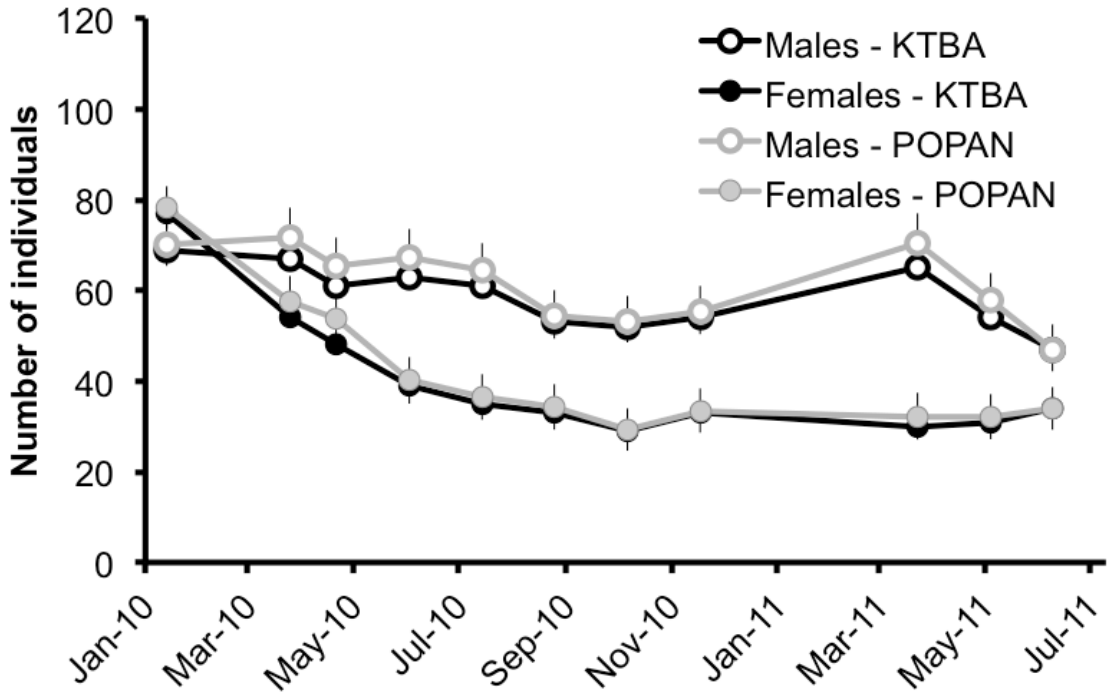

Figure 31. Population estimates for golden bandicoots (*Isoodon auratus*) following release in an enclosure at Lorna Glen in Feb 2010, as estimated by CJS – POPAN analysis and Known To Be Alive (KTBA) calculations.

Discussion

Survivorship

One important consideration for managers undertaking translocations is the short-term survival of animals moved to a new location, in order to calculate the effective starting population of animals moved. It is expected that some immediate post-release mortality will occur due to stress and adjusting to the new habitat in the establishment phase. Stress is an important consideration during translocations, and founder populations need to be of a suitable size to robustly survive the establishment phase (Teixeira et al., 2007; Dickens et al., 2010).

This study provides estimates of apparent survivorship for the first six weeks following release with little to no emigration, effectively enabling me to calculate translocation mortality. For boodies, there was an apparent survival rate of 82.5% immediately following translocation, meaning that approximately 70 of the 85 first released survived the initial phase of translocation. The inclusion of sex or animal origin in the survival models did not improve model performance and hence all boodie estimates were considered jointly. For bandicoots, there was a difference in apparent survival between the sexes, but no difference between founder and new individuals. In the period directly following release, males showed an almost 100% survival rate (77.7 animals) whereas females had an apparent survivorship value of 73% (59.9 animals). Because there was no difference between capture probabilities of sexes for either species, and virtually no emigration outside of the enclosure, we can be confident that these apparent survivorships fairly closely reflect true survivorship. Values of effective founder population are useful for calculations such as population viability analyses (Ottewell et al., 2014) and for managers to plan the sex ratio and numbers of animals to release.

Including animal origin as factor did not improve the survival models for either species. Boodies originated from three locations; Barrow Island, Dryandra and Lorna Glen, bandicoots only two; Barrow Island and Lorna Glen. Boodies from two different translocation origins allow testing of the differing survival rates in the period immediately following release (weeks after release), and in the medium term (1.5 years following release) of animals originating from different conditions. The lack of difference in post-release survival between animals from Dryandra (32° latitude) and Barrow Island (20° latitude) at Lorna Glen (26° latitude) indicates that the captive population from Dryandra performed similarly to the wild animals originating from Barrow Island. This result

validates that the conditions under which the Dryandra captive breeding colony was run were suitable for producing animals fit for translocation.

Importantly, the lack of differentiation in survivorship of animals of Lorna Glen origin means that there was not a significant difference in survival for newly recruited animals compared to founder adults for either species. The animal origin groups incorporate some level of age differentiation, as all newly marked animals were new recruits born on-site, and are therefore younger than the founder Dryandra or Barrow Island animals. This suggests that for boodies and bandicoots in this population, survival from first independence from mothers (at 150-200 days for both species; Tyndale-Biscoe, 1968; Gemmell, 1986) is not significantly different from adult survival. This may be related to two factors; one, that the translocated founders were not a specific age cohort, rather a subsample of a large population, and therefore do not necessarily all share identical survival rates. Two; the enclosure provides protection from some mortality effects, including natural predators such as dingoes and large goannas. Survival as an independent animal appears to be lower than that of pouch young; a study examining survivorship of the northern bettong (*B. tropica*) indicates that approximately 90% of pouch young survive from birth to an age of emergence from the pouch (Vernes and Pope, 2002).

Survivorship differed from session to session for both boodies and bandicoots, indicating that there were temporal factors affecting survivorship. For boodies, this is probably due to the release of an additional 80 animals in August, 2010. The lowest survival between sessions was (0.7 ± SE 0.05) in November 2010, approximately eight weeks after an additional 80 boodies were released from Dryandra. On average, boodie survivorship per six-week period was 0.87 ± SE 0.02.

The decline in apparent survival and population size of boodies was not explained by animal origin, indicating that all animals in the population were affected equally by the release of additional animals. This lends support for the theory that the limiting factor in the size of the population within the enclosure is shelter, in the form of underground warrens, rather than being related to characteristics of either founder population. Boodies dig extensive warrens that provide protection from environmental extremes of temperature and humidity. At Lorna Glen, ground temperatures exceed 70° C in summer and are below zero in winter, whereas temperatures within burrows are greatly buffered, ranging between 4° C and 42° C (J. Dunlop, unpublished data). Omnivorous boodies probably combine their shelter excavation and feeding activities, eating any invertebrates discovered in the soil (Robley et al., 2001). Although warrens provide shelter and some feeding opportunities, they involve time and energetic cost to create, and the addition of extra animals potentially exceeded the carrying capacity of the warrens available at that time.

The temporal factors affecting bandicoot survivorship appear to relate to post-release mortality of females. Survival probabilities stabilised within the first six months for females, to a similarly high rate as that of males. The survival rate of bandicoots remained unaffected by the additional release of boodies in August 2010, indicating that the two species are probably not in direct competition with each other. Although they have been observed to cohabit with boodies, golden bandicoots do not rely on warrens for shelter, and tend to inhabit the dense spinifex clumps in the south and west of the enclosure.

Capture probability and population estimation

Both species maintained a high capture probability throughout the duration of the study. High capture probabilities also reduce the difference between KTBA and MARK population estimates. Program MARK provides additional data resolution than KTBA estimations by calculating the probability that animals existing in the system remain uncaptured. In the case of this study, the probability of eluding capture was less than 35%, adding little to KTBA population estimations.

Population estimates for both species appeared to plateau, regardless of additional releases or high birth rates. This may indicate that at this time, the enclosure had a carrying capacity beyond which additional recruits were not supported. For boodies, this appears to be around 55 males and 40 females, and for bandicoots, 60 males and 40 females (*Figure 30* and*Figure 31*). These estimates of population over time were very similar between the two methods of analysis. For boodies, CJS – POPAN gave slightly different estimates for males, especially after the second introduction. Both methods show declines for the last capture session, probably an artefact of this being the session with the least data to draw from.

Boodie and bandicoot populations in this study both had uneven sex ratios, with more males present in the populations for both species. This bias does not appear influenced by capture probabilities because no difference between the sexes was detected. Trapping of a small number of golden bandicoots on Marchinbar Island (Southgate et al., 1996) and data from Barrow Island fauna monitoring from 1998–2008 (Morris et al., 1999) both revealed parity in sex ratio. The difference in sex bias at the translocation site may be an artefact of establishment, and the sexes may return to parity.

It is likely that both “trap-happy” individuals and trap saturation have the effect of causing population underestimates in this system. Traps were set 200m apart, so individuals continually occupying traps early in the night would have prevented others coming in from further away. This trapping design was limited by the number of animals it was feasible to process in a day with a 76% capture success, but an ideal setup for best estimates may be a grid throughout the entire enclosure.

All of these parameter estimates are useful for informing future translocations. Long and short-term survival, recruitment rate and sex ratios are used in population viability analysis models, which are used to predict the persistence or extinction of a population under different conditions. Knowing the effective founder population size allows planning for retention of genetic diversity over time (Ottewell et al., 2014). Total population size and growth rates enable calculation of potential harvest for translocations outside of the enclosure.

Chapter 7.

General Discussion

Introduction

This thesis closely examined the ecology of two species of Australian marsupials, the boodie (*Bettongia lesueur*) and the golden bandicoot (*Isoodon auratus*) following reintroduction to an area from which they had been locally extirpated. During the release and 18 months of post-translocation monitoring, I examined several aspects of the animals' ecology in detail. Specifically, I studied the host-parasite relationships between the mammals and their ectoparasites and trypanosomes (**Chapter 4**), the morphological change one species went through in its new environment (**Chapter 5**), and changes in mammal population size following release (**Chapter 6**).

Overview of findings, their contribution to knowledge and future questions

Host – Parasite dynamics

In **Chapter 1** of the thesis, I reviewed information from previous translocations regarding the disease management implemented when moving populations of animals to and within Australia. The scale of human-mediated movements of animals is vast; at least 50 different species of terrestrial vertebrates have been introduced to and currently persist on mainland Australia (Bomford and Olsen, 2003). Animals were brought to Australia in a sudden and methodical way; within six months of Captain Phillip's first arrival in 1788, the colony at Sydney included nine species new to the continent, plus, presumably, unintentionally released ship rats, house mice and domestic cats (Trewin, 2001).

Faunal movements in Australia have ranged in intent from deliberate introductions for agriculture (e.g. livestock, poultry), repeated attempts to naturalise animals to make Australia feel more familiar to Europeans (e.g. foxes, hares), biological control (e.g. cane toads), to accidental introductions (e.g. rats and mice). Finally and most recently, native animals have been relocated within Australia for conservation purposes. All of these movements have had the potential to introduce or change the natural distribution of parasites and disease causing agents.

This introduction to the thesis was crucial to understanding the importance of the ecological impacts of parasites on hosts with no acquired immunity, especially species threatened by other stressors. In particular, I discussed trypanosomes, and put forward a hypothesis that details their potential role in Australian faunal declines during the 1880s. Trypanosomes are a potential candidate for epizootic declines of Australian mammals due to their pathogenic nature, ability to infect a variety of host genera and varied transmission modes. Furthermore, when combined with other factors, such as predation and competition effects, trypanosomes are capable of exacerbating extinction of its host and influencing severe fauna declines (Wyatt et al., 2008; Smith et al., 2008; Thompson et al., 2014b). I highlighted the lack of consideration that has been given to Australian trypanosomes and disease transmission throughout Australia's history, and currently, within translocations for conservation purposes. It is worthwhile noting here, that further work is urgently needed to (a) identify other native trypanosome species of Australian animals, (b) investigate the life histories of these parasites within in their vertebrate host, (c) identify their invertebrate vectors, and (d) investigate whether these invertebrate vectors pose any biosecurity threats to Australian mammals and human residents with the inadvertent transmission of exotic human trypanosome species (such as *T. cruzi*, and

Leishmania spp.) from chronically infected international visitors and immigrant to this continent.

General materials and methodologies for the study site, species and translocation of boodies (*Bettongia lesueur*) and golden bandicoots (*Isoodon auratus*) are closely detailed in **Chapter 2**. I give descriptions of the two threatened mammal species examined in this study, and Lorna Glen, the site at which they were reintroduced after being locally extinct for at least 60 years. This reintroduction has added to their limited extant distributions; predator-free islands and in fenced conservation areas. The founder populations consisted of 160 golden bandicoots from Barrow Island, 109 boodies from Dryandra captive breeding facility and 65 boodies from Barrow Island.

Chapter 3 details the DNA extraction and PCR protocols used to detect the two different trypanosome species naturally occurring in the two boodie populations. Using the most sensitive techniques available is particularly important in an ecological context where parasites potentially fluctuate in a population without overt pathogenic symptoms. I established that although automated DNA extraction is an attractive option for high throughput experiments such as this, the efficiency of DNA extraction appears to be lower than for manual extraction methods. Using PCR as a detection tool proved to be highly variable, depending on the methods used. By testing multiple replicates of the same samples, I demonstrated that results vary wildly when using different extraction techniques and primers. This study stresses the importance of testing the sensitivity and efficiency of molecular methods on known samples before relying on them in a diagnostic capacity.

My best method, capable of detecting 221 trypanosomes per mL with 95% confidence, was then used for the parasite analyses in **Chapter 5.** Even this level of

sensitivity is imperfect, and unlikely to show the true oscillations of parasitaemia within an individual or population. This limited the interpretation of factors affecting the change in prevalence of trypanosomes in the boodie population. This study would have benefited from examining prevalence and spillback between the two coexisting mammal species, however I was unable to accurately detect trypanosomes in golden bandicoots using these methods. Successful analysis of the true host-parasite dynamics of this population would require anaesthesia of golden bandicoots to obtain larger volumes of blood, or more sensitive trypanosome detection techniques.

The relatively low trypanosome detection rates on blood samples known to contain trypanosomes precluded any further work testing old specimens and the role of trypanosomes in the epizootic hypothesis (Abbott, 2006). In the future, if suitably reliable trypanosome detection techniques are developed, museum specimens collected before and within the timeframe of the hypothesised epizootic could provide answers about the role of disease in Australia's faunal declines. The introduction of a new trypanosome or other pathogen at the time of European colonisation would lend evidence to the epizootic theory. The different species affected by the declines, and the timeframe of each, could also be examined. Work such as this would require destructive sampling of incredibly valuable museum specimens, so techniques and scientific questions should be well designed, preferably testing several potential pathogens at the same time, and an optimised and verified DNA extraction technique for ancient DNA samples from preserved specimens.

The host-parasite dynamics of translocated boodies and bandicoots are examined in **Chapter 4.** This work was unique in that I used experimental manipulation of ectoparasites to test the temporal dynamics of blood parasites and resulting survivorship of the host mammals. Due to high recapture success, antiparasitic treatment of animals in the treated group was ongoing throughout the study. I examined ectoparasite and trypanosome

prevalence for animals that were experimentally treated with an antiparasitic, compared to those that were not. Experimental manipulation and post-release monitoring are aspects of translocations that have been recognised as important, but lacking, in the field of parasite and disease monitoring (Cunningham, 1996; Kock et al., 2010; Sainsbury and Vaughan-Higgins, 2012). I intensively monitored individuals of both mammal species at regular time intervals after release, creating a picture of the response of ectoparasites and trypanosomes to translocation.

Key findings from this work included observation of the transmission of *T. vegrandis* to the population of boodies that were previously not infected with this species, and to offspring born into the population. Several ectoparasite species did not survive the translocation. I also found application of the antiparasitic to have no impact on ectoparasite or trypanosome prevalence, or subsequent host survival in the translocated population between the treated or untreated groups, despite regular reapplication. However, burrow sharing and contact between animals may have confounded the distinction between treated and untreated groups. Translocation and treatment of a proportion of the population had a substantial impact on the parasite community of translocated hosts, and this impact persisted into the next generation of hosts born at the translocation site, creating a host-parasite community different to that of either source site.

Management recommendations resulting from this work would not support the additional cost and effort of repeatedly treating animals post-release. The circumstances of this study were unusual; translocation programs rarely have such frequent monitoring of a released population or such high recapture rates to facilitate these efforts. As the antiparasitic appeared ineffective in these circumstances, it is unlikely to be effective with fewer recaptures, although this may vary amongst species and locations. As demonstrated by McGill et al. (2010), best practise techniques should involve control, rather than total

eradication, of parasites. This can be achieved by treating a proportion of the population at the time of release (Hardman, 2007) or quarantining animals in low density prior to release (Mathews et al., 2006). Unless specifically pathogenic parasites (e.g chytrid fungus, Skerratt et al., 2007; Daly et al., 2008) are identified prior to release, it is not necessary to completely remove parasite fauna in the translocation process. However, identifying such parasites requires insights into the life cycle and pathogenicity of Australia's native parasites at a greater level than is currently understood (Thompson et al., 2010).

Further work in this area should examine the paradigm of pathogens having an exacerbated impact on stressed animals during translocations (Teixeira et al., 2007). Under what conditions does stress have an impact? What level of pathogenic load is important? Which pathogens should managers be particularly concerned about? Questions such as this can be addressed by quantifying stress levels in a population (e.g via hair, Koren et al., 2008) and examining how this relates to the relative survivorships of animals with differing parasite loads. The ecology of parasites and their impact on translocated hosts are evident only by careful longitudinal investigation of host and parasite populations (Armstrong and Seddon, 2007; Schultz et al., 2011). However, answering questions about the ecology of threatened species is always challenging due to the low numbers of animals available for experimental manipulation. For example, the further experimental investigation involving these two vulnerable species (boodie and golden bandicoot) to answer such questions would require highly justified scientific validity to fulfil the numerous ethical issues of potential lower survivorship.

Translocation ecology

The concept of island dwarfism and how it relates to translocations is discussed in **Chapter 5**. Although there has been much theoretical work on comparing islands with similar fauna and information derived from the fossil record, this study is new in experimentally observing the effect of moving an insular species to a new mainland and island sites. Insular dwarfism or gigantism (Adler and Levins, 1994) has been observed in many species, with the general trend of larger (>700g) animals becoming smaller on islands (Lomolino, 2005; but seeMeiri et al., 2008). In my study, animals in empty niches responded quickly by increasing in body size and mass, reproductive output, and producing larger young in better condition. Animals comprising the original founder population increased in weight in the 18 months following release, but not body size, suggesting that final adult size is related to sub-adult nutritional intake. I give evidence to support the hypothesis that Barrow Island bandicoots are not true 'island dwarves'; the Barrow Island population appears to be environmentally constrained on the island and individuals never grow to their full size potential.

There are several possible interpretations of this study on the concept of insular dwarfism as it applies to golden bandicoots:

1. The concept of the island syndrome is flawed, and all insular species retain phenotypic plasticity to revert to 'normal' size within a few generations once pressures are removed.
2. The island syndrome, caused by natural selection, is a real concept, but golden bandicoots are not true island dwarves due to the fact that they can return to 'normal' body size.

3. Island dwarfism/gigantism by natural selection is a real concept and golden bandicoots are island dwarves, but 8000 years of geographic separation is not long enough for the trait to become irreversible. In essence, we are observing early stages of speciation occurring.

Further questions regarding the relevance of the island syndrome are raised when considering the response to translocation over a longer term. If the resource-limitation hypothesis is true, perhaps there will be a point in the future where the golden bandicoot population on Hermite Island is at 'capacity' and food resources become scarce. Will we see a gradual reversion to dwarf size approaching this point? Or will they maintain the new larger size, due to the lack of interspecific mammal competition on Hermite Island?

The driver for body size change was not explored in this thesis, but I suggest it is due to high population density of golden bandicoots plus several other mammal species in a low productivity ecosystem. This hypothesis is supported by the low reproductive output of females on Barrow Island compared to both of our translocation sites. Golden bandicoots breed according to availability of resources, rather than season, therefore a consistently low reproductive output suggests that resources are consistently limited. Ideally, this study should have been paired with an investigation into resource availability at the source and destination sites across the course of the translocation. Alternatively, this hypothesis of resource-driven morphological change is testable by measuring size change for a population of Barrow Island golden bandicoots with experimentally increased food availability, or by undertaking an analysis of food availability and diet throughout a Barrow Island golden bandicoot translocation to a new site. Furthermore, the response to translocation of the Hermite Island bandicoots in the long-term is of particular interest:

will this insular population eventually suffer from resource depletion and return to a smaller average size and lower fecundity?

Finally, this work raises questions about the taxonomic identity of the golden bandicoot, *I. auratus*. There has been much debate about whether *I. auratus* should truly be considered a species separate from the southern brown bandicoot, *I. obesulus*. The major morphological distinction between the two species is size, with some researchers attributing any genetic distinction to geographical isolation rather than species boundaries (Tate, 1948; Lyne and Mort, 1981; Pope et al., 2001; Westerman et al., 2011). Most recently, Westerman et al. (2011) redefined *I. auratus* as a species separate from *I. obesulus* in their recent genetic review of the family Peramelidae, on the basis of both nuclear and mitochondrial DNA sequences. They recommend that the *I. auratus* (and subsequently the two subspecies *I. auratus barrowensis* and *I. auratus auratus*) retain their species status, separate from *I. obesulus*. In this study, I demonstrated that the size of *I. auratus* is plastic, and likely resource-dependent. Although the golden bandicoots in this study did not reach comparable size compared to the southern brown bandicoot (up to 700 g, cf. up to 1600 g, respectively, McKenzie et al., 2013), perhaps *I. auratus* could achieve a larger body size in a more temperate climate. This work raises question about the true morphological differences between the two species and their potential overlap in recent history.

In **Chapter 6** I examined the population dynamics of the two translocated species, analysing their survival rates and capture success based on population characteristics. This chapter lent insights into the short-term success of boodies and bandicoots following translocations. I established that female golden bandicoots have a higher mortality rate than males immediately following release, indicating that future translocated populations should include a female biased sex ratio in order to retain natural parity in the sexes (Southgate et al., 1996; Morris et al., 1999). Boodie survival was not correlated with age or

animal origin, demonstrating that wild animals from Barrow Island and semi captive animals from Dryandra were equally suited to translocation. For both species, animals born on site (new recruits up to 18 months old) did not show survival rates different to that of adult founders. There does not appear to be a separate survivorship cohort for sub-adult individuals, unlike many other species (McMahon et al., 2003; Millsap et al., 2004; Tuberville et al., 2008). This indicates that for both species, once independent of their mother, survival rates are the same as the previous generation of breeding adults. This finding should be taken in the context of the translocation event within a predator-proof enclosure that provides significant protection from feral cats, dingoes and some protection from other predators such as large goannas.

Future work on this enclosed population could further examine the differences in survivorship according to age cohorts in a (now) stable population. Bi-annual monitoring has continued for the Lorna Glen population, meaning that the majority of individuals have unique marks and can be attributed to approximate age classes. A greater resolution of age class may reveal greater differences in survivorship than was possible with the initial wild founder populations, and may provide valuable information to the translocation ecology of these species.

Contribution of this thesis to a greater body of knowledge

Fauna relocations are a commonly used, but not reliably successful, conservation tool for creating new populations of threatened species. Failure to establish may be due to a number of reasons, including the adaptability of the species, the conditions at the release site, the number of animals moved, mortality effects such as predation or disease, and the presence of other environmental factors required for their life cycle. These factors are the

same for invasive species establishing at new locations. The success of translocations for conservation purposes, and the survival of parasite species within the context of their translocated hosts are topics that were examined by this thesis.

Parasites, along with other disease causing agents are often unjustly neglected in translocations of threatened fauna. We have very little knowledge of the impacts (positive or negative) of parasite communities in Australia's native fauna, and therefore lack decision-making options to manage them appropriately for new populations or communities undergoing significant threatening processes. Should parasites be eradicated from conservation significant hosts prior to movement, in order to give them the best chance of survival? Or does a presence of "familiar" parasites protect the host against potentially more pathogenic parasites at the translocation site? There has been a lack of empirical studies examining these questions in order to provide best practice advice. This thesis attempted to answer some questions in this regard, by quantifying the parasite species prevalent in the mammal population and how these changed over time and with experimental manipulation.

This thesis also highlights the value of large, long-term data sets and monitoring populations across the period of an ecological disturbance or conservation action such as a translocation. High quality, long-term ecological research studies increase the probability that population responses will be identified and interpreted, particularly when multiple pressures are operating simultaneously (Paine et al., 1998; Lindenmayer et al., 2010). Ecological “surprises”, such as the unexpected finding of body size change immediately following a translocation presented in this thesis, are critically important in redefining our view of the natural world. Through this body of work, I demonstrated how an integrated approach to reintroduction biology that examines host-parasite dynamics and population ecology will help to improve future threatened fauna translocations.

Publications arising from this and associated research

Peer-reviewed publications

Dunlop, J., Thompson, C.K., Godfrey, S.S. & Thompson, R.C.A. (2014). Sensitivity testing of trypanosome detection by PCR from whole blood samples using manual and automated DNA extraction methods. *Experimental Parasitology*, **146**, 20-24.

Ottewell K, Dunlop J, Thomas N, Morris K, Coates D, Byrne M (2014). Evaluating success of translocations in maintaining genetic diversity in a threatened mammal. *Biological Conservation* **171**, 209–219.

Submitted papers

Dunlop J, Godfrey SS, Thompson RCA (submitted). The Hitchhiker's guide to Australia: a parasitological perspective on native and exotic fauna translocations. Submitted to *International Journal of Parasitology: Parasites and Wildlife*.

Thavornkanlapachai R, Mills H, Dunlop J, Morris K, Donaldson F, Kennington, J (submitted). Asymmetrical introgression between genetically distinct populations of the burrowing bettong (*Bettongia lesueur*) in a newly established translocated population. Submitted to *Journal of Molecular Ecology*

Manuscripts in Preparation

Dunlop J, Godfrey SS, Thompson RCA (in prep). Host-parasite dynamics of Trypanosomes and ectoparasites on a translocated population of Australian marsupials. To be submitted to *Parasites and Vectors.*

Dunlop J, Morris KD (in prep). Environmental determination of body size in mammals: rethinking 'island dwarfism' in the golden bandicoot. To be submitted to *Proceedings of the Royal Society B.*

Dunlop J, Williams MR, Morris KD (in prep). Getting close to the MARK: An empirical test using captive populations of boodies and golden bandicoots. To be submitted to *Conservation Biology.*

Conference Presentations

Dunlop J, Morris KD, Thompson, RCA (2013). Trypanosomes and their ectoparasite vectors on a translocated population of Australian marsupials (ABSTRACT). In *Conference Handbook: 24th International Conference of the World Association for the Advancement of Veterinary Parasitology Hosted by the Australian Society for Parasitology Inc. 25–29 August 2013, the Perth Convention and Exhibition Centre.*

Dunlop J, Morris KD, Thompson RCA (2012). Translocation removes island dwarfism in the golden bandicoot (*Isoodon auratus*) (ABSTRACT). In *Conference Handbook: Ecological Society of Australia, 2012 Annual Conference, Ecology: Fundamental Science of the Biosphere: 3-7 December 2012, the Sebel Albert Park, Melbourne, Victoria* p. 185.

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Other publications during candidature

Burrows N, Dunlop J, Burrows S (2012). Searching for signs of bilby (*Macrotis lagotis*) activity in central Western Australia using observers on horseback. *Journal of the Royal Society of Western Australia* **95**, 167-170.

Dunlop J, Morris KD (2012). *Chuditch, Dasyurus geoffroii, recovery plan 2008-2018.* Department of Environment and Conservation, Kensington, WA. *Western Australian Wildlife Management Program* **54**, 39 p.

Miller E, Dunlop J, Morris KD (2010). *Rangelands restoration: fauna recovery at Lorna Glen, Western Australia: progress report, August 2008-June 2010.* Department of Environment and Conservation, Woodvale, WA.

Dunlop J, Morris KD (2009). Into the wild: restoring rangelands fauna. *Landscope* **24(4)**, pp. 52–58.

Appendix

Coauthored peer-reviewed manuscript

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Evaluating success of translocations in maintaining genetic diversity in a threatened mammal

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ABSTRACT

The effectiveness of fauna reintroduction programs has been limited by the availability of source animals and the lack of follow up monitoring to assess whether viable populations have been successfully established, particularly in terms of conserving genetic diversity. Here we present genetic assessment of the translocation of golden bandicoots (*Isoodon auratus*) from a large source population on Barrow Island off the north-west coast of Western Australia to two other island sites and a mainland fenced enclosure. We assessed the genetic diversity of animals translocated to each site and their wild-born progeny, and whether wild-born animals showed evidence of genetic bottlenecks or genetic drift from the source population. Encouragingly, we found no significant loss of genetic diversity in any of the wild-born populations compared to the source population and no significant increase in inbreeding or relatedness amongst wild-born individuals compared to founder populations two years post-translocation. However, we detected an approximately 10-fold reduction in effective population size between founding and wild-born populations. We found no apparent differentiation between wild-born populations and the original source population, or between wild-born animals and their respective founders. Population viability modeling predicts that each of the translocated populations is susceptible to loss of genetic diversity over time. Taken together these results suggest that the golden bandicoot reintroduction program has been initially successful as a result of large founding sizes and high reproductive rates; however, ongoing augmentation will be required to prevent genetic erosion and maintain evolutionary potential in the long-term.

1. Introduction

The reintroduction of extirpated fauna to parts of their former native range is a key conservation tool used by wildlife managers to increase effective population size and spatial representation of threatened species, and reduce the risk of extinction. Reintroductions are also expected to have a greater benefit on ecosystems by restoring some level of ecosystem function in the species' former habitat; for example, bandicoots are considered 'ecosystem engineers', having an important role in soil turnover and nutrient cycling (Valentine et al., 2012). The ultimate aim of reintroduction programs is to establish viable, self-sustaining populations (IUCN, 2012), though the criteria used to judge their success or failure are often not clearly defined, such that there is still no clear agreement on what constitutes a successful reintroduction (Moseby et al., 2011; Seddon, 1999). For example, Bajomi (2010) summarises four different definitions of success, including breeding of the first wild-born generation, positive population growth rate over three generations or 10 years, the use of population viability analysis indicating a self-sustaining population and population persistence over a defined period of time.

Globally, reintroductions have been attempted for a large number of vertebrate species (primarily mammals and birds) but have had only what is viewed as limited success in establishing viable, self-sustaining populations in the medium to long-term (Fischer and Lindenmayer, 2000; Sheean et al., 2012; Short, 2009). Habitat suitability and quality, and the failure to control or remove threatening processes (such as predation) are frequently identified as the reasons for the failure of reintroduced populations (Moseby et al., 2011; Sheean et al., 2012), though others may include naivety of

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captive-reared individuals, too few individuals released or disease (Short, 2009). Whether successful or not, the genetic viability of reintroduced populations is rarely investigated despite widespread recognition of the negative impacts of inbreeding and genetic drift in small populations (but see Jamieson, 2011; Mock et al., 2004; Reynolds et al., 2013; Weiser et al., 2013). To avoid or ameliorate these effects it is important that genetic issues be incorporated early in the design of reintroduction strategies, and also in the post-release monitoring, since they are key aspects of both short-term (e.g. inbreeding depression) and long term (e.g. erosion of genetic diversity) population sustainability. These issues have been highlighted recently, with consideration of the selection of founders, maintaining genetic diversity and monitoring genetic diversity in reintroduced populations included within the IUCN species reintroduction guidelines (IUCN, 2012).

Typically, reintroduced populations are established from small numbers of founder individuals due to the rarity of wild populations and the high costs associated with translocation and captive breeding programs, leading to a founding population of small effective size that may be genetically bottlenecked (Fischer and Lindenmayer, 2000; Jamieson, 2011; Tracy et al., 2011). Further, it is becoming more commonplace to establish conservation sites that physically separate vulnerable species from their threatening processes, such as on predator- or disease-free islands or in fenced enclosures (Abbott, 2000; Hayward and Kerley, 2009), resulting in the isolation of these populations from extant ones. Small, isolated populations such as these are likely to be highly susceptible to the loss of genetic variation through random genetic drift and inbreeding, which can impact on long-term population adaptation and persistence (Brook et al., 2002; Frankham, 2005; Jamieson et al., 2006). In addition, in the shorter term, inbreeding depression resulting in lower survival or fitness of offspring may further reduce demographic population sizes contributing to population decline or failure (Gilpin and Soule, 1986). The rate of inbreeding is likely to be affected by the mating patterns and dispersal behavior of the species, which determines the within-population spatial genetic structure. Low density of founding populations may contribute to non-random mating if animals have low dispersal and mate more frequently with closely-located individuals. Thus, ideally, founding populations should be large and genetically diverse to overcome small population inbreeding effects and to retain longer-term adaptive capacity.

There have been extensive declines in the mammal fauna of Australia since European settlement began in 1788 (Burbidge et al., 2008a), with 94 species currently listed as critically endangered, endangered or vulnerable under Australian legislation (*Environment Protection and Biodiversity Conservation* (*EPBC*) *Act* 1999). Today, several of these species persist only on islands or in remote areas where exotic predators (foxes, cats, rats) or competitors (goats, rabbits) are absent. Barrow Island is a large island (23 400 ha) approximately 70 km off the north-western coast of Western Australia that is free of exotic predators. This important nature reserve supports 13 native mammal species that are extinct or rare on the mainland, including the golden bandicoot (*Isoodon auratus*) that is currently listed as Vulnerable under the *EPBC* Act. Golden bandicoots were once widespread across Australia's arid zones prior to European arrival but have suffered severe declines throughout much of their range as a result of predation by exotic predators and loss of habitat (Burbidge et al., 2008b). In Western Australia, golden bandicoots occur in large numbers (estimated population size of 20 000–50 000) on Barrow Island, with smaller and sparser populations on the mainland and coastal islands of the Kimberley region (McKenzie et al., 2008). The Barrow Island population was used as the source population for translocation of golden bandicoots to three conservation sites: to two nearby islands, Doole and Hermite Island, and to the Australian mainland within a fenced enclosure at Lorna Glen proposed conservation reserve (DEC, 2010, 2011). The translocations to Hermite Island and Lorna Glen are considered reintroductions as there is sub-fossil evidence of golden bandicoots at these locations in the recent past (Baynes, 2006; Montague, 1914); however, golden bandicoots are not known from Doole Island and this site is considered a conservation introduction. Due to the large size of the Barrow Island population, large numbers of animals (92–165 animals) were able to be sourced and released to each translocation site; greater numbers than are typically used in threatened species reintroduction programs (Fischer and Lindenmayer, 2000; Short, 2009).

This operational scale translocation of golden bandicoots from a large population to two smaller islands and a fenced reintroduction site provides an ideal opportunity to explore the interacting effects of founder population size on maintenance of genetic diversity and long-term persistence in effectively closed populations. We surveyed the genetic diversity of source and reintroduced populations of the golden bandicoot to determine whether a large founder size contributed to the initial success of reintroductions and to predict future patterns of genetic diversity. Specifically our aims were to: (1) compare the genetic diversity of founding and wild-born offspring at each translocation site to assess how diversity was conserved during the establishment phase; (2) determine whether there was any evidence for inbreeding in the established populations, which may lead to a reduction in fitness in the longer term; (3) assess effective population size of source and reintroduced populations and whether there is any evidence of genetic drift amongst populations; and (4) use modeling approaches to determine whether founding numbers were sufficient to maintain genetic diversity over time or whether further intervention (genetic augmentation) is required to maintain genetic diversity in these populations.

2. Material and methods

2.1. Study species and location

Two subspecies of the golden bandicoot *I. auratus* have previously been recognized (McKenzie et al., 2008). *Isoodon a. auratus* is currently restricted to four islands and several mainland sites along the north-west Kimberley coast of Western Australia, and Marchinbar Island in the Northern Territory. The Barrow Island subspecies, *I. a. barrowensis,* was until recently restricted to Barrow and Middle Islands off the Pilbara coast of Western Australia. The two sub-species are differentiated on morphological grounds though there appears to be little genetic support for the division (Westerman and Krajewski, 2000). *Isoodon a. barrowensis* is slightly smaller and has slightly darker fur than the mainland subspecies, and weighs between 250 and 600 g when mature (McKenzie et al., 2008). The species is mainly solitary, although home ranges overlap and may alter by seasons, usually increasing in size in drier seasons (McKenzie et al., 2008). Females give birth throughout the year, with up to five pouch young possible, though typically only one to two young may survive to weaning (J. Dunlop, pers. comm.).

2.2. Translocation history

The Western Australian Department of Parks and Wildlife (DPaW) have successfully established populations of *I. a. barrowensis* at three locations within their former range that are free of exotic predators: Lorna Glen proposed conservation reserve, Hermite Island and Doole Island (Fig. 1). Animals were sourced from the large Barrow Island population (BI, 20°51′ S, 115°24′ E) by trapping several areas on the island over a four week period. Animals were sexed, weighed and measured before being transported to each

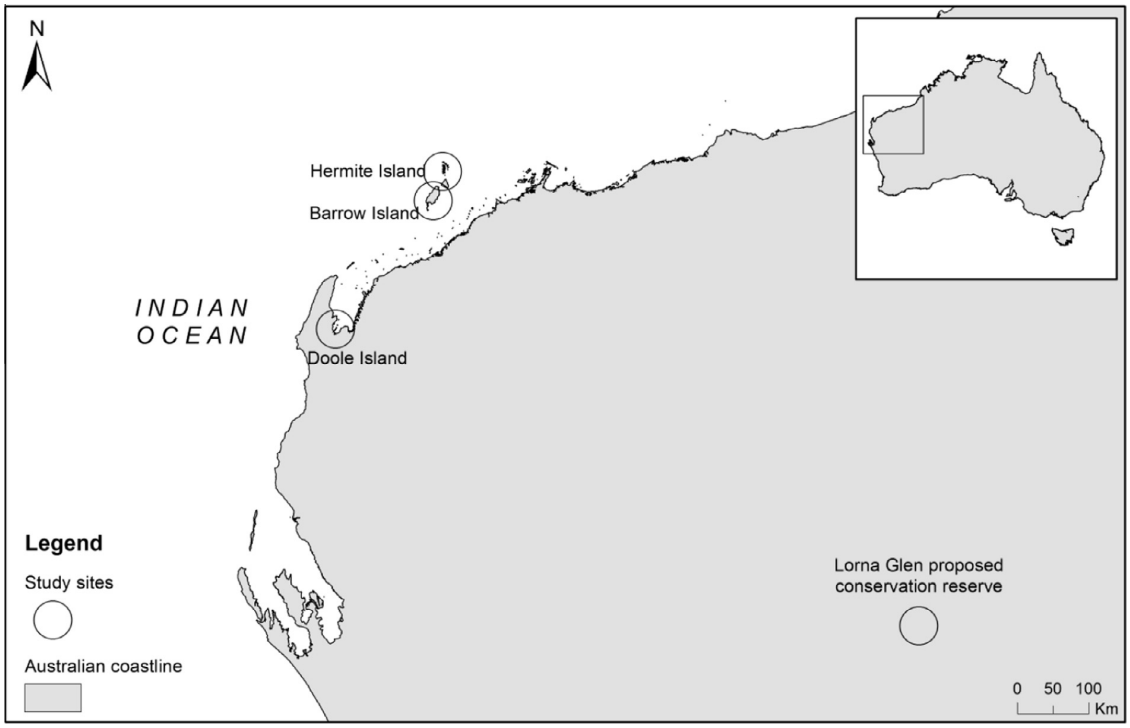

Fig. 1. Map showing location of *Isoodon auratus barrowensis* source population (Barrow Island) and translocation sites (Hermite Island, Doole Island and Lorna Glen proposed conservation reserve).

translocation site via helicopter and fixed wing aircraft, and released within 24 h of capture. All individuals were tagged with a unique identifier PIT (passive implant transponder) tag. Bandicoots were released at Lorna Glen proposed conservation reserve in central Western Australia (LG, 26°13′ S, 121°33′ E), a 244 000 ha ex-pastoral lease now managed by DPaW in partnership with the Martu people from the Wiluna Aboriginal community, and the site of the Rangelands Restoration project. In this translocation, 160 animals (78 males/82 females) were released into an 1100 ha predator proof enclosure in February 2010. The enclosure consists of sandy spinifex grassland (*Triodia basedowii*) and open mulga (*Acacia aneura*) woodlands with a rocky clay substrate. Hermite Island (HI, 20°29 S, 115°31 E) is the largest island in the Montebello group located approximately100 km off the Pilbara coast and 25 km north of BI. The island is 1020 ha in size, from which cats and black rats have been eradicated (Burbidge, 2004) and consists of dense spinifex (*T. wiseana* and *T. augusta*) on a rocky limestone substrate. At HI, 165 *I. a. barrowensis* were released in February 2010 (82 males/83 females). The third translocation site was Doole Island (DI, 22°27′ S, 114°09′ E), a 261 ha island in Exmouth Gulf, approximately 214 km southwest of BI. Ninety-two *I. a. barrowensis* (49 males/43 females) were released onto DI in July 2011. No exotic predators are known on DI. Using mark-recapture analysis, the current population size is estimated at 249 animals within the fenced enclosure at LG (J. Dunlop, unpubl. data) but total population sizes are not known for HI or DI. At HI, mark-recapture analysis of data from two trap lines close to the release site (estimated to represent ~20% of the island's area) estimate population size to be 280 animals (N. Thomas, unpubl. data).

2.3. DNA sampling and microsatellite genotyping

Ear punch biopsies were taken from animals caught in standard cage (Sheffield Wire Products, Welshpool, WA) or Elliott (Elliott Scientific, Upwey, Victoria) traps using a sterilized commercial 1–2 mm ear punch tool during regular monitoring surveys at each location. Samples were obtained from 57 founders (released February 2010) and 67 progeny (trapped 2010–2012) at LG; 38 founders (released February 2010) and 44 progeny (2010–2012) at HI and 49 founders (released July 2011) and 39 progeny (2012) at DI. Ear biopsies were stored in 80–100% ethanol until DNA extraction. We extracted genomic DNA from biopsy samples using a standard 'salting out' extraction procedure. Polymerase Chain Reaction (PCR) amplification was conducted for 12 microsatellite loci sourced from previously published studies (Li et al., 2013; Zenger and Johnston, 2001) (details in Appendix A) using the Qiagen Multiplex Kit, following reaction conditions specified by the manufacturers with an annealing temperature of 58 °C. Amplification products were separated on an ABI PRISM 3100 capillary sequencer using a commercial service (WA State Agricultural Biotechnology Centre) and fragment sizes determined using an internal size standard (LIZ500) in the program Genemapper (Applied Biosystems). We genotyped approximately 10% of samples twice to calculate genotyping error rates.

2.4. Genetic data analysis

Genotyping data quality was assessed by calculating the allele-specific and locus-specific genotyping error rates (Pompanon et al., 2005) and conducting null allele analysis in MICROCHECKER (Van Oosterhout et al., 2004). We tested for Hardy–Weinberg equilibrium (HWE) in each population/locus combination using GENALEX v6.5 (Peakall and Smouse, 2012) and used corrected alpha values determined by False Discovery Rate (FDR) analysis using the online calculator available at http://users.ox.ac.uk/~npike/fdr/ (Pike, 2011). Since we found significant disequilibrium at locus Ioo2 (see Results) we removed this locus from genetic diversity and differentiation analyses. Using the remaining 11 loci, we calculated

standard population genetic parameters using GENALEX (N_a, number of alleles per locus; A_e, effective number of alleles per locus; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient) for both the original source population (BI, by pooling data for each of the founding translocated populations, $n = 144$) and the founding and wild-born populations (separately) at each translocation site. We calculated allelic richness (A_R) in HP-RARE (Kalinowski, 2005) standardized to the smallest population size ($n = 38$). We tested for significant differences in these descriptive population statistics between the source population and all wild-born populations, and the founding and wild-born populations at each translocation site, using a randomized block design ANOVA (with locus as the blocking factor) with acrsine transformed data where appropriate (H_O, H_E). Mean population pairwise relatedness was calculated in GENALEX using the Lynch and Ritland (1999) estimator of r. We assessed the homogeneity of allele frequencies between founding and wild-born populations within and between translocation sites using exact G tests for genic differentiation in GENEPOP (Raymond and Rousset, 1995).

We tested for genetic differentiation between the source population (BI) and wild-born populations, and between the founding and wild-born populations at each translocation site using two metrics (F_{ST} and D_{est}) calculated within GENALEX. The significance of observed values was tested using permutation testing ($n = 999$). We used the single-sample estimator implemented in the software package LDNE (Waples and Do, 2008) to estimate effective population size (N_e) for the source population and each of the translocated populations with founding and wild-born populations combined. All of our population samples consist of overlapping generations. We used a random mating model and estimated linkage disequilibrium amongst alleles using only alleles with frequencies >2%, as this is expected to give the best balance between precision and bias in the N_e estimator (Waples and Do, 2010).

We calculated allele rarefaction curves for the BI source population using the package POPGENKIT v1.0 (Paquette, 2012) implemented in R v2.15.1 (R Development Core Team, 2011). Allele rarefaction curves were estimated for each of the 11 individual microsatellite loci using (1) all rare and common alleles and (2) only common alleles with >5% frequency. From each of these curves we determined, per locus, the number of individuals that would need to be translocated to capture 95% of allelic diversity present in the BI source population and present the mean and range of these estimates.

2.5. Population viability analysis

Demographic and genetic models of population viability for the LG and HI translocated populations were constructed using the software VORTEX v9.99c (Lacy and Pollak, 2012). We ran the models with 1000 iterations and for 100 years using the default model of population growth and our site-specific genetic and demographic parameters as input. Since locus Ioo2 was in HWE in the LG and HI founder populations from which genetic input parameters were taken, this locus was retained for analysis. Demographic parameters on reproductive rates were derived from unpublished field studies conducted at LG (three years data, J. Dunlop), HI (three years data, N. Thomas) and BI (10 years data, K. Morris) (Table 1, Appendix B). Data is not currently available for the DI population and we did not develop a model for this site. We modeled genetic diversity change firstly using the initial number of founders at each site (LG = 160, HI = 165) and using the mean number of founders required to capture 95% of allelic diversity of the source population estimated from the allele rarefaction analysis ($N = 75$). We modeled several management scenarios to maintain heterozygosity and allelic richness in the LG and HI translocated populations including, (a) increasing fence size at LG to increase carrying capacity ($K = 600–1000$); (b) supplementation at periodic intervals to maintain genetic diversity in LG and HI populations (augment with 20/30/50 animals every 5/10 years); and (c) follow-up supplementations at HI to boost initial population growth (augment with 80–160 animals at 5 and 10 years). Full details of the demographic parameters, management scenarios and assumptions made in the model are provided in Appendix B.

3. Results

3.1. Microsatellite genotyping quality

Across the *I. a. barrowensis* genetic dataset the allele-specific and locus-specific genotyping error rates were 0.001 and 0.017, respectively. Overall, the 12 microsatellite loci were variable across the study population with the number of alleles ranging from 3 to 14 alleles per locus and mean observed heterozygosity from 0.11 to 0.85 (Appendix A). All loci were in HWE when analysed across the entire data set, with the exception of Ioo2. Tests of HWE per population/locus combination resulted in significant deviations from HWE in 11 out of 78 tests. Once FDR analysis was applied to correct for multiple tests only five population/locus combinations remained significant, three of these involving locus Ioo2. Indeed, null allele analysis in Microchecker suggested null alleles were present (significant excess of homozygotes) at locus Ioo2 in two of the wild-born populations (Hermite Island, Lorna Glen) and one of the founder populations (Doole Island). For this reason Ioo2 was excluded from the following genetic analyses.

3.2. Genetic diversity in founder and wild-born populations

Overall, genetic diversity was moderately high and similar in all founder and wild-born populations of *I. a. barrowensis* at each of the translocation sites (Table 2). When animals translocated to the three different locations (founder populations) were pooled into a single 'population' representative of the source, BI ($n = 144$), a significantly lower number of alleles were detected in individuals sampled from each of the wild-born translocated populations than the original source population (Table 2, ANOVA $F = 8.11$, $df = 3$, $p < 0.001$). However, when allelic richness was standardized for differences in sample size (A_R), there were no significant differences between source and wild-born populations for rarefied allelic richness or any other measure of genetic diversity (A_e, H_O, H_E).

When analysed per translocation site, expected heterozygosity of animals ranged from 0.64 to 0.67 and allelic richness from 7.13 to 7.99 (Table 2). Slightly higher, but not significantly different (ANOVA, $p > 0.05$), levels of genetic diversity (N_a, A_r, A_e) were detected in founder compared to wild-born populations of *I. a. barrowensis* at all locations (Table 2). Expected and observed heterozygosity were not significantly different in all founder and wild-born populations also. The inbreeding coefficient (F_{IS}) was near zero in all populations, with the exception that inbreeding was significantly higher in wild-born HI animals than wild-born LG animals (ANOVA, $F = 3.46$, $df = 3$, $p = 0.028$). Estimates of pairwise relatedness amongst individuals in each population showed individuals on average were unrelated in each of the founder populations, but were more related than random in each of the wild-born populations (DI $p = 0.041$; HI $p = 0.016$; LG $p = 0.028$, Table 3). There was a non-significant trend for higher relatedness amongst wild-born animals compared to founders at each of these sites (standard errors overlap).

3.3. How representative are wild-born populations of their source?

Allele frequencies were mostly consistent between the sample of animals from the initial source population (BI) and each of the

Table 1
Demographic and life history parameters used in population viability models of *Isoodon auratus barrowensis* translocated populations at Lorna Glen and Hermite Island, and the sources of data used. Full details and justification of the parameters used is provided in Appendix B.

Parameter	Lorna Glen		Hermite Is
Breeding system		Polygynous[a]	
Inbreeding depression		Recessive Lethals (8 Lethal equivalents)[b]	
Adult males in breeding pool		97.8%[c]	
% Males successful		50%[c]	
Mean no. mates per male		1.4[c]	
Age of first reproduction (Females)		4 Months[d]	
Age of first reproduction (Males)		4 Months[e]	
Max. age of reproduction		5 Years[d]	
No. litters/yr		3[e]	
Sex ratio at birth (in% males)		50%[e]	
Max. no. progeny/litter	5[d]		5[d]
% Adult females producing:			
0 Young	31%[d]		48%[d]
1 Young	11%		19%
2 Young	35%		31%
3 Young	20%		1%
4 Young	3%		0.5%
5 Young	1%		0.5%
Mortality of females and males			
0–1 Years of age		50%[e]	
>1 Years of age		10% p.a.[e]	
Population carrying capacity (K)	300[e]		1500[e]
Dispersal between pops	30% juveniles disperse outside fence p.a.		None, closed pop
Initial population size	160		165
Years modeled		100	
No. iterations		1000	

[a] McKenzie et al. (2008).
[b] O'Grady et al. (2006).
[c] Data from congener, *I. obesulus*.
[d] J. Dunlop, N. Thomas, K. Morris, unpublished data.
[e] Assumption made – see Appendix B for rationale.

Table 2
Mean and standard error of genetic diversity measures from the source population at Barrow Island, and founder and wild-born populations of *Isoodon auratus barrowensis* at each of three translocation sites at Doole Island, Hermite Island and Lorna Glen proposed conservation reserve. N = Number of individuals genotyped; N_a = Number of alleles per locus; A_R = Allelic richness (rarefied to $n = 38$); A_e = Number of effective alleles per locus; P_A = Number of private alleles; H_o = Observed heterozygosity; H_e = Expected heterozygosity; F = Wright's inbreeding coefficient. Superscripts (a,b) denote significantly different ($p < 0.05$) groups determined by Tukey's post hoc multiple comparisons test following ANOVA testing for differences amongst (1) the Barrow Island source population and wild-born animals and (2) all founding and wild-born populations.

Population	N	N_a	A_R	A_e	P_A	H_o	H_e	F
Source population	142.5 ± 0.76	9.09 ± 1.04[a]	7.76 ± 0.91	4.40 ± 0.27	0.818	0.655 ± 0.080	0.663 ± 0.083	0.010 ± 0.012[ab]
Doole Island								
Founder	47.5 ± 0.76	8.18 ± 0.99	7.99 ± 0.97	4.32 ± 0.59	0.273	0.638 ± 0.078	0.662 ± 0.081	0.028 ± 0.018
Wild-born	38.8 ± 0.12	7.27 ± 0.92[b]	7.25 ± 0.91	4.14 ± 0.59	0.000	0.657 ± 0.080	0.654 ± 0.080	−0.016 ± 0.022[ab]
Hermite Island								
Founder	38.0 ± 0.00	7.82 ± 1.09	7.82 ± 1.09	4.27 ± 0.57	0.091	0.672 ± 0.078	0.667 ± 0.077	−0.005 ± 0.031
Wild-born	42.6 ± 0.43	7.36 ± 0.80[b]	7.27 ± 0.79	4.09 ± 0.58	0.182	0.631 ± 0.079	0.651 ± 0.079	0.045 ± 0.031[a]
Lorna Glen								
Founder	57.0 ± 0.00	7.91 ± 0.86	7.42 ± 0.81	4.22 ± 0.60	0.273	0.659 ± 0.087	0.648 ± 0.086	−0.024 ± 0.023
Wild-born	66.6 ± 0.20	7.55 ± 1.00[b]	7.13 ± 0.94	4.15 ± 0.61	0.000	0.665 ± 0.088	0.643 ± 0.086	−0.040 ± 0.018[b]

wild-born populations at the different translocation sites. Exact tests for differentiation of allele frequencies between populations detected significant variation in allele frequencies at only one locus, Ioo10, whilst allele frequencies were homogeneous across the remaining loci (Appendix C). Similarly, genetic differentiation between the source population, BI, and wild-born populations of *I. a. barrowensis* was low ($F_{ST} = 0.003–0.008$) (Table 4A), although wild-born animals from HI showed significant differentiation from wild-born LG animals ($F_{ST} = 0.007$, $p = 0.043$). When populations were analysed as founder and wild-born populations at each location, we also found that DI founders were significantly differentiated from the LG founder population ($F_{ST} = 0.007$, $p = 0.044$), though wild born animals on DI were not differentiated from LG founder or wild-born animals (Table 4B).

3.4. Conserving genetic diversity

A total of 100 alleles were observed in the 144 adult animals translocated from BI; of these, 58 alleles were present in frequencies above 5% and considered "common" alleles (Table 5; Appendix C). Using rarefaction analysis of allelic diversity including all rare and common alleles, we found it would be necessary to source between 39 and 121 animals to capture 95% of the allelic diversity present in our sample of the source population, BI (Mean ± SE = 74.9 ± 8.8 individuals; Table 5). When rare alleles (frequency <5%) were removed from the dataset, significantly fewer animals would be required to conserve 95% of allelic diversity (Mean ± SE = 14.3 ± 1.4 individuals; Range 5–21).

Table 3
Mean and standard error of estimates of pairwise relatedness (r) amongst founder and wild-born individuals of *Isoodon auratus barrowensis* at three translocation sites at Doole Island, Hermite Island and Lorna Glen proposed conservation reserve.

Population	Founder	Wild-born
Doole Island	−0.002 ± 0.002	0.002 ± 0.003
Hermite Island	−0.004 ± 0.003	0.002 ± 0.002
Lorna Glen	0.000 ± 0.001	0.001 ± 0.001

3.5. Effective population size (N_e)

Based on the single sample estimator of Waples and Do (2008), we found that N_e was greatest in the source population, BI (N_e = 1124) (Table 6). The program LDNe was unable to resolve the upper confidence limit on this estimate, which suggests N_e may be even larger. In contrast, each of the translocated populations experienced approximately 5–10-fold reduction in N_e, with estimates ranging from approximately 100 individuals on DI (the smallest translocation site) and LG, to 212 individuals on HI.

3.6. Population modeling

Demographic modeling suggests that the population of golden bandicoots at LG reached carrying capacity very quickly (r = 0.213, λ = 1.237), within two years. Despite rapid population growth, both expected heterozygosity and the mean number of alleles declined at a linear rate when the population had reached and was maintained at carrying capacity (Fig. 2a and b). After running the model for 100 years, mean H_e declined from 0.65 to 0.54 (27% decline) and the mean number of alleles declined from 7.6 to 4.3 (43% decline). Rare alleles were lost at a higher rate than common alleles (mean probability of retention for alleles <1% frequency = 0.08; 1–5% frequency = 0.28; 6–10% frequency = 0.58). We found that if we had used an initial founding population of 75 individuals as predicted from the allelic richness analysis above (i.e. the number of individuals required to capture 95% of allelic diversity), the rate of decline of genetic diversity would have been similar (Fig. 2a and b). A proposal to double the size of the enclosure at LG to increase carrying capacity (K = 600–1000) leads to a much slower decline in genetic diversity with time; when K = 1000, H_e is maintained at >95% without supplementation, although the number of alleles still declines to approximately 80% of the number in the founding population (Fig. 2a and b). Loss of genetic diversity can also be ameliorated by supplementing the population with more animals. Through modeling we found that supplementing the population with larger numbers of individuals (30–50 animals) at greater intervals (10 years) was more effective than smaller numbers of individuals (20 animals) at more frequent intervals (five years). We also found that the number of alleles declined at a greater rate than heterozygosity and that the addition of even larger numbers of animals (50 animals/10 years) would be required to maintain 95% of the allelic diversity present in the founding population, compared to 20 animals/10 years for expected heterozygosity (Fig. 2c and d).

The translocated population on HI had lower reproductive rates than LG and a slower rate of intrinsic population growth (r = 0.024, λ = 1.024). The HI population did not reach carrying capacity within the 100 years modeled without supplementation (Fig. 2e). In this scenario, the population grew to approximately 900 individuals but genetic diversity declined from H_e = 0.66 to 0.60 (10% decline) over the 100 year period and N_a declined from 7.7 to 5.61 (27% decline). Over this time there was also a 16% probability of extinction. Though not shown, we modeled a scenario of using an initial population size of 75 individuals to capture 95% of BI allelic diversity, which resulted in a high probability of extinction (82%). We found that supplementing the populations early (at 5 and 10 years) was sufficient to increase population sizes and to maintain genetic diversity above 95% (Fig. 2e and f). Alternatively, supplementing small numbers of animals frequently (20 animals/5 years) or a slightly larger number of animals less frequently (30 animals/10 years) was sufficient to maintain genetic diversity (Fig. 2g and h).

4. Discussion

The assessment of both demographic and genetic data has demonstrated that the translocation of golden bandicoots to three secure conservation sites in Western Australia has resulted in the successful establishment of populations with positive trends in population recruitment and persistence. Use of large founder sizes has led to maintenance of genetic diversity in wild-born populations of *I. a. barrowensis* over six generations. However, modeling shows management intervention through periodical supplementation of animals is likely to be required to maintain genetic diversity over longer time-frames. Importantly, our study highlights the benefit of evaluating long term success of translocated populations through monitoring genetic diversity change in source and reintroduced populations. It also makes the case for incorporating genetics in population viability analysis to explore factors that affect success of translocation strategies and management interventions on both short and long term extinction risk for threatened species.

Table 4
Pairwise genetic differentiation between (a) source and wild-born populations of *Isoodon auratus barrowensis*, and (b) founder and wild-born populations at each location. F_{ST} values below the diagonal and D_{est} values above; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

	Barrow Is. (source)	Doole Is. wild-born	Hermite Is. wild-born	Lorna Glen wild-born
Panel A				
Barrow Is. (source)	–	0.000	0.000	0.000
Doole Is. wild-born	0.004	–	0.008	0.007
Hermite Is. wild-born	0.004	0.008	–	0.008*
Lorna Glen wild-born	0.003	0.007	0.007*	–

		Doole Is.		Hermite Is.		Lorna Glen	
		Founder	Wild-born	Founder	Wild-born	Founder	Wild-born
Panel B							
Doole Is.	Founder	–	−0.007	0.001	0.002	0.007*	0.006
	Wild-born	0.004	–	0.004	0.008	0.007	0.007
Hermite Is.	Founder	0.008	0.006	–	−0.011	0.002	0.001
	Wild-born	0.007	0.006	0.004	–	0.010*	0.008*
Lorna Glen	Founder	0.007*	0.006	0.007	0.005*	–	−0.002
	Wild-born	0.007	0.007	0.008	0.006*	0.003	–

Table 5
The number of individuals required to capture 95% of allelic diversity at each locus when (a) all rare and common alleles are included and (b) only common alleles occurring at greater than 5% frequency are included.

Locus	No. rare and common alleles	No. Individuals	Common alleles > 5% frequency	No. Individuals
B7-2	9	62	6	15
Ioo8	9	44	5	11
B34-2	11	101	7	21
Ioo6	7	121	4	15
B20-5	13	52	7	20
B3-2	4	116	2	10
Ioo10	14	95	5	16
Ioo4	11	70	7	18
B34-1	11	74	7	14
Ioo16	3	39	1	5
Ioo7	8	50	7	12
Mean ± SE	9.1 ± 1.0	74.9 ± 8.8	5.3 ± 0.6	14.3 ± 1.4

Table 6
Effective population size (N_e) estimated from genetic data from the source population of *Isoodon auratus barrowensis* on Barrow Island and at each of three translocation sites, Doole Island, Hermite Island and Lorna Glen proposed conservation reserve.

	Harmonic mean No. individuals	No. independent comparisons	Estimated N_e	95% CI (jackknifing loci)
Barrow Is. (source)	141.0	2381	1124	322-Infinite
Doole Is.	84.9	2242	91	67–134
Hermite Is.	79.6	2110	212	119–659
Lorna Glen	123.3	2257	108	73–179

To date, relatively few studies have incorporated genetic diversity assessment in PVAs (Allendorf and Ryman, 2002; but see Haig et al., 1993; Jamieson, 2011; Weiser et al., 2013).

4.1. Genetic diversity in translocated populations

Interestingly, the source population of golden bandicoots on Barrow Island retains moderately high levels of genetic diversity, in contrast to what has been noted for numerous other island populations of animals (Boessenkool et al., 2006; Eldridge et al., 1999, 2004). It is likely that the large population size of golden bandicoots on Barrow Island has buffered this population from genetic diversity loss following isolation from mainland populations. We found that there was no significant loss of genetic diversity between wild-born animals and their founding populations at each of the translocation sites up to two years post-translocation. Indeed, each of the wild-born populations remain genetically representative of the initial source population on Barrow Island, in terms of both allelic diversity and allele frequencies, with the exception of the Hermite Island wild-born population which showed low but significant genetic differentiation from the Lorna Glen population. The wild-born populations appeared to effectively result from random mating, with little evidence for inbreeding in the newly-established populations. Despite maintaining high levels of genetic diversity in the wild-born golden bandicoot populations, there was a five- to 10-fold reduction in effective population size within each translocated population compared to the larger source population on Barrow Island reflecting the reduction in the number of animals contributing to breeding at the newly established sites. At both the smaller translocation sites, Lorna Glen and Doole Island, effective population size was estimated at ~100 individuals, compared to 212 individuals on Hermite Island and >1000 individuals on Barrow Island. These results indicate that the reintroduction process has left a genetic signature of a population bottleneck and that these populations will be susceptible to loss of genetic diversity over time if population sizes remain small and the populations remain effectively isolated.

Over the longer term, population persistence is obviously one measure of translocation success but several authors have suggested genetic criteria also be used in evaluating the viability of populations, most commonly with the goal of retaining at least 90–95% of heterozygosity over 100–200 years (Allendorf and Ryman, 2002; Soule et al., 1986). Since each of the golden bandicoot translocation sites are effectively closed populations, we predicted they would be susceptible to genetic erosion with time as a result of genetic drift. Indeed, using the demographic parameters gained from field monitoring at two of the translocation sites, Hermite Island and Lorna Glen, the population viability models showed that despite maintenance of genetic diversity in the initial reintroduction stages, both populations lost genetic diversity over time. The modeled rate of loss was highly dependent on population size, with Lorna Glen suffering greater declines in expected heterozygosity and number of alleles over 100 years than the larger Hermite Island population. Increasing demographic population size through expansion of the enclosure (Lorna Glen) or through low levels of population supplementation (Hermite Island), led to minimal rates of genetic diversity loss over the time frame modeled. Modeling predicted that if the current small size of the enclosure at Lorna Glen is maintained, frequent periodic supplementation of animals will be required to avoid the sustained erosion of genetic diversity. Our findings have implications not only for golden bandicoot translocations, but for other translocations that involve effectively closed populations that might be susceptible to genetic erosion and indicate that periodic supplementation of animals may be an ongoing action that is a necessary part of those translocation programs (Jamieson, 2011; Jamieson and Lacy, 2012; Weiser et al., 2013).

Consistent with population genetic theory, we found that allelic richness declined in our translocated populations at a more rapid rate than heterozygosity and that this was primarily due to the loss of rare alleles (alleles <5% frequency had a much lower rate of retention than common alleles). Arguably it is allelic richness rather than heterozygosity that reflects the long-term evolutionary potential of a population, since it is the number of genetic variants in a population that determines the material available for selection to act upon (Allendorf, 1986; Tracy et al., 2011; Weiser et al., 2013). In addition, whereas heterozygosity can be recovered following a population bottleneck, novel alleles can only arise through

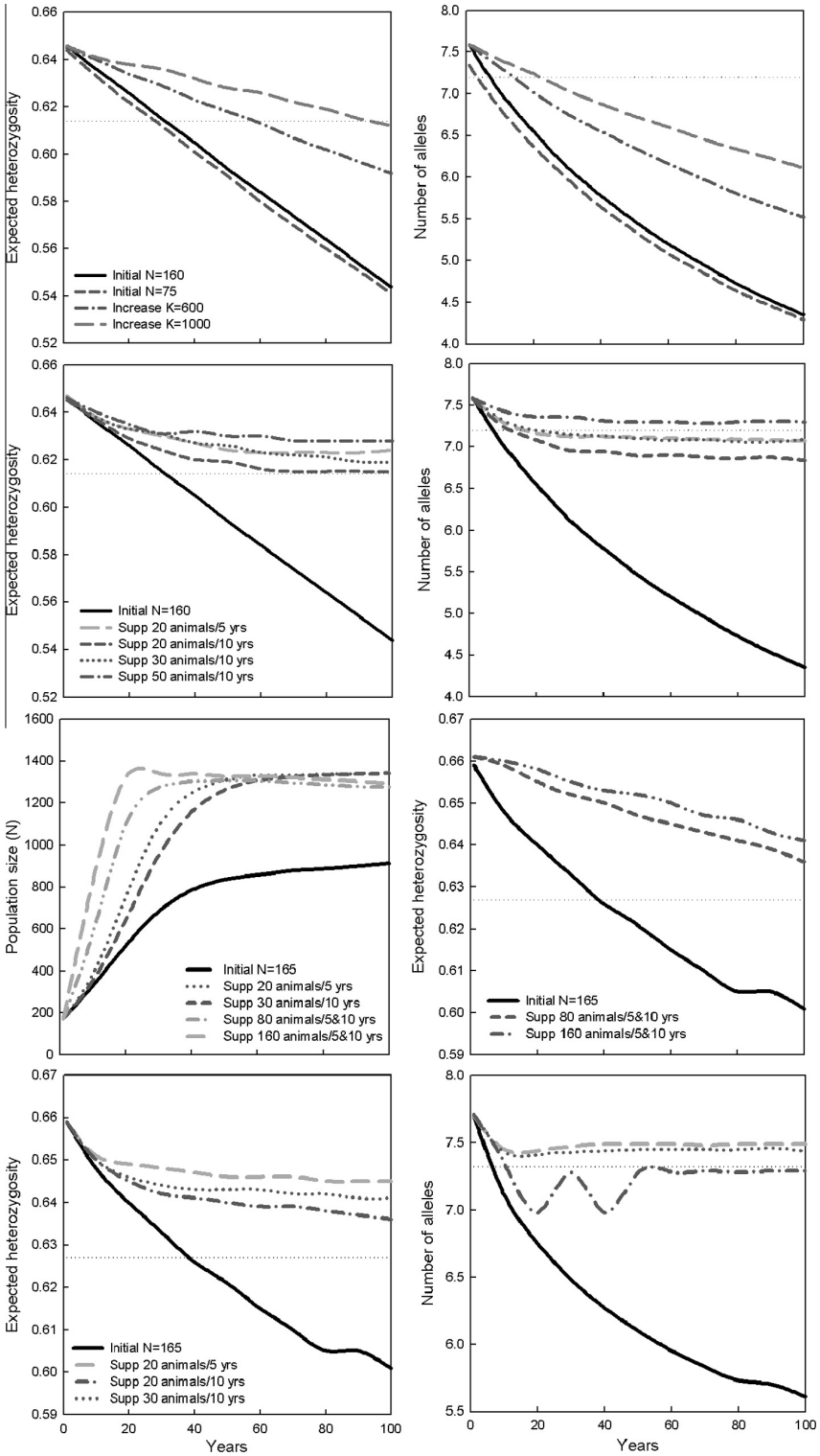

Fig. 2. Change in expected heterozygosity and number of alleles of *Isoodon auratus barrowensis* translocated populations modeled over 100 years using population viability analysis with input parameters as described in Appendix B. (a–b) Lorna Glen, change in H_e and N_a with initial founding population $N = 160$; with founding population $N = 75$ to capture 95% allelic diversity (from rarefaction analysis); with increased carrying capacity $K = 600–1000$ as a result of expansion of enclosure. (c–d) Lorna Glen, change in H_e and N_a with different supplementation strategies (20, 30, 50 animals at continuous 5 or 10 year intervals) to prevent genetic diversity loss. (e) Hermite Island, change in population size with initial population size $N = 165$ and different supplementation strategies (small numbers of animals at continuous intervals or larger numbers of animals as early supplementations). (f) Hermite Island, change in H_e with early supplementation strategies. (g–h) Hermite Island, change in H_e and N_a with initial founding population $N = 165$ and with different continuous periodic supplementation strategies. Horizontal dotted lines represent 95% H_e or N_a.

mutation or migration. Recent meta-analyses have shown that the loss of diversity at neutral loci following a population bottleneck is often accompanied by an equivalent or greater loss of diversity at other highly variable adaptive genetic loci, such as major histocompatibility complex (MHC) genes that are important in disease resistance (Radwan et al., 2010; Sutton et al., 2011). If this is the case, our results suggest managers will have to make concerted efforts to maintain allelic richness in translocated populations to promote their resilience to future environmental change or novel disease risk and ensure their long-term viability.

4.2. Retrospective analysis on the number of animals for translocation

Analytical reviews of reintroduction success rates have suggested that translocations employing release group sizes of $n > 100$ leads to greater establishment success (Fischer and Lindenmayer, 2000; Short, 2009). Large numbers of golden bandicoots were released in the translocation investigated in this study (DI $n = 92$; HI $n = 165$, LG $n = 160$) and a mean sample size of 75 animals was sufficient to capture 95% of total allelic diversity (at the 11 microsatellite loci used in this study) of the Barrow Island population. At Lorna Glen there was no difference in the rate of genetic diversity decline between a founder group of 75 compared to 160 animals, potentially a result of buffering due to random mating and the high reproductive rate at this site. This implies that a cost-saving could potentially be made in future reintroductions by reducing the number of founders, as long as it was to be reasonably expected that mortality rates of the released animals would not be excessive and that the population size would increase quickly so that a large population size can be established and maintained. The effect of inadequate founder size was demonstrated in our Hermite Island population model where a lower reproductive rate led to a higher probability of population extinction when the smaller founder size was used. In this situation, larger founder sizes were required to ensure population persistence.

4.3. Genetic viability and management of translocated populations

Each of the translocated populations in this study is considered a closed population as they are either on islands or in a fenced enclosure. With no migration, our population viability modeling showed a signature of genetic diversity decline over time unless population numbers were high. Loss of genetic diversity can be mitigated through population supplementation at regular intervals. Modeling found that the small population of golden bandicoots in the fenced enclosure at Lorna Glen required the most intensive management but that genetic diversity loss could be ameliorated through regular translocations of ~50 animals every 10 years. It is expected that the enclosure size at Lorna Glen will be expanded to approximately 5000 ha within the next decade, which clearly will benefit the long-term management of golden bandicoots if population sizes expand accordingly to >1000 animals. Genetic augmentation is not likely to be required if population size is increased and sustained at >1000 animals.

Reproductive rates of golden bandicoots were lower on Hermite Island than on the mainland and consequently population size was slow to increase. In this case, either early supplementation of a large number of individuals (>100) or supplementation of only a small number of individuals at regular intervals (30 animals/10 years) was required to increase population sizes and maintain genetic diversity at 95% of the founders. We could expect that once Hermite Island reaches carrying capacity ($K = \sim 1500$) the population should be self-sustaining in the longer-term. While we did not model changes in genetic diversity at the second island translocation site, Doole Island, we would expect that if the population was sustained at less than ~1000 animals, as is likely due to the small size of the island, ongoing genetic augmentation will be required.

The population viability models as we have presented here provide insights into the management of translocated populations to maintain genetic diversity. However, the models were paramaterised with limited information on survival rates and with little knowledge of the true rate of inbreeding depression (though we have used a higher rate than commonly applied; O'Grady et al., 2006) or environmental variation in carrying capacity and how this changes with time. Recent experience has shown that translocated populations may achieve high population growth in initial stages but decline sharply some years after (e.g. Pearson, 2012; Smith et al., 2008). Additionally, we have not modeled for demographic changes that may result from catastrophes, such as fire, disease outbreak or predator invasion. Incorporating this type of information could provide a more realistic model of the long-term demographic and genetic viability of these populations, particularly as closed or island populations may be especially vulnerable to deterministic and stochastic phenomena.

4.4. Effective population size

Populations with a large effective population size are resistant to loss of genetic diversity through genetic drift and inbreeding, thus it is important in conservation to establish populations that are capable of growing and maintaining a large population size. In addition, inbreeding depression may occur in small newly established populations in the short term, so that initial population size may be important even if the population increases rapidly.

It is estimated that minimum viable population sizes should be ~5000 to prevent species extinction in the long-term (i.e. over a time-frame of several hundred to 1000 years) (Clements et al., 2011; Traill et al., 2010). In the immediate term, for management of recovering populations it may be appropriate to follow the 50/500 rule instead, where effective population size should not be less than 50 in the short term and should be greater than 500 in the long term to maintain genetic diversity (Franklin, 1980; Jamieson and Allendorf, 2012). If we use these numbers as a guide, the initial reintroduction of golden bandicoots to mainland and island sites has been successful in maintaining an effective population size greater than 50 at all sites. With the short generation time and high reproductive rate of golden bandicoots, it is likely that population expansion will occur rapidly (in the absence of catastrophic events or artificial constraints to population growth) to maintain an effective population size >500 at Lorna Glen and Hermite Island, at least.

4.5. Conclusions

Studies have shown that many reintroduced populations show reduced genetic diversity compared to source populations (Maudet et al., 2002; Mock et al., 2004; Sigg, 2006; Vernesi et al., 2003), leading to problems with genetic drift and increased inbreeding in the establishing populations, even when large numbers of founders are used (e.g. Stockwell et al., 1996). However, we found here that using a large founder size of 92–165 animals contributed to successful conservation of genetic diversity between the source population of golden bandicoots and wild-born progeny approximately six generations post-translocation at each of the conservation sites. Nonetheless, population models predicted that these populations would be susceptible to erosion of genetic diversity over time with no immigration, particularly the smaller populations in the Lorna Glen fenced enclosure and presumably the small Doole Island site, though this was not modeled. A program of periodic genetic augmentation is required to prevent the loss of genetic diversity over time if translocated population sizes remain at less

than ~1000 animals. Supplementation of animals to maintain evolutionary potential is typically not explicitly included, or budgeted for, in reintroduction plans (Jamieson and Lacy, 2012). Thus, we highlight the use of genetic diversity assessment and incorporation into PVA to determine the interacting factors contributing to population persistence, and the evaluation of potential actions required to ensure viable populations to greater assist in conservation planning. The incorporation of genetic information into the reintroduction process at an early stage is a critical aspect in evaluation of translocation success beyond monitoring of demographic parameters, especially since early intervention to address problems is more likely to contribute to long-term success.

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Chevron Australia provided financial and logistical support for translocation of animals from Barrow Island to reintroduction sites and for follow-up monitoring through the Gorgon Gas Development environmental offset program. Employees of DPaW conducted all aspects of the study. Chevron Australia was not involved in the study design, collection, analysis or interpretation of data, or in the writing of this report for publication.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biocon.2014.01.012.

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