Reintroduction as a tool for the recovery of warru (*Petrogale lateralis* MacDonnell Ranges race) on the Anangu Pitjantjatjara Yankunytjatjara Lands of South Australia.

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Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution. To the best of my knowledge and belief, it also contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Cover image: Warru Recovery Project logo © APY Land Management

Warru Recovery Team Logo by Dora Haggie, Traditional Owner, Pukatja.

This dissertation is dedicated to the Warru Recovery Team.

The Warru Recovery Team (WRT) formed in 2007 with the overarching vision to recover populations of warru (black-footed rock-wallaby, *Petrogale lateralis* MacDonnell Ranges race) on the A<u>n</u>angu Pitjantjatjara Yankunytjatjara (APY) Lands in South Australia and, in doing so, provide training and employment opportunities for A<u>n</u>angu, the traditional owners of the APY Lands. Over the last six years this multi-stakeholder team has made significant progress towards meeting these objectives. The research contained within this dissertation sits within the broader context of the warru recovery project and would not have been possible without the extensive work conducted by the WRT both prior to, and during, my involvement. Furthermore, the team's members have provided extraordinary amounts of support since I joined the project in 2010.

I hope that the work contained within this thesis will assist your continuing commitment to ensure that warru remain on the APY Lands.

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GLOSSARY

Pitjantjatjara/Yankunytjatjara language used in this thesis:

An̲angu	people of the An̲angu Pitjantjatjara Yankunytjatjara Lands
An̲angu Pitjantjatjara Yankunytjatjara	the traditional land owned by An̲angu in north-western South Australia
inma	ceremony, song, dancing
it̲i	baby, used to refer to warru pouch young
itjaritjari	marsupial mole, *Notoryctes typhlops*
kanyal̲a	euro, *Macropus robustus*
kapi	water, rain
kulini	listen to, used by warru rangers to refer to radiotracking
mina	water
minyma	a mature woman
ninu	greater bilby, *Macrotis lagotis*
palya	good, fine, alright
pintji	fence, specifically the warru pintji - the 97-ha exclosure on the APY Lands
Pitjantjatjara	one of two major languages spoken on the APY Lands (the other is Yankunytjatjara)
pun̲u	living/growing tree or bush
tjukurpa	encompasses An̲angu law, stories and beliefs.
warru	black-footed rock-wallaby *Petrogale lateralis* MacDonnell Ranges race
wayuta	brush-tailed possum *Trichosurus vulpecula*
wiya	no

ABSTRACT

Captive breeding for reintroduction is widely used as a tool for threatened species conservation. While captive populations have been successfully established for many species, the failure rates of subsequent reintroductions have been high. Often this is because managers are required to make reintroduction decisions without adequate knowledge of species biology, and little or no understanding of post-release population dynamics. A captive-breeding for reintroduction programme was initiated in 2007 for warru (black-footed rock-wallaby, *Petrogale lateralis* MacDonnell Ranges race (MRR)) of the Aṉangu Pitjantjatjara Yankunytjatjara (APY) Lands of South Australia (SA). A 97-ha predator exclosure (the "warru pintji") was constructed on the APY Lands in 2010 in which to release captive warru to establish a source population for future releases. This thesis presents five data chapters that examine multiple aspects of the soft-release (release from captivity to a fenced area of natural habitat in comparison to a hard release which is directly to the wild) and long-term reintroduction process, with the broad aim to advance the limited knowledge of warru reintroduction biology and assess reintroduction as a potential recovery tool.

Responses to reintroduction and post-release population dynamics were assessed through a 24-month multi-faceted monitoring programme following the release of 16 captive warru. Warru survival rate was 63%. Definitive mortality causes were not determined but their early timing suggested reintroduction-related stress. Blood biochemistry and body mass indicated rapid adaptation to the wild in surviving warru, with maintenance of excellent body condition post-release. This was also evidenced by high female reproductive rates. This work represents the first data on the reintroduction biology of warru.

Behaviours post-release were examined using Global Positioning System (GPS) collars in home range studies of seven reintroduced warru. As knowledge of warru home ranges was lacking, GPS collars were also fitted to nine warru within a remnant population to facilitate comparison. Reintroduced warru displayed rapid selection of hill habitat and established home ranges (8.9 ± 5.9ha) with core areas centred about the rocky outcrop. Reintroduced warru displayed lower fidelity to their home ranges compared to remnant warru, likely due to exploratory behaviours and establishment of dominance hierarchies, although none of these behaviours led to dispersal from the release site. Patterns of male/female overlap were similar to the remnant warru population suggesting the formation of a functioning social system post-release.

Drinking at supplementary water points was significantly predicted by rainfall and plant moisture content for both reintroduced and remnant warru populations. As limited rainfall during the dry winter on the APY Lands is linked to low juvenile warru survival, the results suggest that provision of supplementary water during the drier winter or drought could alleviate resource pressures for warru. This could potentially increase warru survival chances and thus population persistence.

A review of the genetic composition of the three *in situ* warru populations (New Well, Alalka and Kalka), the two genetic captive groups (1-New Well/Alalka and 2-Kalka), and the broader MRR, was undertaken using 14 microsatellite loci and a 672 base pair sequence of mitochondrial DNA. Captive groups had significantly lower genetic diversity than their wild source populations, increasing the likelihood of inbreeding. While microsatellite data presented significant contemporary genetic differentiation among the *in situ* populations, there was a lack of phylogeographic structure within mitochondrial haplotypes, suggesting greater historical connectivity across the MRR. Outbreeding depression was therefore predicted to be unlikely, so this thesis recommends that captive warru populations be managed as one genetic group to increase the adaptive potential of future reintroduced groups. These data provide the first phylogenetic analysis for the MRR and highlight the importance of assessing historical, as well as contemporary genetic structure, for threatened species.

Population viability analysis using post-release demographic rates predicted that the warru pintji population would exhibit a positive growth rate. Supplementation of the pintji population from captive stock in 2014 and delaying the first removal of a release group for new reintroductions until 2015 will enable larger release groups to be removed. This should increase the probability of successful establishment of the released population. Juvenile recruitment is an important component of successful establishment of a reintroduced warru population and will require intensive monitoring to enable adaptive management, such as the release of additional animals or increased predator control, to be implemented if successful recruitment is not observed.

The results of the current study suggest that, in the absence of introduced predators, reintroductions are capable of successfully re-establishing populations of warru. The thesis provides a case study of the reintroduction biology data that can be obtained through appropriate, intensive and targeted monitoring of both reintroduced and remnant populations. The outcomes also highlight the contributions that a long-term soft release can make to assess the suitability of captive breeding for reintroduction as a tool for population recovery. Future monitoring of hard releases of warru to gain comparative reintroduction biology data will ultimately determine the role that reintroduction can play in warru recovery on the APY Lands.

The Warru Recovery Team at their annual meeting in Yulara, February 2013. At this meeting, discussions commenced on the next phase of reintroduction for warru (hard releases from the warru pintji) in light of the findings of this thesis.
Photo: AW NRM Board.

ACKNOWLEDGEMENTS

View out to Wamitjara from the top of the warru pintji hill. Photo: R West

I sat on top of the warru pintji hill to write these acknowledgements, in early October 2013, at the end of our sixth warru pintji trapping week. The warru pintji is the 97-ha predator exclosure on the APY Lands into which captive warru were released in 2011/2012. From the top of the warru pintji hill you can see ‘Wamitjara’. The extinction of the warru population at this site in 2006 initiated the formation of the Warru Recovery Team (WRT). One of the aims of the WRT is to prevent further extinctions by increasing the abundance and distribution of warru on the lands using reintroduction. My PhD project was devised to assist the team in assessing this possibility and recommend appropriate future management. During this week we have confirmed the continued survival and excellent health/condition of ten captive founders, the three recruits from 2012, six new recruits for 2013 and that all adult females are breeding. These results are a fantastic outcome, and combined with the predictive models in this thesis, show great promise for reintroductions to re-establish warru populations. This will fulfil significant aspirations of A<u>n</u>angu to ensure warru remain on their lands. It really did seem like the most appropriate place from which to reflect on all those who have contributed to this research project.

Approval to conduct the research was granted by the APY Lands Executive Board and the University of Adelaide Animal Ethics Committee (S-2011-009). The project was made possible through the financial support of the Sir Mark Mitchell Research Foundation, Nature Foundation SA, Field Naturalists Society of SA, Norman Wettenhall Foundation, Margaret Middleton Fund for endangered terrestrial vertebrates and Duncan Turner at Adelaide Off Road. In-kind and financial support was also generously provided by APY Land Management, Zoos SA and the Alinytjara Wiluṟara NRM Board.

The seven field trips to collect the data within this thesis were made possible by the significant contributions and support of the Traditional Owners of the APY Lands. Firstly I would like to thank the warru minyma; Tjariya Stanley, Nyinguta Edwards, Inpiti Winton and Dora Haggie for their encouragement every step of the way, including Pitjantjatjara lessons around camp, and all the warruku inma and itjaritjariku inma which made each field trip extra special. Fieldwork was conducted with the assistance of 21 Aṉangu warru rangers: Eric Abbott, Bronson Bennett, Marlene Cooley, Anthony Minutjukar, Thomas Tjilya, Margaret Winton, Dale Richards, Melinda Stewart, Sherada Stanley, Grant Nyaningu, Jacob McKenzie; Kym Nelson, Matthew Miller, Cameron Cooper, Jarrod Nelson, Delwyn Watson, Timothy Dalton, Travis Lewis, Raymond Kenny, Russell Kickett and Ethan Dagg. Special thanks to Margaret and Marlene for all their contributions to puṉu work; Bronson, Kym, Cameron and Matthew for enduring habitat assessments in the October heat; Eric and Grant for their infectious laughs and hill races; Sherada for all of his ‘cheeseburger’ moments which kept us much amused; Thomas for many, many jokes; and Ethan for Sonia Dada and sharing Wamitjara with us.

The research project would also not have been possible without the significant support of three APY Land Management warru project staff. Jasmina Muhic facilitated my early field trips and helped to build my relationships with the minyma and rangers; Rachel Barr has provided significant support both on and off the lands over the last two years and never made anything seem impossible; and Simon Booth jumped right in to get things done in the last year and shared a good laugh when fieldwork went a little pear-shaped.

My involvement in the warru recovery project and the development of this PhD project was initiated and encouraged by Matt Ward, chair of the WRT. Matt took me on my first trip to the APY Lands, taught me many important things about working up there, how to tell a good story (and write a good song) and above all has provided a bucket load of emotional support the whole way through. Thank you.

To my supervisors; Drs David Taggart and Wendy Foster. Tags introduced me to the fascinating critters that are the rock-wallabies during a trip to Australia from the UK in 2007 and encouraged me to emigrate to pursue a PhD that involved applied conservation (not welfare of laboratory mice). He also taught me the importance of ‘stickability’, always provided encouragement and supported me during two field trips – one of which was very wet and drama filled. And my co-supervisor, Wendy, who endured many hours of mind mapping over coffee (and tears) in the early days whilst devising the project structure, pushed me to question the story and bigger picture of the thesis and taught me a lot about logical flow and science communication.

The data chapters contained within this thesis have also benefited greatly from the wisdom and knowledge of a number of collaborators. Mark Eldridge taught me that wallies don’t really move much until they do, provided exceptional comments on all of my chapter drafts, and shared his extensive knowledge of the *Petrogale* genus. To Sally Potter, my dearest friend and fantastic genetics mentor who took me on the journey from my first extraction to the final stages of analysis and interpretation and provided constant support both within and outside the PhD. Brian Rich (and the ‘boys in the lab’), who volunteered their time to conduct the blood analyses and to interpret the results. Tom Prowse supported my first steps into the world of PVA and never showed frustration - even when I sent panicked emails claiming the model was all wrong, only to realise they were obvious mistakes on my part. And to Steve Delean, who also provided guidance on the PVA aspects of the project, was always available to assist with statistical analysis and taught me to love the power of ‘*R*’.

My involvement in this project was also continually supported and encouraged by a number of other members of the WRT. Pete Copley constantly reminded me that I was doing a ‘great job’. John Read always made my brain hurt but pushed my thinking to the next level. The Monarto Zoo captive team, particularly Mick Post and Heidi Baker,

organised the health checks for warru prior to release and shared in the delights when ‘barren’ Sandy was documented with her first iṯi in the warru pintji. Zoo veterinary nurses Rebecca Probert and Paula Modra organised equipment for the pintji trapping trips and assisted with the releases, and zoo veterinarians Ian Smith and Lynley Johnson conducted preliminary health checks, attached collars and fitted ear-tags prior to release.

My field trips were made all the better for the attendance of brilliant volunteers and friends who gave up their precious time to share this experience. Thank you to Teagan McKillop, Jen Molyneux, Colin Bailey, Liberty Olds and Meryl Jenkins.

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To all of you, thank you.

CHAPTER 1: GENERAL INTRODUCTION

APY Land community members, the Warru Recovery Team and its stakeholders, and media representatives gather to witness the first release of captive warru into the 97-ha predator exclosure on the APY Lands, March 2011.

> *'Warru has been here for a long time, before us, and they should be in our lands because they are Traditional Owners too. We want to see them back in all places where they used to be.'*
>
> *Frank Young, Traditional Owner, APY Lands*

1.1 Conservation of threatened species

Globally, just over 1.7 million species have been described (IUCN 2013) and although we know very little about many of these species, an increasing number are found to be facing a high risk of extinction, such that we are experiencing an unprecedented biodiversity crisis. The International Union for Conservation of Nature (IUCN) has spent four decades working with its partners to assess the conservation status of the world's species, to highlight those that are threatened with extinction, and catalyse action for biodiversity conservation. In 2013, species assessed against the IUCN Red List criteria numbered 70,289 with 30% of these listed as threatened, i.e. they were assessed to be either 'Critically Endangered', 'Endangered' or 'Vulnerable' to extinction (IUCN 2013). These three conservation status categories are assigned when reductions in population size/extent of occurrence, and the demography/area of occupancy of the remnant populations suggest that the species is unlikely to persist without human intervention (IUCN 2012). Halting this global biodiversity crisis therefore requires identification of the causes of decline and selection of appropriate management actions to reduce extinction risk for threatened species.

Many of the drivers of species decline are related to human activity and can be broadly categorised into the 'evil quartet': (1) overkill from hunting practices, (2) habitat destruction and fragmentation, (3) impacts of introduced species, and (4) chains of extinction that result in the extinction risk of one species being exacerbated by the prior extinction of another species (Diamond *et al.* 1989). More recently, these drivers/threats to biodiversity have been further specified to create 11 key categories, nine of which relate to direct human action (residential development, agriculture and aquaculture, energy, production and mining, transportation and service corridors, biological resource use, human intrusions and disturbance, natural system modifications, invasive species and genes, and pollution) but also includes categories of catastrophic geological events and climate change/severe weather (Salafsky *et al.* 2008). Direct perturbation, as a result of factors such as habitat clearance, hunting, and predation by introduced species, is said to be the primary driver of species extinction (Purvis *et al.* 2000; Schipper *et al.* 2008). Despite the use of a range of *in situ* conservation management actions such as habitat protection through reserve creation, enforcement of legislation for hunting and trade, and habitat restoration (including the removal of introduced species), no action has yet succeeded in offsetting any of these extinction drivers for vertebrates (Hoffmann *et al.* 2010). For many species, extinction is most likely to be caused by a synergy of threats (including intrinsic biological traits) which means that conservation actions will only be successful if they target multiple threats in order to address these synergies (Brook *et al.* 2008). *Ex situ* options, such as the establishment of captive insurance populations, become necessary for some species when these multiple threats cannot be addressed within either an appropriate timescale or landscape scale to reverse the declining population trend (Caughley and Gunn 1996). A growing number of conservation management strategies are now focusing on the protection of *in situ* populations whilst running concurrent programmes to re-establish populations at former sites within the species range.

Species movements to assist persistence are termed 'conservation translocations' and are placed into two main classes: those of '*population restoration'*, and those of '*conservation introductions'* (Seddon 2010). Methods to restore populations occurring within the species indigenous range involve either '*reintroduction*' of a species to an area from which it is extinct or '*re-enforcements*', the release of individuals into an existing population. Re-enforcements (also referred to as supplementations) are usually conducted to increase population growth, induce artificial dispersal when barriers exist to natural dispersal, or augment genetic diversity (Seddon 2010). For example, wild populations of the globally threatened marbled teal (*Marmaronetta angustirostris*) were re-enforced with individuals from captivity that had been rescued from the wild as ducklings (Green *et al.* 2005). Conservation introductions, on the other hand, are movements of individuals outside of their native range and may be used to replace an ecological role of an extinct species or move a species to habitat where the threats to their natal habitat do not exist. All of these translocation methods are now widely used as conservation tools, with an increasing number of individuals released as part of *reintroduction* projects each year (Seddon *et al.* 2012).

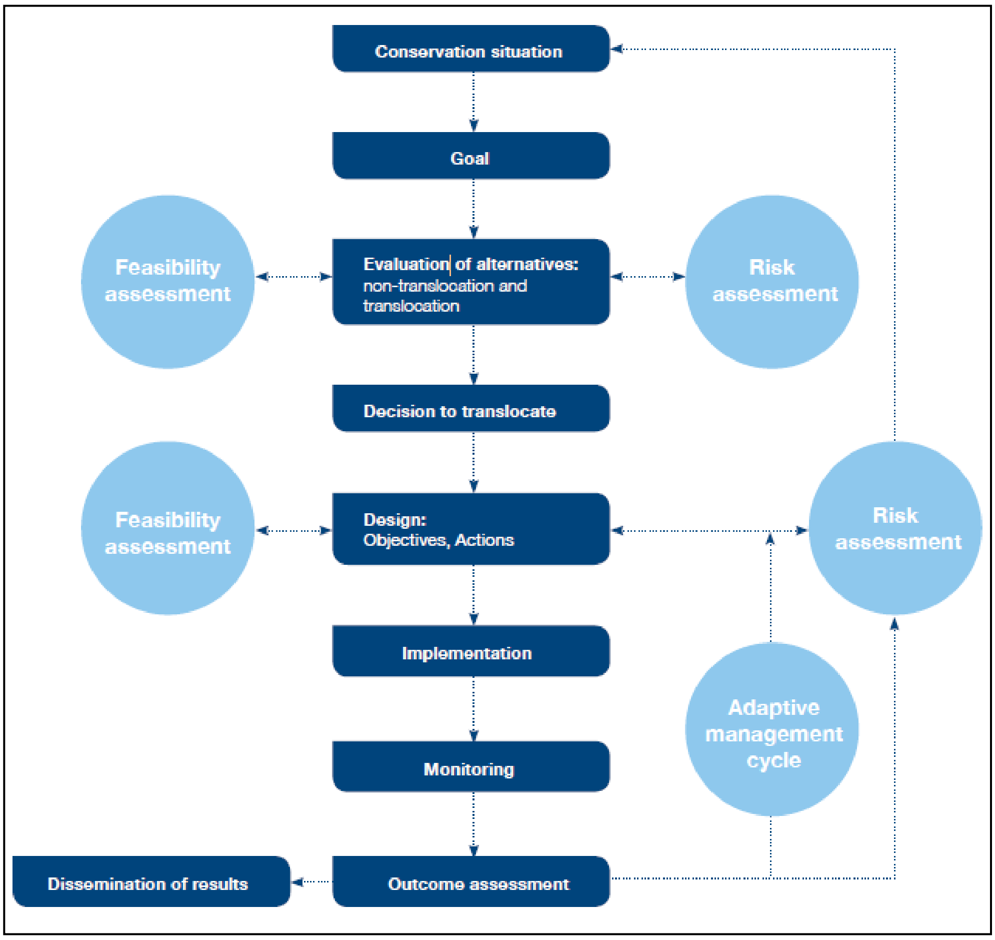
1.2 Reintroduction as a conservation tool

Reintroductions are defined as 'the intentional release of an organism into a part of its native range from which it has disappeared or become extirpated in historic times' (IUCN 1998). They are most often implemented when it is determined that the wild population size or genetic diversity requires augmentation (Caughley 1994). They are therefore an important conservation tool for a wide range of threatened species across the world (Hodder and Bullock 1997; Rout *et al.* 2009), including plants (Godefroid *et al.* 2011), although there is a bias within the reintroduction literature towards vertebrate translocations, particularly mammalian and avian (Bajomi *et al.* 2010). The popularity of reintroduction as a conservation tool is evidenced by 454 papers on conservation reintroductions that were published between 1990 and 2005 (Seddon *et al.* 2007).

Guidelines for reintroduction

In response to an increasing number of reintroduction projects across the globe, the IUCN Species Survival Commission (SSC) established a Reintroduction Specialist Group (RSG) in 1998 with the purpose to create a set of policy guidelines for reintroduction, to help ensure each attempt could achieve its intended conservation outcomes (IUCN 1998). These guidelines were revised between 2010 and 2012 and released in August 2013 (IUCN/SSC 2013). The guidelines state that every reintroduction project should follow a logical process from design to implementation (Figure 1.1). This process should include the development of a clearly defined goal with a thorough evaluation of whether alternative strategies can be used to meet these goals. As reintroduction projects are highly risky in terms of their ecological, social and economic impacts, the outcome of feasibility studies and risk analyses must conclude that the intended benefits outweigh the presumed risks. Where reintroduction is considered an appropriate option, projects should then progress to define a series of objectives to achieve the outlined goal and precise measurable actions that realise these objectives. The guidelines also advocate the use of an adaptive management cycle to improve reintroduction design. Adaptive management (AM) for reintroduction involves using learning from monitoring post-release to adjust pre-release protocol in order to improve reintroduction outcomes (Rout *et al.* 2009). The important role that AM can play in decision making in reintroduction programs has been recently highlighted (see Converse *et al.* 2013; Runge 2013).

Figure 1.1. The conservation translocation cycle, as published in the IUCN '*Guidelines for Reintroduction and Other Conservation Translocations*' (IUCN/SSC 2013)**.**



Measuring success of reintroduction

Reintroduction aims to establish a viable population of a threatened species within its former range that will require minimal intervention in the future (IUCN/SSC 2013). To assess the success of a reintroduction program in achieving this aim, monitoring is integral (Nichols and Armstrong 2012). Effective monitoring is multi-faceted and should include assessments of population growth and demographic performance, genetic diversity, adaptation to novel environments through behavioural monitoring, health post-release in terms of welfare considerations, the broader ecological impacts of re-establishing the species, and the social, cultural and economic impacts of release (IUCN/SSC 2013). Translocation success is therefore based on a matrix of these inter-related factors (Griffith *et al.* 1989) which has made 'success' in itself, particularly hard to define. There is still no agreement on the best method to determine success, with managers utilising a variety of benchmarks (Seddon 1999). Definitions range from measures such as breeding by the first wild born generation (Sarrazin and Barbault 1996) through to unsupported wild populations of a pre-determined size (Beck *et al.* 1994). Issues arise with the fact that initial recruitment and growth, often used to determine ‘success’, does not always equate to long-term persistence (Seddon 1999) and/or monitoring does not continue for the time period required to fully evaluate the outcomes against the pre-determined performance indicators. This has resulted in a number of cases in which success was claimed prematurely. For example, a review of reintroduction project dynamics found that 5% of projects declared successful had declining populations six years later (Wolf *et al.* 1996). It has therefore been suggested that a more appropriate assessment method should be to evaluate a reintroduction project against three key stages: (1) survival of founders, (2) breeding of founders and their offspring, and (3) persistence of the established population (Seddon 1999). Post-release monitoring can assess success at each of these stages, which will not only determine the timing of failures, but with appropriate monitoring, establish the likely causes of failure (Nichols and Armstrong 2012).

Factors affecting the success of reintroductions

Despite their concept as a tool for threatened species conservation, reintroductions have a poor track record of success (Fischer and Lindenmayer 2000; Griffith *et al.* 1989; Wolf *et al.* 1996). Reviews of previous reintroduction attempts around the world have reported average success rates of between 11% (Beck *et al.* 1994) and 26% (Fischer and Lindenmayer 2000) with lower success rates for avian reintroductions (7%; Cade and Temple, 1995). A wealth of factors are attributed to causing success/failure of these projects including: the source of the founders (Kleiman 1989), the demography and size of the release group (Green *et al.* 2005; Le Gouar *et al.* 2008; Maran *et al.* 2009; Sjöåsen 1996), genetic diversity of the released species (Godefroid *et al.* 2011), selection of inappropriate habitat for resource provision (Armstrong and Perrott 2000; Cheyne 2006), and a failure to remove previous threats (Moseby *et al.* 2011; Short and Turner 2000). Of these factors, those associated with habitat quality at the selected release site are most often cited as the main causes of success or failure (Cook *et al.* 2010; Osborne and Seddon 2012; Short 2009; Wolf *et al.* 1996).

Habitat quality encompasses all environmental features that impact survival and reproduction of a species including food resources, predators and parasites (Hall *et al.*

1997). In the context of reintroduction, the selection of a high quality release site should therefore ensure that the factors determined to have caused initial declines have been removed and that the site supports the current and future habitat requirements of the species. In line with the IUCN guidelines, the release site for a reintroduction should also be within the species historic range (IUCN/SSC 2013). The selection of a suitable release site therefore requires a thorough understanding of a species' ecological habitat requirements but this information is rarely available for threatened species (Short 2009). Therefore, managers will often select habitat which mirrors that in which the remnant populations remain, but this may not represent 'optimal habitat' (Osborne and Seddon 2012). To further compound this, the acceptance of a chosen site by release individuals can be affected by the prior experience of the founders. For example, captive individuals have been reported to seek areas of habitat similar to their captive enclosures (Stamps and Swaisgood 2007). Post-translocation behaviour can also be influenced by the size and composition of the release group (Kleiman 1989), which has been associated with long distance dispersal of some founders and thus significantly reduces the probability of population establishment and persistence (Hardman and Moro 2006).

A number of conservation managers have used experimental approaches in release protocols or post-release provision to determine species habitat resource requirements. For example, the role of food availability in the survival and reproduction of reintroduced hihi (stitchbird, *Notiomystis cincta*) in New Zealand was tested through a series of supplementation experiments post-release and subsequent demographic monitoring (Armstrong and Perrott 2000). Similarly, the release of water voles (*Arvicola terrestris*) into habitats of different quality highlighted the importance of vegetation density in the establishment probability of the release group (Moorhouse *et al.* 2009).

The complete removal of threats can be challenging for reintroduction managers, particularly if the identified threats include the presence of exotic predators within the system. The importance of this is most apparent in a comparison of reintroductions of macropods in Australia that occurred to mainland sites (11% success rate) to those on islands where exotic predators were absent (60% success rate) (Short *et al.* 1992). Of 180 mammalian and avian reintroductions reviewed by Fischer and Lindenmayer

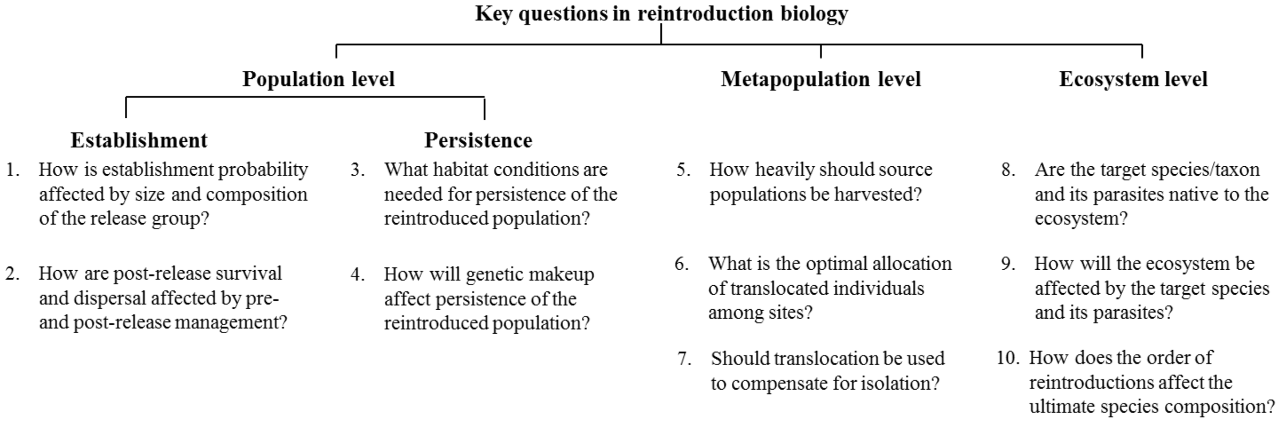
(2000), 50% occurred in areas where predators were still present and only two succeeded. If the control of high densities of predators is ineffective, predator naivety of released individuals, particularly those who have spent a number of generations in captivity, exacerbates the effects (Balmford *et al.* 1996). Methods such as predator training have been used to improve anti-predator skills in a variety of taxa (fish - Brown & Laland 2003; birds - McLean *et al.* 1999; and mammals - Griffin *et al.* 2002; McLean *et al.* 2000), although the influence of this training on reintroduction success requires further testing (McLean *et al.* 1996; Griffin *et al.* 2002; Moseby et al. 2012). In some instances, releases within predator exclosures have been determined to be the most appropriate reintroduction protocol for species at high risk of predation (de Tores and Marlow 2012). For example, in the light of previously failed reintroduction attempts, a 170-ha predator exclosure was constructed in which to re-establish populations of mala (*Lagorchestes hirsutus*) at Uluru-Kata Tjuṯa National Park in the Northern Territory of Australia and this has been deemed to be successful (Clayton 2011).

In addition to the factors directly related to whether individuals will survive post-release, a contributing factor to these repeated failures has been the lack of research dissemination from previous reintroduction attempts. This has limited the opportunity for decisions in future projects to be made from a reliable evidence base (Bajomi *et al.* 2010).

1.3 The science of reintroduction biology

The field of *reintroduction biology* (research undertaken to improve the outcomes of reintroduction) is relatively new (Ewen and Armstrong 2007) and emerged during the 1980s in recognition of a lack of learning from repeated failures of reintroduction programmes (Armstrong and Seddon 2008). Since the release of the first set of reintroduction guidelines in 1998 (IUCN 1998), and advocacy of a research approach to monitoring and assessment, the success rates of reintroduction projects appear to have increased (Soorae 2008). In an attempt to continue this trajectory of improved success and to provide a more strategic and integrated approach to reintroduction biology, Armstrong and Seddon (2008) proposed a conceptual framework for future reintroductions, underpinned by a series of key questions that operate at three system levels (Figure 1.2).

Figure 1.2. The ten key questions within reintroduction biology as proposed by Armstrong and Seddon (2008).



1. How is establishment probability affected by size and composition of the release group?
2. How are post-release survival and dispersal affected by pre- and post-release management?
3. What habitat conditions are needed for persistence of the reintroduced population?
4. How will genetic makeup affect persistence of the reintroduced population?
5. How heavily should source populations be harvested?
6. What is the optimal allocation of translocated individuals among sites?
7. Should translocation be used to compensate for isolation?
8. Are the target species/taxon and its parasites native to the ecosystem?
9. How will the ecosystem be affected by the target species and its parasites?
10. How does the order of reintroductions affect the ultimate species composition?

The concept advocates extending the scope of reintroduction monitoring in order to address those key questions which are identified *a priori* as lacking in knowledge for that particular species or system (Armstrong and Seddon 2008). This will improve communication between researchers conducting releases on similar species and thus increase the capacity to transfer knowledge between projects and across the globe (Armstrong and Seddon 2008). Poor monitoring and a failure to disseminate information into the public domain have been major criticisms of previous reintroduction programs (Nichols and Armstrong 2012). The integration of this framework should, ultimately, enable refinement of the techniques of reintroduction and a better understanding of the likely outcomes, so that reintroduction can be confidently used to conserve threatened species.

1.4 The role of reintroduction in Australian threatened species conservation

Reintroduction has been recommended as a conservation strategy for one-third of Australian vertebrates (Serena and Williams 1994). This is in response to severe range contractions, population declines and local extinctions of many taxa (Burgman *et al.* 2007; Murray and Hose 2005), in particular Australian mammals (Johnson 2006; Short and Smith 1994). Of the documented global mammal extinctions prior to the 1990s, 50% of these were from Australia (Short and Smith 1994), the most severe mammalian extinction rate of any continent. Although major historic extinctions (such as those of the mammalian megafauna) occurred during the Pleistocene and Holocene, changes associated with European settlement (introduction of disease, herbivore competitors, altered fire regimes, habitat destruction, hunting and introduced predators), combined with periodic droughts on the continent are thought to have overwhelmed the remaining species, resulting in large scale declines and extinctions in the last 200 years (Johnson 2006). These extinctions have also been concentrated in the arid interior regions of Australia and have occurred at a higher rate in the ‘critical weight range’ (CWR) mammals (35g to 5.5kg) (Burbridge and McKenzie 1989; Short and Smith 1994), thought to be the result of an interaction between life-history traits and body size (McKenzie *et al.* 2007).

As a result, translocations to restore populations of mammal species and decrease their conservation listing are increasingly used in Australia (Short and Smith 1994). Of the 54 Australian species relocation programmes documented by Sheean *et al.* (2012), 25 were for threatened or rare mammal species. Examples include: reintroductions to fenced enclosures such as those for the greater bilby, *Macrotis lagotis* and greater stick-nest rat, *Leporillus conditor*, in South Australia (Moseby and O'Donnell 2003; Moseby and Bice 2004), and the numbat, *Myrmecobius fasciatus* and quokka, *Setonix brachyurus* in western Australia (de Tores and Marlow 2012); and reintroductions to unfenced but managed sites such as those of the tammar wallaby, *Macropus eugenii*, to Innes National Park, South Australia (Kemp 2011) and brush-tailed bettongs, *Bettongia penicillata,* to Yathong Nature Reserve in western New South Wales (Priddel and Wheeler 2004). Although better than the global trend for reintroduction, Australian reintroduction project success rates have still been poor (46%) (Sheean *et al.* 2012).

The most significant factor to impact the success of mammal translocations in Australia has been predation by introduced species (Short 2009). The red fox (*Vulpes vulpes*) and feral cat (*Felis catus*) are recognised as major drivers of increased extinction risk for a large proportion of Australia’s mammal species (Johnson 1999), and both species have been implicated in the decline and subsequent failure of restoration attempts for a number of Australia’s macropodid species (Short *et al.* 1992). There have been 109 separate macropodid translocation attempts since 1969, with a sharp increase in the number of attempts in the last two decades (Clayton 2011). In addition, intensive *in situ* recovery actions have been implemented for multiple macropodid species (WWF 2011). Despite this, only two sustained improvements in IUCN conservation status for Australian macropodids have been recorded: a down listing from ‘Vulnerable’ to ‘Near

Threatened' for the boodie (*Bettongia leseuer*), and for the tammar wallaby a change from 'Near Threatened' to 'Least Concern' (WWF 2011). This poor rate of improvement in conservation status, despite considerable resource investment, outlines the clear need to improve the scientific rigour of reintroductions in Australia to advance reintroduction biology knowledge and improve project outcomes (Sheean *et al.* 2012).

1.5 Reintroduction and *Petrogale*

Petrogale, the rock-wallabies, of the family Macropodidae, have colonised rocky habitats in a range of environments across Australia as result of a unique set of ecological, morphological, and behavioural adaptations (Eldridge 2011). The 16 recognised species can be found in tropical, temperate and arid regions of the mainland and demonstrate an exceptional level of chromosomal diversity (Eldridge 2011). However, their naturally fragmented distribution, combined with a wide array of threats, causing localised extinctions and declining population sizes, has resulted in high extinction risks for *Petrogale,* with two 'Endangered', five 'Near Threatened' and one 'Data Deficient' species on the IUCN Red List (IUCN 2013). This array of known and possible threats includes: predation by introduced predators (Kinnear *et al.* 1988), shelter and dietary overlap with competitor species (Copley 1983; Creese 2007), habitat destruction and degradation (Johnson *et al.* 2003), altered fire regimes (Tuft 2010), disease (Lunney *et al.* 1997), climate change (Pearson 2012), and poor population viability due to small population size (Hazlitt *et al.* 2006). In response, a variety of recovery options have been explored/implemented for *Petrogale* which has made them useful models in the study of wildlife management and conservation biology (Eldridge *et al.* 1999; Kinnear *et al.* 2010; Potter *et al.* 2012).

Recovery plans are published for: the brush-tailed rock-wallaby (*Petrogale penicillata*) (Menkhorst and Hynes 2010), the Proserpine rock wallaby (*Petrogale persephone*) (DERM 2010), populations of the black-footed rock-wallaby in South Australia (*Petrogale lateralis* MacDonnell Ranges race) (Read and Ward 2011a), and a national recovery plan for 5 *Petrogale* species (*Petrogale lateralis, Petrogale brachyotis, Petrogale burbidgei*, *Petrogale concinna* and *Petrogale rothschildi*) (Pearson 2012). Recommended recovery actions include: conservation status assessments for those species for which survey data is lacking, feral predator and competitor control, habitat management to permit successful dispersal, the use of re-enforcement (supplementation) translocations to augment small colonies, and reintroductions to increase the number of extant sub-populations (Pearson 2012).

Rock-wallaby translocations have occurred since the 1960s, with the earliest documented movement being of six *P. lateralis pearsoni* individuals between the north and south Pearson Islands in 1960 (although accidental and not for conservation purposes) (Lennon *et al.* 2011). Island translocation strategies have since been utilised as a conservation management tool for two *Petrogale* species. A group of 15 *P. rothschildi* were translocated from Enderby Island to West Lewis Island in 1982 in response to threats of sand mining, and with a subsequent supplementation, successfully established a population (Abbott and Burbridge 1995). In 1998, a conservation *introduction* to Hayman Island (outside of the species former range) was conducted using 27 captive bred *P. persephone* (Johnson et al. 2003). Deaths attributed to eagle predation lead to an adjustment in release protocol to a 'soft' strategy where individuals were held in a fully enclosed steel pen, constructed within rock-wallaby habitat, for three months prior to release. This appeared to increase survival rates (Johnson *et al.* 2003) and the introduction has been deemed to be a success (WWF 2011).

On the mainland, *Petrogale* translocation success rates have been more variable. Whilst 23 separate translocations of wild *P. l. lateralis* in Western Australia are believed to have been successful (Pearson 2012), some movements of wild *P. penicillata* have failed to establish (Soderquist 2011). This variability in response has also been seen in reintroductions using captive-bred rock-wallabies. The best outcomes have been documented in the reintroduction of the two subspecies of the yellow-footed rock-wallaby (*Petrogale xanthopus*). In 1996, 12 captive *P. x. xanthopus* were reintroduced to Aroona Sanctuary in the arid lands of South Australia (Lapidge 2001). This population has since successfully established with annual trap rates remaining high and good recruitment rates (a total of 81 'new' individuals have been marked since inception) (Zoos South Australia, pers. comm.). In 1998, 24 captive individuals of the sub species, *P. x. celeris* were released onto a pastoral station in the semi-arid Wallaroo Ranges of southwest Queensland, and following intensive monitoring for two years, were also deemed to have established (Lapidge 2001). However, this same level of success has not been realised in reintroductions of captive *P. penicillata* in the Grampians National Park, Victoria. Between 2008 and 2011, three releases of 22 captive *P. penicillata* (from the southern evolutionarily significant unit (ESU)) took place but failed to establish a self-sustaining population, with only six animals known to be alive at the end of 2011 (Weeks 2012). A review of the program suggested that inbreeding depression as result of a lack of genetic diversity within the captive founders was strongly influencing fecundity and thus recruitment in the population (Weeks 2012). An attempt to supplement the population was conducted in 2012 through the release of a further 18 captive individuals (including some central ESU animals) but high mortality rates post-release have also failed to establish a population (seven individuals remain) (Brush – Tailed Rock - Wallaby Recovery Team, pers. comm.). Fox predation combined with a slow onset of reproduction appear responsible for these failures, with other unexpected mortalities from factors such as rock falls, disease and injuries further limiting initial establishment (D. Taggart, pers. comm.).

The *P. penicillata* case study in the Grampians and the variable responses from other rock-wallaby translocations and reintroductions (Soderquist 2011), suggest that managers are still a long way from understanding best practice for reintroductions of *Petrogale*. As translocation is also listed as a potential recovery action for *P. concinna* (‘Data Deficient’) and *P. sharmani* (‘Near Threatened’) (IUCN 2013), targeted reintroduction biology research within all future *Petrogale* translocation efforts is essential to help improve the success rates of reintroduction.

1.6 Recovery of *Petrogale lateralis* in South Australia and the role of reintroduction

Species description and conservation status

The black-footed rock-wallaby (*P. lateralis*) is characterised by a distinct white cheek stripe, prominent black and white lateral stripes, and black feet and tail tip (Figure 1.3), with adult males weighing a maximum of 5.0kg and females 3.9kg (Eldridge and Pearson 2008). First described by Gould in 1842, the species consists of three subspecies (*P. l. lateralis*, *P. l. hacketti* and *P. l. pearsoni*) and two chromosomal races (“West Kimberley” and “MacDonnell Ranges race”) (Eldridge and Pearson 2008), all of which are listed as ‘Vulnerable’ under the national EPBC Act (1999). The MacDonnell Ranges race (*P. lateralis MRR*) used to occupy the largest distribution of any *P. lateralis*, extending from south of Lake Eyre South in South Australia (SA), throughout the central region of Western Australia (WA) and as far north as the Tanami

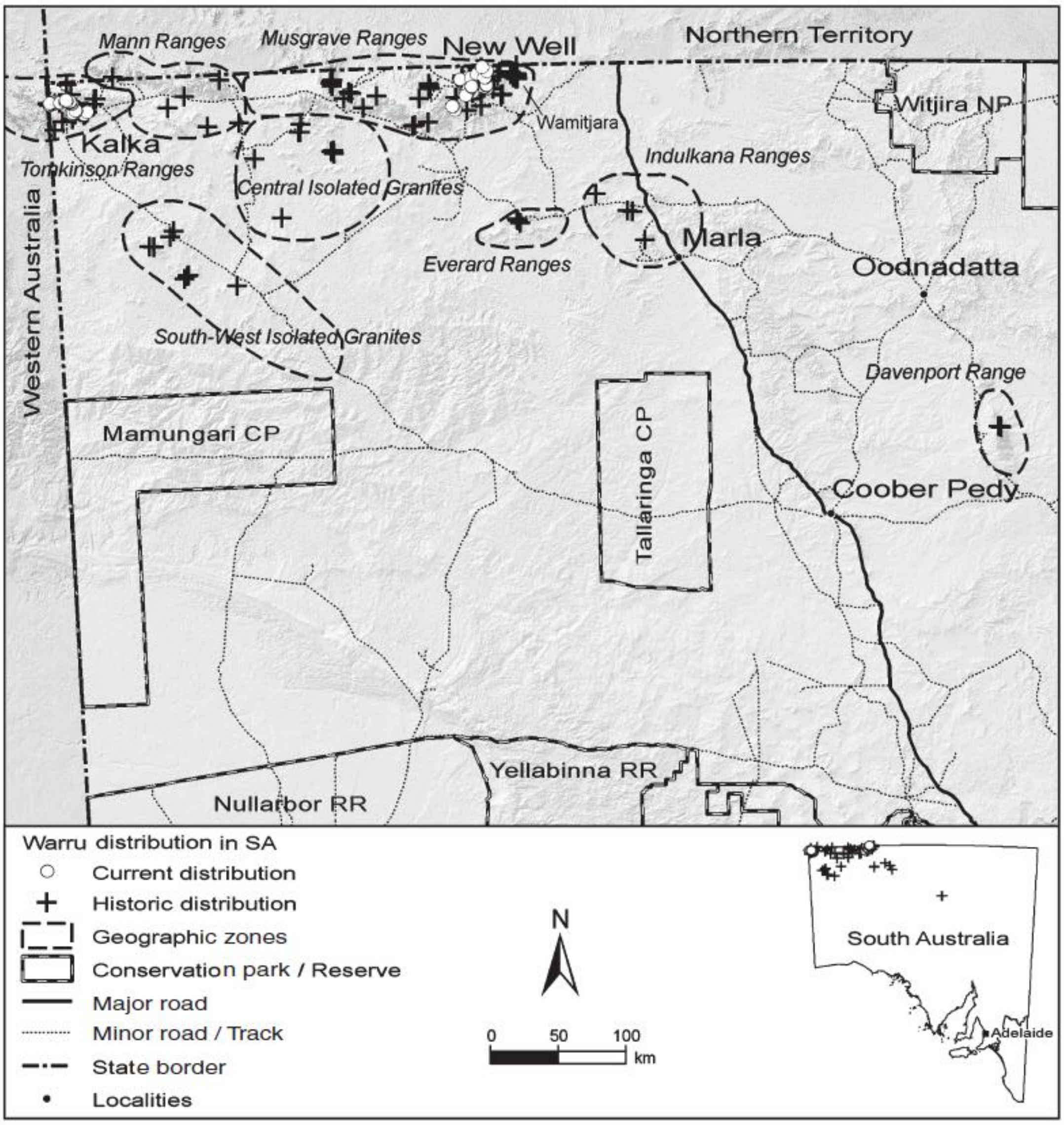
Desert in the Northern Territory (NT) (Read and Ward 2011a). Whilst populations appear to remain widespread and common in the NT, populations in SA and WA have experienced dramatic declines in range and abundance (Pearson 2012). Population estimates of fewer than 250 individuals within SA are confined to two metapopulations on the arid A<u>n</u>angu Pitjantjatjara Yankunytjatjara (APY) Lands (Figure 1.4), where the species is known as 'warru' to traditional owners (Pitjantjatjara for 'rock-wallaby'). This has resulted in the classification of warru as 'Endangered' under the SA National Parks and Wildlife Act (1972).

Figure 1.3. A captive female warru at Monarto Zoo, South Australia, showing the distinctive white cheek stripe, lateral stripes, black feet and black tail tip of *Petrogale lateralis* MRR. Photo: The Advertiser

Decline of warru in SA

Warru were described as 'swarming' the hills of the APY Lands between 1932-35 (Finlayson 1961), with A<u>n</u>angu informants also reporting that the species was once very common (Nesbitt and Wikilyiri 1994). Today on the APY Lands, warru are known to exist in a metapopulation of about 100 animals in the eastern Musgrave Ranges and at three locations in the Tomkinson Ranges (Figure 1.4), with a combined population of less than 50 animals (Ward *et al.* 2011). This represents a 93% decline in the extent of occurrence of their former range in SA (Ward *et al.* 2011). The feral cat (*Felis catus*) and the fox (*Vulpes vulpes*) have been implicated in the decline of *P. l. lateralis* populations in WA (Kinnear *et al.* 2010) and so their arrival on the APY Lands, presumed to be in the late 1880's (Abbott 2002; Robinson *et al.* 2003) is proposed to have contributed significantly to the decline of warru (Read and Ward 2011a). In addition, interruption to traditional burning practices which has led to more uniform vegetation assemblages, exotic plant invasions such as those of buffel grass (*Cenhrus ciliaris*), competition from herbivores such as the euro (*Macropus robustus*) and changes to water availability are all thought to have contributed to the decline of warru and remain as current threats to populations (Read and Ward 2011a).

Figure 1.4. Current and historic distribution of warru (*Petrogale lateralis*, MacDonnell Ranges race) in South Australia. Taken from Ward *et al.* (2011)**.**



Recovery actions in SA

Following extinction of one of the last known populations at Wamitjara in 2006, the SA Warru Recovery Team was formed in 2007 with the aim of achieving a downgrading in the conservation status of warru (Read and Ward 2011b). A summary of the recovery actions and related milestones to date are provided in Table 1.1. The dramatic decline and few known localities led to the decision to initiate an *ex situ* captive insurance population of warru (Read and Ward 2011b). This commenced in 2007 through the cross-fostering (Taggart *et al.* 2010) of pouch young from wild mothers on the APY Lands to surrogate *P. xanthopus* mothers at Monarto Zoo, SA. The recovery team formation also led to additional warru distribution surveys and the implementation of poison baiting, predator monitoring and annual trapping at the *in situ* colonies. However, the small size of the remaining colonies and lack of connectivity also lead to the conclusion that these *in situ* populations were likely to require supplementation to reduce their extinction risk, and that long-term persistence of the species would require an increase in the distribution of sub-populations of warru across the APY Lands (Read and Ward 2011b). Reintroduction was therefore considered a key conservation tool for the recovery of warru populations in SA (Read and Ward 2011b).

Although a number of successful wild-to-wild translocations have occurred for *P. lateralis* in WA (Pearson 2012), this was the first use of captive warru, which created a level of uncertainty in the outcomes. As introduced predators have been implicated in the decline of warru, and predation is the most significant contributor to reintroduction failure of a suite of mammal species (Short 2009), it was determined that initial releases of captive warru would take place in a soft-release predator-free enclosure. In consultation with A<u>n</u>angu warru rangers and elders, 26 sites were assessed for the potential to construct a predator-proof warru ‘pintji’ (Pitjantjatjara for ‘fence’) (Ward *et al.* 2010). Site assessments combined traditional ecological knowledge with scientific methods to examine historical presence of warru, current habitat suitability, population capacity, feasibility of construction and subsequent ability to manage the enclosure (Muhic *et al.* 2012; Ward *et al.* 2010). Each site was scored against a set of ranked criteria (Appendix 1A), and resulted in the selection of a site at Donald’s Well, approximately 17km south east of the extant New Well population. This site scored highest against the site selection criteria (Appendix 1B) and was confirmed by traditional owners to be an historic warru site that previously supported a large population of warru (Ward *et al.* 2010). The warru pintji is 97-ha in area (Muhic *et al.* 2012), enclosing a discrete granite outcrop ~17ha in size with a mean height of 50m (Figure 1.5 and Figure 1.6). The fence, modelled on that of the Arid Recovery project in SA, was completed in late 2010 (Muhic *et al.* 2012).

Table 1.1. Summary of survey and recovery actions for warru (*Petrogale lateralis* MacDonnell Ranges race) in South Australia 1985 – 2012.

1985	First biological survey of the APY Lands highlights absence of warru in previously known locations
1994	Survey of 14 sites where Aṉangu knew warru used to exist, two found evidence of warru - New Well and Wamitjara
1996	1080 ground baiting commenced at New Well and Wamitjara
1998	Scat quadrats established at New Well and Wamitjara
1999	Aṉangu rangers employed by APY Land Management to assist warru conservation
2000	Biannual scat and spotlighting surveys commence at New Well and Wamitjara
2001	Biannual scat counts commence at Kalka
2004	1080 aerial baiting of the Eastern Musgrave Ranges instigated
2005	Alalka colony in Musgrave Ranges found
	Annual warru trapping established at the 3 remnant colonies - New Well, Alalka and Kalka
2006	Wamitjara colony declared extinct
2007	Formation of the Warru Recovery Team
	Captive colony commenced at Monarto Zoo, SA through cross fostering of pouch young from New Well, Alalka and Kalka (n = 16)
	VHF collars fitted to adult warru at New Well to monitor survival
2008	Further survey of the Musgrave Ranges
	Captive colony supplemented through additional cross fostering (n = 7)
	34 ground bait stations established around New Well, baited monthly
2009	Further survey of the Musgrave Ranges (western side)
	Captive colony supplemented through further cross fostering (n = 3)
2010	Construction of the 'warru pintji' (a 97-ha predator exclosure) commenced at Donald's Well
	Further survey of the Tomkinson Ranges
	1 PY abandoned during trapping hand reared to add to captive colony
2011	11 captive warru returned to the warru pintji (March = 5, July = 6)
	Warru Recovery Team awarded the National NAIDOC 2011 Caring for Country Award
	Warru Recovery Plan 2010-2020 launched
	First warru recovery team Annual Report
	1 PY abandoned during trapping hand reared to add to captive colony
2012	5 captive warru supplemented to the warru pintji (July)
	Survey of the Mann Ranges in conjunction with Central Land Council

Figure 1.5. Aerial view of the 97-ha predator exclosure (known as the ‘warru pintji’) on the A̲n̲angu Pitjantjatjara Yankunytjatjara Lands. Photo: J Muhic

Figure 1.6. View of the south-eastern end of the warru pintji hill demonstrating the availability of complex boulder piles and food resources. Photo: R West

1.7 Thesis aims

The current thesis focuses on the reintroduction biology of an arid zone, CWR mammal, the warru (black-footed rock-wallaby, *Petrogale lateralis* MacDonnell Ranges race). As many species of arid zone, CWR mammals have suffered dramatic declines in range and abundance within Australia, the reintroduction of warru provides a useful model in which to examine the role of captive breeding and soft-release in re-establishing threatened species populations. The thesis implements the recommended multi-faceted monitoring approach within the IUCN *Reintroduction Guidelines* (IUCN/SSC 2013), and using the conceptual framework of Armstrong and Seddon (2008) (Figure 1.2), aims to address the first five of their ten questions in the context of warru reintroduction biology:

1. How is establishment probability affected by size and composition of the release group?
2. How are post-release survival and dispersal affected by pre- and post-release management?
3. What habitat conditions are needed for persistence of the reintroduced population?
4. How will genetic makeup affect persistence of the reintroduced population?
5. How heavily should source populations be harvested?

The questions are addressed across five data chapters presented as standalone manuscripts, written in a style for publication following thesis submission. As a result, some repetition in the Introduction and Methods for these chapters was unavoidable.

- Chapter 2 presents data on post-release survival, reproduction and health (Questions 1, 2 and 3) and an assessment of various monitoring techniques.

- Chapter 3 presents an assessment of the adaptability of captive warru to the wild and the selection criteria used to choose the release site, through examination of habitat selection and home range behaviours post-release (Questions 2 and 3).

- Chapter 4 examines the role of supplementary resources (water) in the provision of suitable habitat conditions (Question 3).

- Chapter 5 utilises historical and contemporary markers to examine the genetic structure of *in situ* and captive/reintroduced warru populations and thus examines the impact of captive management on the genetic viability of the reintroduced populations (Question 4).

- Chapter 6 uses the data from preceding chapters to construct a population viability analysis model which is used to make predictions about the impact of group size on establishment probability (Question 1). It also extends this beyond the population level, to examine metapopulation level considerations in terms of harvesting the soft-reintroduced group for future hard releases (Question 5).

- Chapter 7 synthesises the findings of Chapters 2 to 6 to present the progress that the current study has made in addressing the key reintroduction biology questions, provides an evaluation of the potential role of reintroduction as a tool for warru recovery, and highlights the implications of this research to the field of reintroduction biology, monitoring and management. Areas of future research pertinent to both warru recovery and other arid zone reintroductions are highlighted. The results contained within this thesis have also directly contributed to the adaptive management of warru, including the engagement and training of Aṉangu Warru Rangers during the reintroduction process, and thus these aspects are also outlined for the benefit of managers of similar community focused reintroduction projects.

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CHAPTER 2: REINTRODUCTION BIOLOGY OF WARRU: MONITORING FOR ADAPTIVE MANAGEMENT.

Warru Rangers Eric Abbott and Quentin Stanley release 'Nyi Nyi' into the warru pintji, July 2012. Photo: L Olds.

2.1 Preamble

This chapter examines the outcomes of a multi-faceted post-release monitoring approach that was used to gain information on the reintroduction biology of warru. Radio tracking, trapping, remote cameras and blood analyses were used to determine the survival of individuals, condition, health, reproductive output and recruitment post-release. The chapter provides recommendations for the most appropriate monitoring schedules for future releases to enable assessments of the factors driving establishment success and thus facilitate adaptive management.

2.2 Statement of Authorship

Title of paper	**The reintroduction biology of captive warru; monitoring for adaptive management.**							
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Name of Principal Author	**Rebecca West**		
Contribution to the paper	Design/developed the work. Collected all data/samples and conducted statistical analyses. Wrote manuscript.		
Signature		Date	24/10/2013

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Contribution to the paper	Supervised design and development of work.		
Signature		Date	24/10/2013

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2.3 Abstract

Reintroduction projects face problems with monitoring the fate of released individuals. The risk of reintroduction failure is likely to be particularly high when the founding individuals are captive-born or reared. Early survival rates are determined by the quality of habitat (including predator densities) at the release site and the ability of captive individuals to adapt to the wild. Monitoring is therefore essential for interpreting the causal factors of establishment success and can provide fundamental knowledge of species reintroduction biology, on which to base adaptive management and future reintroduction decisions. The current study used trapping, radiotracking and remote cameras to examine the first release of 16 captive warru (black-footed rock-wallaby *Petrogale lateralis* MacDonnell Ranges race) into the ‘warru pintji’ (a 97-ha predator exclosure) on the Aṉangu Pitjantjatjara Yankunytjatjara (APY) Lands of South Australia. Warru survival rate post-translocation was 63% with no effect of age, sex or weight at release on survival. Definitive causes of death were not determined, but the early timing of the six mortalities (within nine weeks of release) suggested translocation-induced stress played a role. For the surviving warru, blood biochemistry and change in body mass analyses indicated that captive warru rapidly adapted to a wild diet and maintained excellent body condition post-release. Surviving female warru conceived within six months of release and reproductive rates were high throughout the study period. Continued monitoring will be required to determine if recruitment of independent juveniles matches the reproductive output observed. The next phase of reintroduction will involve the release of groups of warru to outside the warru pintji (hard - release). It is recommended that future releases implement a similar multi-faceted monitoring approach to determine survival, health and fecundity post-release. Comparative reintroduction biology of warru under these two management scenarios will enhance the interpretation of factors influencing the successful establishment of released groups, and will provide the data necessary to implement effective adaptive management for future warru reintroductions.

Keywords: reintroduction biology, soft-release, *Petrogale*, monitoring, adaptive management.

2.4 Introduction

Reintroduction refers to the movement of organisms into parts of their native range from which they have become extinct (IUCN 1998). *Reintroduction biology* is the term used to refer to research that is undertaken to improve the outcomes of these reintroductions (Ewen and Armstrong 2007). While movements of species to facilitate reintroduction have occurred for over 100 years (Kleiman 1989), the field of reintroduction biology is relatively new (Armstrong and Seddon 2008). Its development came during the 1980s in recognition of poor reintroduction success rates and a lack of learning from these failures (Armstrong and Seddon 2008; Griffith *et al.* 1989; Sarrazin and Barbault 1996). This was attributed in part to insufficient monitoring, which prevented determination of the causes and timing of failures (Seddon *et al.* 2007) and thus limited the opportunity for adaptive management (Griffith *et al.* 1989; Nichols and Armstrong 2012; Sarrazin and Barbault 1996). Since the publication of the first *'Guidelines for Reintroductions'* by the International Union for Conservation of Nature (IUCN 1998), the level of reintroduction-related monitoring has increased significantly (Seddon *et al.* 2007), with a ten-fold increase in the number of published journal articles on the topic since the 1990s (Armstrong and Seddon 2008).

Monitoring prior to release is required to identify suitable release sites and usually focuses on assessment of habitat quality factors such as food resources, availability of shelter/nesting sites and predator abundance (Johnson *et al.* 2003; Taggart *et al.* 2008). The majority of monitoring reported in the literature is conducted during the initial period post-release (Nichols and Armstrong 2012). Multiple survey methods such as trapping, radiotelemetry, tracking, cameras traps and genetic analyses are used to determine vital rates (survival, reproduction and recruitment) of the newly released individuals (Le Gouar *et al.* 2008; Moseby and O'Donnell 2003). In some cases these data supplement gaps in basic species biology (Short 2009) in addition to contributing to an understanding of reintroduction biology (Ewen and Armstrong 2007). Physiological measures may also be deployed to determine the adaptation of released individuals to their new environment (Mathews *et al.* 2006), which in turn can be used to evaluate the quality of the selected habitat (Homyack 2010). Monitoring data may also be used to study survival as a function of various management actions, such as release methodology (Bright and Morris 1994; Hardman and Moro 2006), pre-release experience (Frair *et al.* 2007) and release group composition (Maran *et al.* 2009).

Effective monitoring therefore provides the raw data with which to assess progress towards the project objectives and improve reintroduction protocols. It can also be used to develop and subsequently refine models of population persistence under varying management scenarios (Ewen and Armstrong 2007). In addition to evaluation and management of the focus population, this monitoring can provide valuable information for future projects and the larger decision processes governing reintroduction management (Nichols and Armstrong 2012; Sarrazin and Barbault 1996). This is particularly important for new projects that may be required to make management decisions without adequate knowledge of species biology and little or no experience of post-release population dynamics and release strategies (Nichols and Armstrong 2012; Runge 2013). For example, lessons learnt from the reintroduction of Andean condors (*Vultar gryphus*) were utilised in the subsequent captive breeding and release of Californian condors (*Gymnogyps californianus*) (Toone and Wallace 1994). However, many other projects have been criticised for ineffective monitoring which fails to gather the information required, or if information is collected, it is unreported. For example, the outcome of 40% of vertebrate translocations within Australia could not be determined due to a lack of long-term monitoring and reporting (Short 2009). This limits the ability for prior experience to be used in adaptive management processes, which can be essential to improving the success of reintroduction programs (Converse *et al.* 2013; Runge 2013).

Reintroduction was determined to be a key conservation tool for warru (black-footed rock-wallaby, *Petrogale lateralis* MacDonnell Ranges race) on the Aṉangu Pitjantjatjara Yankunytjatjara (APY) Lands in South Australia (SA) (Read and Ward 2011). This chromosomal race of *P. lateralis* is listed as ‘Endangered’ in SA (NPSW Act, 1972) with estimates of less than 250 individuals remaining in the wild (Ward *et al.* 2011b). Despite reintroduction being recommended as a conservation tool for warru, reintroduction biology knowledge of the species is sparse (Read and Ward 2011). While a number of previous translocations of *Petrogale* species have occurred, including *P. lateralis* (Pearson 2012), the results have been highly variable, making their application to new translocations difficult (Soderquist 2011). In addition, this project utilised captive individuals of this species for reintroduction, for which adaptability to the wild had not yet been assessed. The decision of the Warru Recovery Team (WRT) to commence with an initial soft-release (reintroduction into a fenced area of natural habitat) provided the opportunity to collect specific information on post-release behaviours and vital rates for this species. Releases of captive warru into a 97-ha predator exclosure commenced in 2011 (Muhic *et al.* 2012). This study implemented a multi-faceted monitoring programme undertaken during the first 24 months of the soft-reintroduction, to address the following *a priori* questions to enhance reintroduction biology knowledge for warru:

1. What percentages of released warru survive and is age, weight or sex an explanatory factor of survival?
2. What are the causes of mortality for released warru in the absence of introduced predator pressure?
3. Does the growth and health of released warru reflect adaptation to wild resources and habitat suitability?
4. What is the reproductive rate of released female warru?
5. What is the recruitment rate of the population?
6. How does monitoring method affect the population estimate of the released group?

2.5 Methods

2.5.1 *Establishment of the captive population*

The captive warru population was established between 2007 and 2009 through the cross-fostering (Taggart *et al.* 2010) of 22 warru pouch young (PY) from three wild colonies (New Well, Alalka and Kalka) on the APY Lands (Read and Ward 2011) to yellow-footed rock-wallaby (*Petrogale xanthopus*) surrogates at Monarto Zoo, SA. Genetic data at the time indicated gene flow between Alalka and New Well but not Kalka (Ruykys 2011), so two breeding groups were maintained at the zoo: a New Well/Alalka group (n = 17) and a Kalka group (n = 5). Between 2009 and 2011, New Well/Alalka pairs were bred, resulting in ten births. A further PY from Kalka was added to the captive population after being abandoned by its mother during annual trapping in 2010. At the start of 2011 the captive warru population consisted of 26 individuals (21 New Well/Alalka warru and 6 Kalka warru) (WRT 2012).

2.5.2 *Release site selection and preparation*

The WRT selected a soft-release method as the first phase for reintroduction of warru to the APY Lands. This involved the construction of a fence to exclude introduced predators (feral cat - *Felis catus*, red fox - *Vulpes vulpes*) and dingo (*Canis lupus*) from an area of natural warru habitat (Read and Ward 2011). In consultation with A<u>n</u>angu warru rangers and elders, 26 sites were assessed for the potential to construct the predator-proof warru pintji (Pitjantjatjara for ‘fence’), in which to release captive warru (Ward *et al.* 2010). Site assessments combined traditional ecological knowledge with scientific methods to examine historical presence of warru, current habitat suitability, population capacity, feasibility of construction and enclosure management (Muhic *et al.* 2012; Ward *et al.* 2010). Site attributes were scored and summed to determine the most appropriate site (the one with the highest score) (Ward *et al.* 2010).

The selected site was at Donald’s Well [26.184^{0}S, 132.345^{0}E], approximately 17km south-east of the extant New Well population. This site was confirmed by traditional owners to be an historic warru site that previously supported a large warru population (Ward *et al.* 2010). The warru pintji was completed in late 2010 and is 1.8m high with a 600mm external floppy overhang and a 300mm buried skirt, enclosing an area of 97-ha

(Muhic *et al.* 2012). Inside the pintji is a discrete granite outcrop (~17ha) with multi-entrance caves, deep fissures and an abundance of fig (*Ficus brachypoda*) and spearbush (*Pandorea doratoxylon*) plants. The outcrop is surrounded by open mulga woodland (*Acacia aneura*) with *Aristida spp.* and *Triodia spp.* grasslands.

Following fence closure, two sessions of three nights spotlighting followed by diurnal track monitoring walks of the fence perimeter (inside and out) were conducted with no introduced predator presence or incursion detected (J Read, pers. comm.). Attempts were made to remove euros (*Macropus robustus*) and European rabbits (*Oryctolagus cuniculus*) (suspected warru competitors: Geelen (1999), Ruykys (2011)) from inside the enclosure. It was estimated that two to three adult euros and a number of rabbits still remained at the time of warru release but these were removed by September 2012. Anangu Warru Rangers reported that two perentie (*Varanus giganteus*) were inside the pintji fence and a wedge-tailed eagle (*Aquila audax*) nest was located ~1km from the pintji site, both of which are potential native predators of warru (Read and Ward, 2011). Daily intensive track monitoring and spotlight survey methods were conducted in the ten days prior to the first release to ensure there had been no introduced predator incursions. Three 8-L chicken water feeders (Horsley Wholesale, Australia) monitored by remote cameras (Scoutguard KG680V, Faunatech Austbat, Australia) were installed prior to the first release (ref. Ch. 4 for full specifications of water points and cameras). This was increased to five water points for subsequent releases (Figure 2.1).

2.5.3 *Release protocol and group composition*

In order to maintain the genetic segregation of the captive breeding groups, it was determined that only New Well/Alalka captive warru would initially be reintroduced to the warru pintji. A total of 16 captive warru (8 male, 8 female) were released into the pintji between March 2011 and July 2012 in three release groups (Table 2.1). The composition and size of each release group was determined by age (>12months), genetic representation within the retained captive population and temperament (hand raised warru (n = 3) were too tame and thus considered unsuitable for release). In addition, the first release used only captive born individuals, to test the suitability of the release site without the risk of losing captive founder genetics.

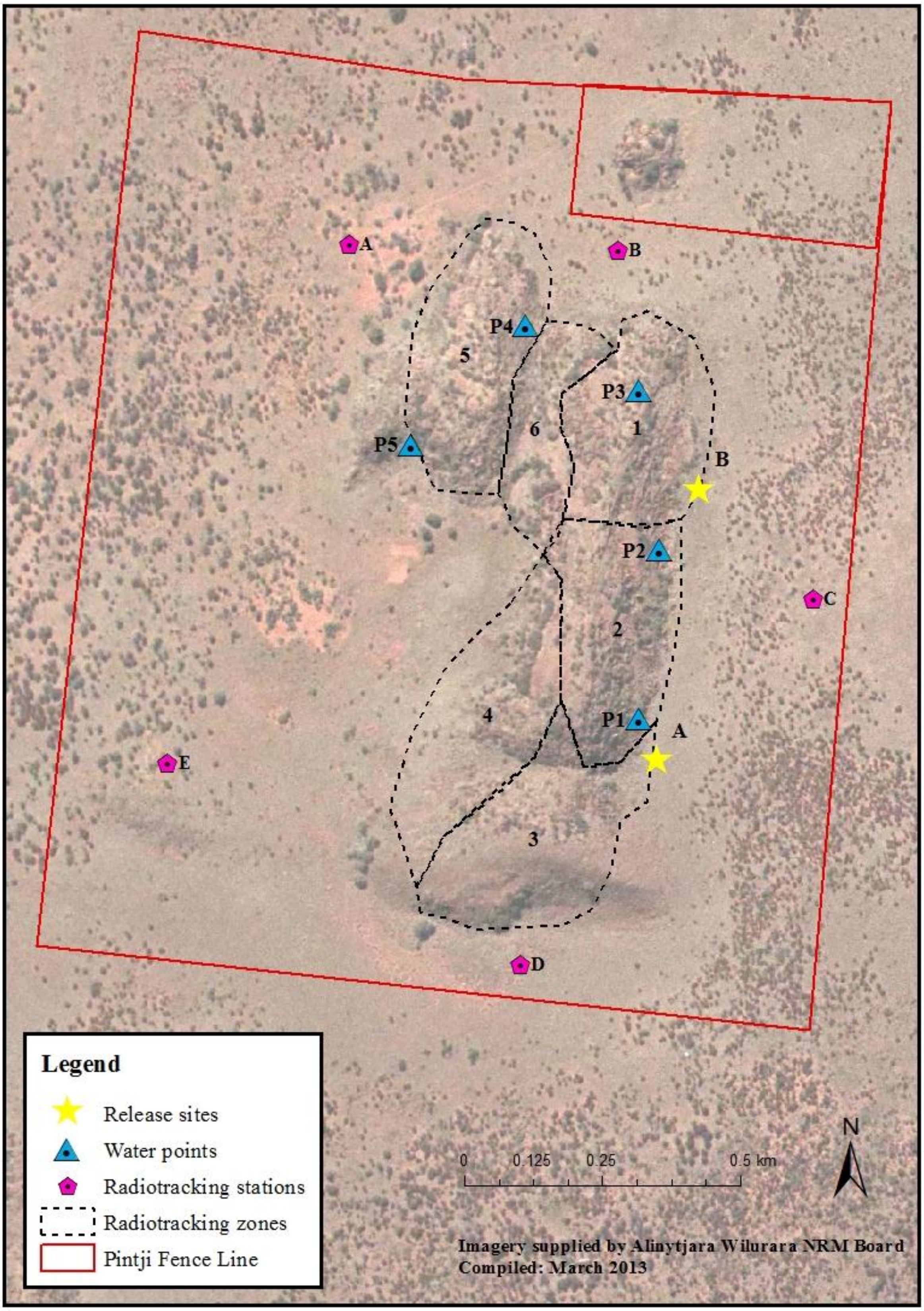
In the week prior to release, all warru underwent a thorough medical examination by zoo veterinarians to confirm suitability of health for release. This included taking a blood sample to provide baseline biochemical values for later comparison. During the health check, warru were fitted with either a Global Positioning System (GPS) or VHF radio collar (Sirtrack, New Zealand) (ref. Ch. 3 for specifications). Black and white heat-shrink tubing was used to uniquely mark the antenna of each collar to enable individual identification via infrared remote camera. Some individuals were also fitted with combinations of black or white ear-tags (males-right ear, females–left ear) to further assist identification. All warru were also microchipped (Trovan Ltd.).

On the morning of the release day, captive warru were captured from their zoo pens and given an intramuscular injection of vitamin E at 0.02ml kg^{-1}, in an attempt to reduce capture myopathy (Vogelnest and Woods 2008). Warru were then placed individually into pet packs lined with straw, driven to the airport (~one hour) and transported to the APY Lands by aeroplane, a journey of approximately five hours. Warru were released into the pintji at dusk from two release sites at the base of the hill (Table 2.1 and Figure 2.1).

Table 2.1. Sex, age and weight of captive warru (black-footed rock-wallaby, *Petrogale lateralis* MacDonnell Ranges race) at the time of soft-release to a 97-ha predator exclosure on the APY Lands, SA. Date of release and site of release (A or B – see Figure 2.1) are indicated.

Warru ID	Sex	Release age (years)	Release weight (kg)	Release date	Release site
Marura	M	1.3	2.9	March 29 2011	A
Itunpa	M	1.9	3.6	March 29 2011	A
Itatura	M	1.8	2.6	March 29 2011	A
Ngangala	F	1.5	2	March 29 2011	A
Tjalpu Tjalpu	F	1.7	2.3	March 29 2011	A
Ula	M	4.6	4.7	July 25 2011	B
Kalinya	F	3.6	3.6	July 25 2011	B
Aliyan	F	4.3	2.6	July 25 2011	B
Minnie	F	4.3	3.5	July 25 2011	B
Mingkiri	M	4.3	3.7	July 25 2011	B
Kupinya	M	3.4	4	July 25 2011	B
Tiltin	F	5	3.7	July 16 2012	B
Sandy	F	5	3.7	July 16 2012	B
Kaanka	F	1.1	1.5	July 16 2012	B
Nyi Nyi	M	5	5	July 16 2012	B
Puti	M	4.2	3.9	July 16 2012	B

Figure 2.1. Map of the warru pintji, the 97-ha predator exclosure on the APY Lands, SA, showing the release sites (A and B), water points (P1 – P5), radiotracking stations (A – E) and radiotracking zones (1 – 6).



2.5.4 Monitoring protocol post-release

2.5.4.1 *Radiotracking*

The VHF signal for both radio collar types (GPS and VHF) was programmed to latch into a mortality mode if no movement was detected for 12 hours (indicated by a doubling in pulse rate of the signal). Warru were radiotracked daily for the first two weeks post-release, then subsequently at weekly intervals for the 24-month study period. All radiotracking post-release was conducted by Anangu Warru Rangers. Radiotracking took place at five stations around the pintji hill (Figure 2.1; A – E). Warru Rangers recorded whether or not the signal was heard and recorded an approximate location of each signal within one of six determined zones (Figure 2.1). If a mortality signal was detected, the warru was immediately located, the body retrieved and if sufficiently fresh, driven to Alice Springs (~425km drive) for a post-mortem examination with a veterinarian.

2.5.4.2 *Trapping*

Warru were trapped on five occasions after the first release in March 2011, in accordance with the WRT standard operating procedure (West and Ward 2012). Trapping occurred in July 2011, February 2012, May 2012, October 2012 and March 2013. Warru were trapped in cage traps consisting of a shade cloth bag supported by a galvanised steel rod frame (Thomas Traps, Sheffield Wire Products, WA). Traps were located in caves and crevices across the pintji hill where warru scat was found, with the number of traps increasing following each release. A total of 21 traps were in place by the October 2012 and March 2013 trapping sessions. Traps were free-fed for three nights prior to trapping with a combination of peanut-butter-oat balls, chopped apples, kangaroo pellets and lucerne hay. Traps were then set for a maximum of four nights and cleared at dawn (number of nights varied from two to four due to weather conditions and Warru Ranger availability).

Captured warru were removed from traps and placed into a hessian sack or cotton processing bag. On first capture, warru were administered with an intramuscular injection of vitamin E at 0.02ml kg^{-1} to reduce capture myopathy (Vogelnest and Woods 2008). New warru were marked with a microchip, sampled via an ear biopsy for genetic analysis, fitted with an ear-tag, and when body mass was ≥2kg, fitted with a radio collar (ref. Ch. 3 for specifications). The following measurements were recorded for all warru: weight, head-length, pes length, tail length, and male scrotal diameter or female pouch status (where PY were present, a head-length and sex were recorded).

In October 2012 and March 2013, 1ml blood samples were taken from the lateral tail vein of all adult warru, placed in to 1ml EDTA tubes, centrifuged in the field and the resultant plasma separated and frozen for analysis.

2.5.4.3 *Remote camera monitoring*

Images from the water point remote cameras were downloaded quarterly. For the purposes of this study, the photographs were analysed to determine the presence of individual warru at each water point, using the unique radio antenna and ear-tag identifiers (analysis of drinking behaviour is presented in Ch. 4). This enabled a ‘last seen on camera’ date to be logged for all warru between trapping sessions, should they not be heard via radiotracking or later trapped. The photographs were also used to note the condition of warru, and the reproductive status of females (presence of bulging pouches or young-at-foot (YAF)), and to record the presence of independent unmarked warru.

2.5.5 *Survival and mortality post-release*

The number of weeks that each warru survived post-release was calculated from a combination of radiotracking, remote camera and/or trapping records. For each warru that died, these data, in addition to field signs, were used to determine a possible cause of death if a conclusive post-mortem was not likely. For warru still known to be alive at the end of this study their survival time equalled their time since release. A Kaplan-Meier survival analysis (Kaplan and Meier 1958; Pollock *et al.* 1989) was conducted (IBM SPSS Statistics ver. 19, 2010 (SPSS)) to determine the overall survival curve of warru post-release.

The sex of the warru that died post-release was compared. Separate logistic regressions in SPSS were used to examine the influence of both age and weight at release on the subsequent survival of warru.

2.5.6 *Condition, male maturity and nutritional health*

2.5.6.1 *Condition*

Differences in the body mass of warru at each trapping session, in comparison to their pre-release body mass, were used as a measure of maintenance of body condition post-release.

2.5.6.2 *Male maturity*

Changes in male scrotal diameter were compared between trapping sessions. Previous studies of *P. lateralis* have noted a mean scrotal diameter of 37.2 ± 2.6mm for sexually mature adult males (Jones 2001), so these values were used to determine the maturation of released sub-adult males.

2.5.6.3 *Nutritional health*

Biochemical analyses of blood samples were performed at the Adelaide Zoo Animal Health Centre. Roche Diagnostic kits and a Cobas Bio analyser (Roche Diagnostics, Switzerland) were used to perform 11 chemistry and antioxidant assays of Ascorbic Acid (vitamin C), Retinol (vitamin A), alpha-tocopherol (vitamin E), total protein, albumin, urea, cholinesterase (ChE), FRAP (ferric reducing ability of plasma), TBARS (thiobarbituric acid reducing substances) and TEAC (trolox equivalent antioxidant capacity). An oxidative stress index (OSI) was then calculated as the ratio of TBARS to the sum of TEAC and FRAP to represent the antioxidant capacity (TEAC+FRAP) to mitigate cell membrane damage (TBARS) (Schultz *et al.* 2011). The OSI index was used as an indicator of how released individuals were coping with stress.

Biochemical parameters were analysed in three groups. As diet and water availability does not fluctuate seasonally for captive animals, all samples taken during zoo health checks were examined as one group, ‘pre-release’. Post–release samples were then divided into two groups in order to examine seasonal variation: post-release spring (collected during trapping October 2012) and post-release autumn (collected during trapping March 2013). Mean values (±s.d.) were calculated for each group and compared to wild reference values from the Musgrave Ranges populations (most proximal of the wild colonies) or if values for these warru populations were unavailable for some biochemical parameters, the reference values for Kalka in the Tomkinson Ranges were used (Ruykys *et al.* 2012).

Linear mixed-effects models were fitted using *R* (R Core Team 2013) and the packages *lme4* (Bates *et al.* 2013) and *languageR* (Baayen 2011) to examine differences between pre- and post-release values for ascorbic acid, albumin, urea, cholinesterase, FRAP and OSI. These values have previously been used to indicate nutritional health in *Petrogale* (Ruykys *et al.* 2012; Schultz *et al.* 2011). Retinol and alpha-tocopherol were not modelled due to a limited pre-release sample size (n = 2) and total protein was excluded due to a significant correlation with albumin (SPSS Pearson correlation *r* = 0.645, n = 34, *P*= <0.001). Linear mixed models (*lmer*) were fitted for each biochemical variable with SEASON (pre-, post-release spring or post-release autumn) and SEX as fixed effects, an interaction between SEX*SEASON and a random effect of ID to account for repeated measurements on the same individuals. Residual plots for each fitted model were examined for normality. The *lmer* model determines the significance of each fixed effect using Markov chain Monte Carlo (MCMC) simulations of the posterior distribution of each parameter. The *P* values of the MCMC simulations were evaluated using the pvals.fnc() of *languageR* (Baayen 2011; Baayen *et al.* 2008). The interaction term between SEX*SEASON was removed from the fitted models if not found to be a significant explanatory effect on a biochemical variable. Where SEASON was identified as a significant effect variable, post-hoc Tukey's all pair comparison tests were implemented using the *R* package *multcomp* (Hothorn *et al.* 2008).

2.5.7 Reproduction and population recruitment

2.5.7.1 *Female reproductive output*

Reproductive status of released and recruited female warru at each trapping session was used to determine the percentage of females breeding. A head-length (HL) was recorded for each PY and HL measurements were used to estimate an age in days using the revised exponential equation of Delaney and De'ath (1990), as outlined in Ruykys (2011):

$$Age = \frac{\ln(1 - HL\,/\,111.88)}{-0.0051} - \ 9.24$$

Age in days was then deducted from the trapping date to provide an estimated date of birth for each PY. Where an elongated, lactating teat was present, it was presumed that the female was nursing a YAF and thus was logged as a birth. Permanent pouch emergence (PPE) occurs at an average 176 days (range 155 – 194) in wild warru (Ruykys 2011) and thus 176 days was subtracted from the mother's trapping date to give an estimated date of birth for these unsighted young. Where female warru (n = 1) were not trapped, remote camera photographs were analysed for pouch bulging or presence of a YAF, however accurate age estimates could not be calculated from this information.

The birth dates of each PY were then used to calculate: 1) the number of months post-release before each female was reproductive and 2) a birthing schedule across the year. In addition a sex ratio of PY was calculated.

2.5.7.2 *Survival of pouch young and recruitment*

Fully-furred PY that were trapped with mothers were microchipped (Trovan Ltd.), ear biopsies were taken, and their survival to PPE was logged. Survival to PPE for all other PY was determined in one of three ways:

1) PY was caught as an independent juvenile (maternity was assigned from 14 microsatellite loci (R West, unpublished data) to match the age of the independent juvenile to the previous PY records of the mother)
2) Presence of an elongated lactating teat in the next trapping session
3) Presence of a YAF on remote camera with a female in the period between trapping events.

Mortality of PY was confirmed if the HL and age estimation for the PY that the mother was carrying in the next trapping session did not match the time required for the previously recorded PY to have been weaned. Recruitment of PY to the population was confirmed when individuals were trapped as independent juveniles or sub-adults.

2.5.8 *Population estimates and influence of monitoring method*

A known-to-be-alive (KTBA) estimate was calculated for each month post-release from the cumulative data of the three monitoring methods (trapping, remote cameras and radiotracking). The number of individuals logged each month by the separate methods was calculated and compared to the KTBA.

2.6 Results

2.6.1 *Survival and mortality*

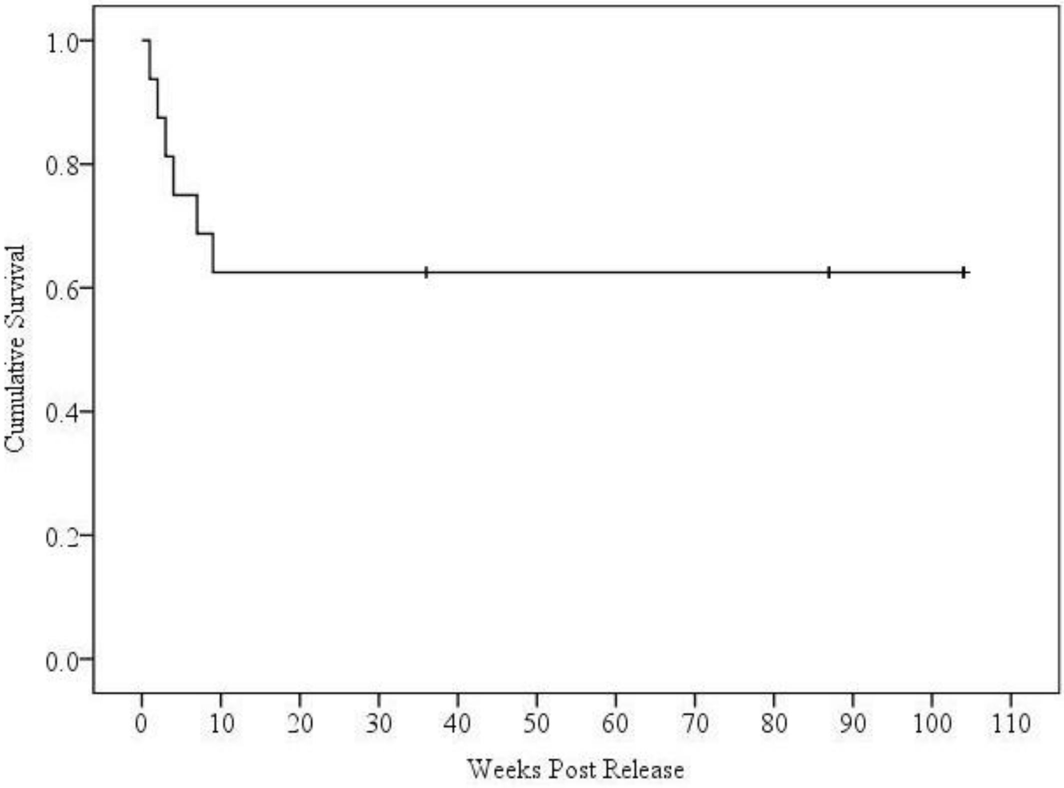
Of the 16 captive warru that were released into the pintji, ten were known to be alive at the conclusion of this study (March 2013), thus 63% of founding individuals had survived. Five corpses were retrieved during the study period, but were not recovered within a suitable time period to determine a definite cause of death (Table 2.2). The sixth warru (Mingkiri) was heard via radiotracking for 51 days post-release, after which the signal disappeared. Although a mortality signal was not detected through radiotracking, this male was also never seen on remote camera or trapped post-release, so is assumed to have died between the two radiotracking sessions (Table 2.2).

Table 2.2. Deaths of captive-reared warru (*Petrogale lateralis*) following soft-reintroduction to a fenced enclosure on the A<u>n</u>angu Pitjantjatjara Yankunytjatjara Lands of South Australia.

Warru	Release date	Circumstances	Approximate date of death	Weeks survived
Tjalpu Tjalpu	29/03/2011	Collar latched into mortality on first night after release, suggesting little movement. Last seen on camera 01/04/2011. Skeleton retrieved 10/07/2011.	7/04/2011	1
Mingkiri	25/07/2011	Last radiotracked 25/08/2011. Not radiotracked in subsequent session on 14/09/2011. Never trapped or detected on remote camera post release.	14/09/2011	7
Kupinya	25/07/2011	Determined from GPS collar fixes in same location. Remote cameras revealed deterioration in condition from 1 month post release with large fur loss on rump possibly due to fighting. Skeleton retrieved 17/10/2011.	29/09/2011	9
Kaanka	16/07/2012	Radio signal recorded in mortality 15 days after release. Skeleton retrieved 15/10/2012.	31/07/2012	2
Tiltin	16/07/2012	Last seen on remote camera 30/07/2012. Remote cameras indicated large loss of rump fur. Skeleton retrieved 15/10/2012.	7/08/2012	3
Puti	16/07/2012	GPS collar unable to record fixes from 14/08/2012. Skeleton retrieved from deep within a cave on 15/10/2012.	14/08/2012	4

All deaths occurred within nine weeks of release, as indicated in the Kaplan-Meier survival curve (Figure 2.2), which demonstrates a consistent survival rate after week ten until the termination of this study at week 104 (for individuals released in group 1), week 87 (for individuals in release group 2) and week 36 (for individuals in release group 3). Kaplan Meier survival analysis therefore estimated a mean survival time of 66.6 weeks (95% confidence interval [CI] 43.0, 90.1) during this study.

Figure 2.2. The Kaplan-Meier survival curve for captive warru following soft-reintroduction (n = 16). The three vertical bars on the line denote the number of weeks post-release that each release group was monitored as part of this study (group 1: 104 weeks; 2: 87 weeks; 3: 36 weeks).



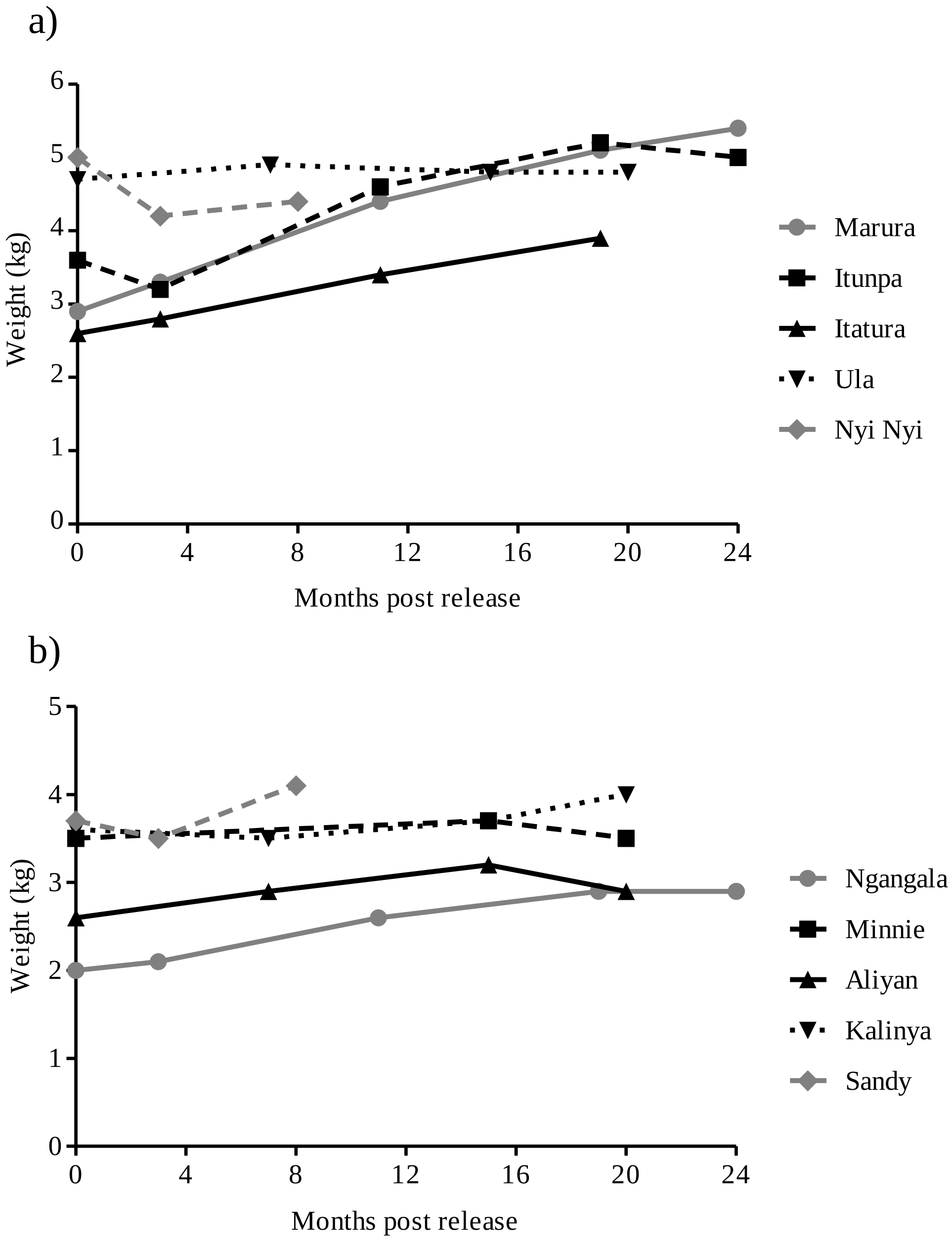
Survival was not sex biased (mortalities n = 6; 3 male, 3 female). Age at release did not predict survival (Odds ratio [OR] = 1.031, CI = 0.512, 2.074, *P* = 0.932) nor did weight at release (OR = 1.327, CI = 0.434, 4.061, *P* = 0.62).

2.6.2 Condition, male maturity and nutritional health

2.6.2.1 *Condition*

Variations in body mass post-release are displayed in Figure 2.3. Minor loss in body mass was noted in four warru in the first trapping session post-release (3 – 7 months post-release; mean -0.38 ± 0.3kg), but subsequent trapping sessions indicated their body mass was increasing (Figure 2.3).

Figure 2.3. Body mass of captive warru prior to soft-release (month 0) and at subsequent trapping sessions a) males b) females



2.6.2.2 *Male maturity*

Maturation of the released sub-adult males (Marura, Itunpa and Itatura) was evident in increases in scrotal diameters (15.1mm ± 0.26) post-release, while the mature adult males reintroduced in the second and third releases (Ula and Nyi Nyi) displayed little change in scrotal diameter (0.75mm ± 1.1).

2.6.2.3 *Nutritional health*

Pre- and post-release biochemical values fell within existing published wild reference ranges for the Musgrave Ranges (Ruykys 2011) (Table 2.3), except for pre-release and autumn cholinesterase levels. However, these values did fall within the separate reference range for the Kalka site in the Tomkinson Ranges (683 – 1,525 IU/L).

No significant interaction between SEASON*SEX was found for any of the biochemical parameters and so it was removed as a fixed effect from the linear mixed models. The final model fitted to each biochemical parameter was therefore SEASON (pre-release, spring 2012 and autumn 2013) and SEX as fixed effects with a random effect of ID. Residual plots for each model displayed normality. SEX did not have a significant explanatory effect on any of the biochemical variables. There was no significant effect of SEASON on ascorbic acid and FRAP values but SEASON did significantly explain variation in albumin, urea, cholinesterase and oxidative stress index (OSI) values and thus Tukey’s post-hoc multiple comparison tests were implemented for these four parameters (Table 2.3). Albumin and urea values were significantly higher pre-release than either of the observed post-release values, but there was no difference between the spring and autumn values (Table 2.3). Cholinesterase and OSI values were significantly higher pre-release than in the first sampling session in spring, but showed no significant difference to pre-release values in the subsequent autumn sampling period (Table 2.3). However, both cholinesterase and OSI indexes showed significant differences in the values between the spring and autumn sampling periods (Table 2.3).

Table 2.3. The effect of soft-reintroduction on the biochemistry of captive warru released to the APY Lands of South Australia (SA). Warru were sampled at Monarto Zoo, SA prior to reintroduction (pre-release) and then on two occasions post-release, October 2012 (spring) and March 2013 (autumn). Mean values are compared to reference values of wild warru in the Musgrave Ranges (Ruykys 2011). Linear mixed effects models were generated to examine differences between pre-and post-release values for six of the biochemical parameters (- = not modelled). Results of post-hoc Tukey contrasts are indicated by significance codes for adjusted *P* values * = 0.05, *** = <0.001 or NS = not significant.

Variable	**Pre-release**			**Post-release SPRING**			**Post-release AUTUMN**			**Wild reference interval**	**Statistical comparison**		
	n	**Mean**	**s.d.**	***n***	**Mean**	**s.d.**	***n***	**Mean**	**s.d.**		**Pre vs Spring**	**Pre vs Autumn**	**Autumn vs Spring**
Ascorbic acid (µM/L)	*5*	68.6	11.79	*10*	85.69	20.97	*10*	77.66	11.63	50.7 - 318.9	NS	NS	NS
Retinol (mg/L)	*2*	0.12	0.01	*10*	0.067	0.025	*10*	0.13	0.03	0.04 - 0.2	-	-	-
a- tocopherol (mg/L)	*2*	3.45	0.35	*10*	3.51	0.97	*10*	6.47	1.16	1.3 - 6.7	-	-	-
Total protein (g/L)	*14*	60.59	7.22	*10*	50.38	3.55	*10*	54.94	5.64	57 - 80	-	-	-
Albumin (g/L)	*14*	35.96	2.14	*10*	34.24	0.74	*10*	34.15	0.91	30.2 - 46.3	***	***	NS
Urea (mmol/L)	*14*	22.04	8.31	*10*	16.12	7.73	*10*	16.51	4.01	11.0 - 27.7	*	*	NS
Cholinesterase (IU/L)	*12*	788.42	222.07	*10*	1155.6	348.72	*10*	812.4	274.07	975 - 2,532	***	NS	***
FRAP (mM/L)	*12*	0.68	0.14	*10*	0.63	0.16	*10*	0.61	0.16	0.55 - 1.53	NS	NS	NS
TEAC (mmol/L)	*12*	2.53	0.05	*10*	2.4	0.06	*10*	2.46	0.004	2.25 - 2.51	-	-	-
TBARS (µmol/L)	*10*	2.65	1.27	*10*	0.77	0.26	*10*	2.03	0.56	0.04 - 3.19	-	-	-
Oxidative stress index	*10*	0.81	0.35	*10*	0.26	0.08	*10*	0.66	0.16	-	***	NS	***

a-tocopherol - alpha tocopherol; FRAP - ferric-reducing ability of plasma; TEAC - trolox equivalent antioxidant capacity; TBARS - thiobarbituric acid reducing substances

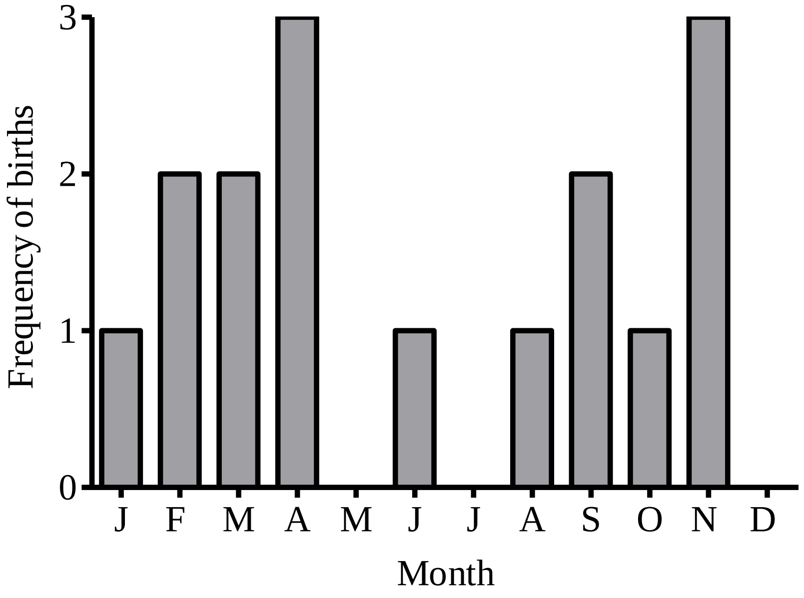
* *P* = 0.05, *** *P* = <0.001

2.6.3 Reproduction and recruitment

2.6.3.1 *Female reproductive output*

All females that survived more than nine weeks post-release gave birth within two to six months. Breeding of a newly recruited female at 11 months of age (Kuḻilypuru) was also documented. A total of 16 births were documented across the females in the warru pintji, with births occurring in each month of the year apart from May, July and December (Figure 2.4). The PY sex ratio was nine males, six females and one of unknown sex (this individual was not sexed as the ‘birth’ was only documented from an elongated teat present at a subsequent trapping session).

Figure 2.4. Monthly frequency of births for soft-reintroduced captive and recruited warru females between March 2011 and March 2013 (n = 16) on the APY Lands, SA.



2.6.3.2 *Survival of pouch young and recruitment*

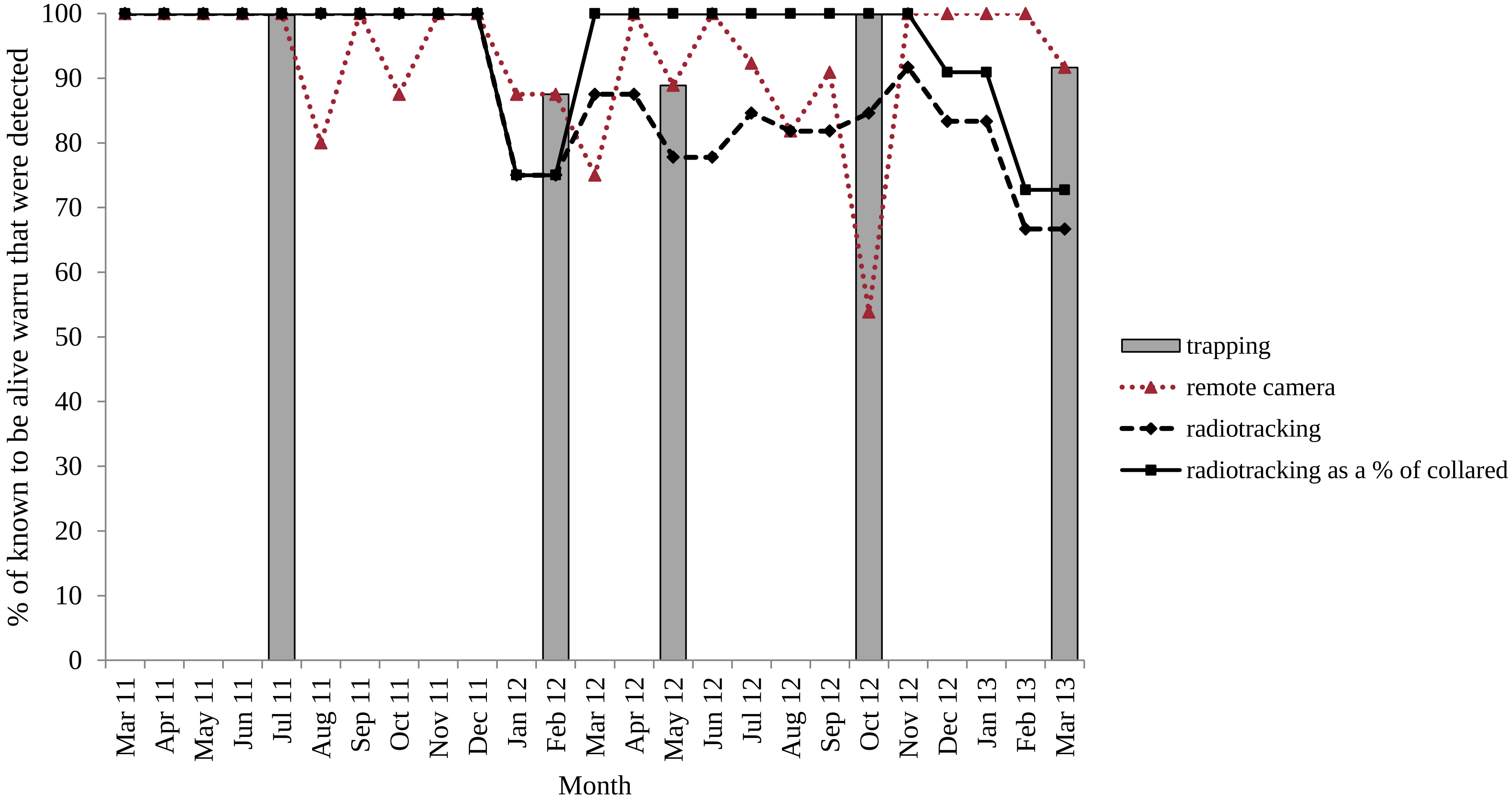
Excluding the PY from the final trapping session (n = 5), survival to PPE was calculated at 82%. Two PY mortalities were confirmed, as the HL of the PY the mother was carrying in the next trapping session did not correspond to the previously recorded PY. One PPE was confirmed by the presence of a YAF with the female on a remote camera in the period between trapping sessions. All other survivals were confirmed via the presence of elongated lactating teats or parentage analyses that matched independent juveniles to previous female trapping records (R West, unpublished data).

Three surviving PY were trapped as independent juveniles in 2012 (one male, two females), hence successfully recruited to the pintji population. One of the recruited females was also nursing a YAF in October 2012, although this female was found dead in early 2013 (survival of the YAF has not been confirmed). The cause of death was not determined. In addition, the final session of remote camera monitoring indicated the presence of two unmarked independent juveniles within the population, although they were not trapped in the final session for this study.

2.6.4 *Population estimates and influence of monitoring method*

Figure 2.5 displays the number of warru detected by each of the three methods as a percentage of the KTBA population estimate. The monthly KTBA estimate was based on marked individuals only and reflects the most recent known status of each individual. At the termination of this study the KTBA estimate was 13. This was based on those marked adults re-trapped in the final trapping session, the marking of one PY at PPE and one marked warru that was not trapped but later confirmed alive via remote camera. This estimate excluded the four PY observed in the final trapping session as they were not marked. No single monitoring method consistently detected 100% of marked individuals in any month (Figure 2.5).

Figure 2.5. The influence of monitoring method on the detection of known to be alive (marked) warru following soft-reintroduction to the APY Lands, SA. Two lines are provided for radiotracking. The solid line represents the number of individuals detected as a proportion of those collared, whilst the dashed line represents this as a proportion of the total population size.



2.7 **Discussion**

Detailed monitoring is required during any reintroduction attempt to determine if there is successful establishment of released individuals and can improve knowledge of a species' reintroduction biology (Seddon *et al.* 2007). In particular, the fate of released captive-reared animals is often difficult to track, hence this information is imperative for the adaptive management of the focal population and future releases (Runge 2013). The current study monitored the first soft-release attempt for warru into a predator exclosure on the APY Lands of SA. Survival, health and reproductive rates demonstrated that released warru were able to adapt to wild resources, and that there is potential for rapid population growth post-release.

2.7.1 *Survival post-release*

In the current study, 63% of captive warru survived translocation to the APY Lands. As in other *Petrogale* reintroductions, warru survival was not age-, weight- or sex-biased (Lapidge 2001; Soderquist 2011). Releases of captive and wild *Petrogale penicillata* have experienced survival rates of between 40 and 100% (Soderquist 2011). Some of the deaths in these translocations have been attributed to direct predation, while others have been from causes such as rock falls and trap-related deaths (Soderquist, 2011; D. Taggart, pers. comm.). A definitive cause of death was not determined for any of the mortalities recorded in the current study. While this is disappointing in terms of learning opportunities for adaptive management, it would appear to be a common problem in reintroduction programmes and particularly compounded by remote locations. In a review of over 100 translocations, 90-100% of animal losses were from unknown causes with retrieval of specimens for necropsy uncommon (Wolf *et al.* 1996). Similarly, due to the state of decomposition, a conclusive post-mortem could not be conducted for 67 % of the carcasses retrieved during reintroductions of *Petrogale xanthopus* (Lapidge 2001). Given the absence of introduced predator pressure within the warru pintji (continued track monitoring failed to detect any incursions within the fence), the early occurrence of the deaths within the first nine weeks post-release, and subsequent stabilisation of the survival rate of released adults, suggests that the deaths of these captive warru are likely related to translocation-induced stress, although predation by native predators (wedge-tailed eagles and perentie) cannot be completely ruled out. However, translocation stress can significantly increase the vulnerability of released individuals to mortality factors and decrease their ability to adapt to wild conditions (Dickens *et al.* 2010), hence acting as an ultimate cause of death post-release. Translocation has been shown to alter the stress physiology response of the chukar partridge (*Alectoris chukar*) (Dickens *et al.* 2009) and stress is often implicated as a causal agent in the failure of reintroduction programs (Teixeira *et al.* 2007).

In addition to the cumulative effects of stress induced by capture, medical examination, transport and release, and having to adapt to a new environment, interactions with warru from previous releases may also have affected mortality, particularly for supplemented individuals. Male warru were housed separately at Monarto Zoo and whilst females were housed in groups of two or three, these were not necessarily familiar groups prior to release. Therefore, for some warru, release into the pintji was their first encounter with conspecifics. It has been proposed that the introduction of naive individuals to established groups can disrupt social patterns and thus increase mortality (Kleiman 1989). Remote camera images revealed loss of rump fur (indicative of fighting) and poor condition of two of the warru just prior to death. Whilst reintroductions of *Petrogale penicillata* have demonstrated rapid integration of naive individuals into native groups (Soderquist 2011), staff at Monarto Zoo have reported considerably more aggressive behaviours in warru than captive *P. penicillata*. As the current data are limiting, further supplementations should be conducted and monitored in order to determine the effectiveness of supplementation as a strategy to increase population size within the warru pintji, particularly with increasing population density.

2.7.2 _Condition and nutritional health post-release_

New environments pose many survival challenges and pressures for released captive individuals (Kleiman *et al.* 1994; Teixeira *et al.* 2007), the most immediate being that they must locate appropriate food resources within their new habitat (Mathews *et al.* 2005). The capability of released animals to do this can be affected by their adaptability (Mathews *et al.* 2005) and the quality of habitat at the release site (Cheyne 2006). An assessment of the ability of captive animals to adapt to wild conditions can be made through comparison of condition and nutritional health at pre- and post-release stages. Captive warru were examined by veterinarians at Monarto Zoo, and using a combination of physical and pathological examinations, all selected individuals were deemed of suitable health for release.

The current study found that surviving captive warru maintained or increased their pre-release condition with only four demonstrating a temporary reduction in body mass following soft-reintroduction. The increases in scrotal diameters of the immature sub-adult males matched those reported in wild male *Petrogale lateralis* (Jones 2001), showing that reintroduction as sub-adults did not affect sexual maturation.

Warru also maintained nutritional health post-release based on the 11 biochemical parameters measured. Both captive and post-release values were within established wild reference ranges (Ruykys *et al.* 2012) with no severe contraindications (B Rich, pers. comm.). Although it is well-documented that biochemistry parameters can be heavily influenced by a number of non-dietary variables, including reproduction, weather and age (Stirrat 2003), cautious interpretation can enable them to be used to assess nutrition (Robert and Schwanz 2013). Plasma albumin levels were indicative of adequate protein intake (Schultz *et al.* 2011) and although these decreased post-release, this most likely reflects the higher protein concentration of pellets consumed in captivity (Lapidge 2001). Ascorbic acid (Vitamin C) and alpha-tocopherol (vitamin E) levels, presumed to be biosynthesised by *Petrogale* (Schultz *et al.* 2011), were maintained post-release as were levels of FRAP, which is absorbed from vegetation and contributes to the antioxidant capacity of plasma (Ruykys *et al.* 2012). The cholinesterase (ChE) levels observed in post-release warru were not indicative of infection or liver malfunction (Schultz *et al.* 2011). However, the values observed in autumn were below the reference range for the Musgrave Ranges populations and similar to wild values taken at Kalka in the Tomkinson Ranges, which was suggested to be because Kalka warru were consuming plants containing ChE inhibitors (Ruykys *et al.* 2012). However, seasonal variation in ChE levels (this study and Schultz *et al.* (2011)), suggests this requires further investigation.

The significant decrease in urea concentrations post-release demonstrates that reintroduced warru had adequate water intake and a good plane of nutrition, as urea levels increase during periods of dehydration or starvation (Lapidge 2001; Ruykys *et al.* 2012). It must be noted that although there is no permanent water source within the warru pintji, water was provided quarterly during the study period (ref. Ch. 4) and therefore may have assisted adaptation to fluctuating water availability. Finally, oxidative stress indices below 1.0 indicate that reintroduced warru were coping with the levels of stressors and conditions within the warru pintji (Schultz *et al.* 2011).

Warru that survived translocation were clearly able to adapt to a wild diet, and must have located appropriate and sufficient food resources to enable them to maintain body mass, and not experience under-nutrition (Stirrat 2003). The current study also shows that the warru pintji provided a nutritionally adequate environment, providing support for the methods used to select it as a release site (Ward *et al.* 2010). Effective transition of captive-bred animals to a wild diet within several months of release has been documented in *P. x. xanthopus* reintroduced to the Flinders Ranges, whose dietary composition reflected wild counterparts just two to four months post-release (Lapidge 2000). This transition was even more rapid in captive *P. penicillata*, that demonstrated a dietary switch within 36 hours of release (Soderquist 2011). The body mass and nutritional profiles observed would support similar timeframes of dietary adaption for captive warru, although dietary composition post-release was not directly assessed in the current study.

2.7.3 *Fecundity and recruitment post-release*

The swift adaptation of captive warru to their new environment was also demonstrated by almost immediate conception in some of the females and high female reproductive rates throughout the study period. Of the surviving females, three gave birth within two months of release, with the other two females giving birth within three and six months post-release. A total of 16 births (including a birth from a recruited female) were documented across the study period with births occurring in all months of the year except December and May. The birth rates and frequencies seen in this study are similar to those observed in wild warru populations where female breeding rates are documented between 75 and 96%, with births occurring throughout the year (Ward *et al.* 2011a). Rates of survival of PY to PPE were also high (82%) and similar to rates of PPE documented in wild *P. penicillata* (Wynd *et al.* 2006). Body condition of females has been found to influence the reproductive success and survival of *P. penicillata* PY (Wynd *et al.* 2006). The PPE rates observed in this study therefore provide further evidence for the successful adaptation of captive females to a wild diet, enabling good condition to be maintained post-release.

While the reproductive rates observed in this study suggest released populations of captive warru have the potential to grow rapidly, there remained a discrepancy between the number of observed PY and the number of individuals observed to have successfully recruited into the population. Only three independent juveniles were captured and marked during trapping, suggesting a 73% mortality rate after PPE. This period is documented to be the most vulnerable for juvenile mortality in other macropod species with causes of death often related to predation (Banks *et al.* 2000). In the wild, the majority of warru predation pressure is presumed to come from introduced predators (foxes, *Vulpes vulpes* and cats, *Felis catus*) and wild dogs (dingoes mixed with dogs) with native predators such as wedge-tailed eagles (*Aquila audax*), pythons and large goannas (such as Perentie, *Varanus giganteus*) likely to be occasional predators of warru but with minimal impact to population growth (Read and Ward 2011). Whilst foxes, cats and dogs are absent from within the warru pintji, rangers estimate that there are three large ‘ngintaka’ (Perentie) inside the enclosure (E Dagg, pers. comm.). As *Varanus* diet is known to consist of small-medium sized mammals (James *et al.* 1992) and prey species such as rabbits have been removed from within the enclosure (APYLM, pers. comm.), it is possible that these goannas could pose a threat to recruitment of the warru pintji population (D Pearson, pers. comm.). Removal of these individuals and subsequent monitoring of juvenile recruitment may assist in addressing this question.

An alternative explanation is that juveniles are more trap-shy and less likely to be detected during trapping. At least two unmarked independent juveniles were observed on remote camera but not subsequently trapped. However, as low juvenile survival rates are proposed to be limiting population growth in wild colonies (Ward *et al.* 2011a), continued monitoring will be required within the warru pintji to better understand these post-release population dynamics. This will be important for confirming successful recruitment and population growth prior to removing groups for future releases.

2.7.4 *Monitoring methods and population estimates post-release*

Comparative population estimates from the multiple monitoring methods used in this study suggest that no single method provided an effective estimate. Indeed, a review of macropod translocations found that most programs used two or more methods of post-release monitoring with trapping and radiotracking most popular (Clayton 2011). While radiotracking and trapping provided good estimates of the KTBA population estimate in the initial stages of this warru reintroduction, they both also presented issues. Poor trapping conditions (high winds and/or heavy rainfall) can result in low trap success rate which potentially leads to underestimates of juvenile recruitment. Collar failures and the inability to collar all individuals (due to percentage bodyweight restrictions for animal ethics compliance and the need to trap new recruits in order to fit collars) can also lead to underestimates of population status through radiotracking. Remote photography is a rapidly developing field in ecology (Kucera and Barrett 2011) but has been used rarely as a monitoring technique in previous translocations (see review in Clayton 2011). The current study found that remote cameras placed at water points were a useful tool to supplement radiotracking and trapping data, when these methods did not identify all known individuals. In addition, the remote cameras provided information on body condition, reproductive status and the presence of YAF, information that would otherwise have been unavailable.

2.7.5 *Future monitoring for adaptive management*

A successful reintroduction will meet three key objectives: 1) survival of the release generation; 2) breeding of the release generation and their offspring; and 3) persistence of the established population (Short 2009). The reintroduction biology data gained in the current study suggests that the soft-release of captive warru has met the first objective and is progressing towards meeting the second objective. As the aim of the soft-reintroduction is to create a source population for future reintroductions of warru, monitoring now needs to focus on determining the population growth rate and the influencing factors. It is therefore recommended that biannual trapping and remote camera monitoring be prioritised in order to monitor progress towards objective three. It is also important to note that density dependent habitat quality has been identified as a cause of failure for other programs using predator exclosures (see review of Clayton 2011). As the warru pintji also restricts the area of habitat available to warru, continued monitoring will be necessary to determine carrying capacity effects on the growth of the warru pintji population (Read and Ward 2011).

The next stage of reintroduction will involve the transfer of groups from within the pintji to unfenced sites on the APY Lands (hard-release), where warru will experience introduced predator and competitor pressure in addition to potential variations in habitat quality. Monitoring of hard-released animals, to ascertain factors preventing successful establishment, recruitment and dispersal, is listed as a high priority research objective within the National *Petrogale* Recovery Plan (Pearson 2012). This is because an understanding of the responses of released individuals under varying conditions and management strategies is key to implementing active adaptive management, which can maximise the probability of reintroduction success (Armstrong and Seddon 2008) (Converse *et al.* 2013; Runge 2013). The results of the current study can be used as a valuable baseline, from which to compare the response of warru in future hard-releases and/or supplementations to the warru pintji. Analysis of repeated releases could facilitate identification of the predictive variables for survival of translocated individuals (Barnes *et al.* 2008; Mathews *et al.* 2006). In the case of warru, the comparative success of hard- and soft-releases will determine the most appropriate reintroduction technique for their conservation on the APY Lands.

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CHAPTER 3: TURNING WILD - HABITAT SELECTION AND HOME RANGE BEHAVIOURS OF REINTRODUCED CAPTIVE WARRU.

A captive warru fitted with a GPS collar, shortly after release into the warru pintji.
Photo: L Olds

3.1 <u>Preamble</u>

Using data from Global Positioning System collars, this chapter examines the habitat selection and home range behaviours of captive warru following soft-release into the 97-ha enclosure on the APY Lands. To determine whether these behaviours represented adaptability to the wild, the temporal and spatial home range characteristics of reintroduced warru were compared to the home range behaviours of a remnant warru population. These behaviours were also used to assess the suitability of the selection criteria used to choose the release site.

3.2 Statement of Authorship

Title of paper	**Turning wild - habitat selection and home range behaviours of reintroduced captive warru.**							
Publication status	☐	Published	☐	Accepted for Publication	☐	Submitted for Publication	☒	Publication style

Author contributions
By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author	**Rebecca West**		
Contribution to the paper	Designed, developed and implemented the study. Collected data and conducted all analyses. Wrote manuscript.		
Signature		Date	24/10/2013

Name of Co-Author	**Matthew Ward**		
Contribution to the paper	Supervised design/development of work. Commented on a draft manuscript.		
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Contribution to the paper	Supervised development of work and commented on a draft manuscript.		
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Contribution to the paper	Supervised development of work and commented on a draft manuscript.		
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3.3 Abstract

Habitat selection and home range behaviours drive species persistence because they directly impact survival. The development of these ecologically adaptive behaviours are therefore important in reintroduction programmes, particularly those using captive founders, because they play a major role in the establishment of the release group and hence the success of this recovery method. The current study used Global Positioning System collars to examine the habitat selection and home range behaviours of captive warru (black-footed rock-wallaby, *Petrogale lateralis* MacDonnell Ranges race, n = 7) that were reintroduced into a 97-ha predator exclosure, on the An̲angu Pitjantjatjara Yankunytjatjara (APY) Lands of South Australia. To assess whether these behaviours demonstrated adaptation to wild-type habitat selection, a concurrent study of the temporal and spatial characteristics of the home ranges of warru in a remnant (‘wild’) population was conducted (n = 7). Reintroduced warru quickly adapted to using the available rocky outcrops and did not seek habitat similar to the low scrub in their captive enclosures. As with wild warru, core areas were centred on the rocky outcrop and few fixes were located on the flat grasslands surrounding each site. Wild warru used mean home range areas of 22.8 ± 11.8ha (90% kernel) and reintroduced warru used mean home range areas of 8.9 ± 5.9ha. In wild warru, male home ranges (34.5 ± 10.8ha) were larger than female home ranges (16.2 ± 5.9ha) and overlapped multiple females, supporting the genetic evidence for a polygamous mating system in this species. Similar overlap patterns in reintroduced warru home ranges shows promise for the establishment of a functioning social system and high reproductive output in reintroduced populations. Reintroduced warru displayed lower fidelity to their home range locations than wild warru, most likely due to exploratory behaviours in a new habitat but these did not lead to dispersal from the release site. Future monitoring will be required to determine the impact of increasing density within the exclosure on habitat selection and home range behaviours. The current study suggests that in the presence of effective introduced predator control, reintroduced captive warru are capable of recolonising the hill habitats of their former range across the APY Lands.

Keywords; home range, habitat selection, *Petrogale*, reintroduction

3.4 Introduction

In order for a species to persist, individuals must select high quality habitat that provides the resources critical for survival and reproduction (Caughley 1994; Hall *et al.* 1997). Failure to do this can result in an ‘ecological trap’ whereby selection of low-quality habitat leads to reductions in fitness and hence survival probability (Rantanen *et al.* 2010). Knowledge of habitat selection and utilisation behaviours are therefore critical to informing the management of threatened species (Finlayson *et al.* 2008; Norris 2004). Understanding these behaviours is also important for the translocation of individuals to novel habitats, as they play a major role in the likely establishment of the release group (Moehrenschlager and Macdonald 2003; Stamps and Swaisgood 2007).

Successful establishment of a reintroduced population is driven by the survival and reproduction of its founders. It is essential, therefore, that release sites provide the habitat conditions necessary for population growth, although assessment of these required conditions can be challenging (Osborne and Seddon 2012). Released individuals must settle in habitat near the release site, locate appropriate resources within the area and be able to interact with their conspecifics (Armstrong and Seddon 2008). For reintroductions using captive founders, this often requires individuals to learn a repertoire of behaviours they have not developed in captivity (Kleiman 1989). Adaptations to captivity can pose major obstacles to the establishment of a release group if individuals reject the habitat at the release site and instead search for areas similar to their captive enclosures, known as natal habitat preference induction (NHPI) (Stamps and Swaisgood 2007). This can be especially problematic if movement away from the release site is also sex biased. For example, 32% of reintroduced hare-wallabies (*Lagorchestes hirsutus* and *L. fasciatus*) left the release site within the first four weeks post-release, with all but one of those individuals being male (Hardman and Moro 2006). Reintroduction failure has regularly been attributed to the inability of animals to establish home ranges at the release site in good quality habitat, and/or travel long distances before settling (see review in Le Gouar *et al.* 2011).

Monitoring the habitat selection and home range behaviours of reintroduced individuals can be used to assess the ability of naive animals to develop behaviours akin to their wild counterparts, the timeframe in which this occurs, and thus the effectiveness of reintroduction as a conservation strategy (Kleiman 1989). Comparison of behaviours exhibited by reintroduced captive and wild bank voles (*Clethrionomys glareolus*) demonstrated that captive individuals failed to develop a number of key habitat selection characteristics, concluding that pre-release environmental training would be required in future releases to improve survival probabilities (Mathews *et al.* 2005). In contrast, the selection of dune habitat and high fidelity to burrows by reintroduced greater bilbies (*Macrotis lagotis*) was seen as evidence that captive bilbies could successfully recolonise parts of their former range in the absence of introduced predators (Moseby and O'Donnell 2003). Such observations can also be used as a proxy measure for the provision of suitable resources at the release site. Replicated releases of water voles (*Arvicola terrestris*) into different habitat types indicated that habitat quality strongly influenced post-release survival and subsequent population densities (Moorhouse *et al.* 2009). While these comparisons are exceptionally useful for refinement of reintroduction protocols, wildlife managers are often hampered by a lack of baseline data on the natural wild behaviours of the focal threatened species (Short 2009).

In the A<u>n</u>angu Pitjantjatjara Yankunytjatjara (APY) Lands of South Australia (SA) a recovery team is aiming to use reintroduction methods to re-establish populations of the black-footed rock-wallaby (*Petrogale lateralis* MacDonnell Ranges Race), known to traditional owners as ‘warru’ (Read and Ward 2011). The distribution of warru on the APY Lands has contracted dramatically in the past 80 years (recent surveys estimate <250 animals remain) (Ward *et al.* 2011) , and so warru are classified as ‘Endangered’ in SA (National Parks and Wildlife Act, 1972). A captive population at Monarto Zoo, SA was established between 2007 and 2009 from the three known remnant (‘wild’) populations in SA (Read and Ward 2011). In 2011, soft-releases of captive warru into a 97-ha enclosure (known as the ‘warru pintji’) on the APY Lands (Muhic *et al.* 2012) provided the opportunity to assess the habitat selection and home range establishment behaviours of captive warru prior to hard reintroduction. Knowledge of wild warru habitat behaviours was lacking, consisting of only one study of den site selection (Read and Ward 2007) and one home range dataset from a Global Positioning System (GPS) collar (Ruykys *et al.* 2011). Using GPS collars, the current study aimed to provide baseline data on the habitat selection and home range behaviours of wild warru to facilitate an assessment of the adaptability of captive warru to wild habitat post-release.

3.5 Methods

3.5.1 *Study sites*

The study was conducted at two sites on the APY Lands between July 2011 and May 2012. ‘Wild’ studies were conducted in a remnant warru population at New Well (26.080ºS, 132.215ºE), a ~590ha granitic hill with an elevation range of 624 – 830m, in the eastern Musgrave Ranges, SA. There is an abundance of fig (*Ficus brachypoda*) and spearbush (*Pandorea doratoxylon*) plants across the hill slopes and the hill is surrounded by open mulga woodland in combination with spinifex grasslands (Robinson *et al.* 2003). Warru are known to inhabit the granite cliff faces and boulder piles of the slopes and have been trapped annually at this site since 2005, with trapping records for 2012 providing a known-to-be-alive estimate of 33 individuals (West 2012).

Studies of the reintroduced population took place at the warru pintji (26.184ºS, 132.345ºE), the 97-ha enclosure into which captive warru were released, located ~17km south east of the New Well hill. The hill within the pintji is a discrete granite outcrop about ~17 ha in size with an elevation range of just 50m (649 to 699m). The pintji hill consists of multi-entrance caves, deep fissures and vegetation that reflects the assemblages seen at New Well (Ward *et al.* 2010).

The APY Lands experiences two main seasons: a cold, dry winter (March – October) and a warm, wet summer (Robinson *et al.* 2003). During the study period, mean maximum temperatures were lowest in July 2011 (18.2 ºC) and reached a peak of 34.4ºC in January 2012 (Bureau of Meteorology (BOM), Pukatja station #016097, ~18km from both sites). A total of 259mm of rainfall fell from October 2011 to April 2012, similar to the annual winter average (291.8mm, BOM records Pukatja, 1971-2000). During the study period, a concurrent supplementary water provision experiment was conducted (ref. Ch. 4), so 8-L water points were installed at New Well (n = 6) and in the warru pintji (n = 5). These water points were filled in August and November 2011, and Feb and May 2012 with water persisting for 2 – 4 weeks following each top up. The first group of captive warru (n = 5) were released into the warru pintji in March 2011 and a supplementation of six adults occurred at the commencement of this study in July 2011.

3.5.2 *Collar design*

GPS collars were used to study the habitat and home range behaviours of warru because they enable data to be gathered remotely which reduces site and hence animal disturbance. They are also capable of collecting larger, and potentially more accurate datasets than traditional VHF triangulation techniques which can be particularly restricted in poor weather conditions and in rocky habitats (Tomkiewicz *et al.* 2010). The temporal programming of GPS fix collection also permits an array of ecological questions to be addressed (Tomkiewicz *et al.* 2010) although designs need to reflect the trade-offs between fix frequency and collar longevity, versus the necessary battery power and collar weight. GPS collars used in the current study were manufactured by Sirtrack®, New Zealand. Two different transmitter designs were used to reflect differences in body mass between wild and captive warru, and the number and frequency of fixes required to assess behaviours at each site. University of Adelaide Ethics Committee approval (S-2011-9) was granted for a maximum collar weight of 3% of an individual's body mass.

The collar design for wild warru weighed 118g and emitted a VHF signal (not duty cycled) with a mortality latching mode that emitted a pulse double the rate of the normal VHF pulse if no movement was detected for 12 hours. To determine seasonal home ranges in wild warru, the GPS component operated on a duty cycle of 30 days on, 60 days off for 12 months. During each 30 day period, fixes were collected every three hours from 1900 to 1000 hours, with each location attempt lasting for a maximum of two minutes. As *Petrogale* typically shelter in rocky refuges during the day, diurnal fixes were avoided because such habitats significantly decrease the likelihood of obtaining a GPS fix and instead drain battery life during the attempt (Lewis *et al.* 2007). This enabled four x 30 day sessions of GPS fixes to be collected for wild warru across the study period with two datasets for each season (August 2011-winter; November 2011-summer; February 2012-summer and May 2012-winter).

To ensure as many of the released captive warru as possible could carry a GPS collar, a smaller battery and different program was used for the 'reintroduced' GPS collar. The reintroduced collar design weighed 96g and the GPS component was programmed to operate every alternate day, recording a location fix every three hours between 1700 and 0800 hours with a maximum searching time of two minutes. This produced an expected GPS lifespan of 180 days. The VHF component operated on a duty cycle of 0900 to 2100 hours each day and also incorporated a mortality latching mode.

3.5.3 *Collar deployment and retrieval*

As part of the Warru Recovery Team (WRT) annual trapping session, wild warru were trapped for four nights at New Well in July 2011, following three nights of free feeding, in concordance with the WRT standard operating procedure (West and Ward 2012). Traps were baited with a mixture of peanut butter, rolled oats and apples and checked at dawn each day. Captured warru were removed from the trap, placed in a hessian sack or cotton bag for handling and prior to processing, given an intramuscular injection of vitamin E at 0.02ml kg^{-1} to reduce capture myopathy (Vogelnest and Woods 2008). Ten GPS collars were fitted opportunistically to adult warru that met the minimum weight requirement of 3.9kg. Collars were fitted with two fingers spacing between the warru neck and collar band and were secured with a buckle fastening that was reinforced with a mini cable tie (West and Ward 2012). In total, six wild males and four wild females were fitted with GPS collars. VHF signals were monitored weekly by A_n_angu Warru Rangers and any collars found in mortality mode were tracked and retrieved. Remaining collars were removed during the July 2012 annual trapping session.

Of the captive warru released in March 2011, four were trapped within the warru pintji across three nights in July 2011 (ref Ch. 2). Despite attempts to decrease the weight of the GPS collars for reintroduced warru, only the three males were of a suitable weight for collaring (>3.2kg) (ref. Ch. 2). Five of the six captive warru released into the pintji on July 25th 2011 were also of suitable weight to be fitted with a GPS collar. These collars were fitted during pre-release veterinary health checks at Monarto Zoo and switched on at 1700 hours on the day of release. Processing and collar fitting was conducted as described above except the collars of reintroduced warru were fastened with a nylon nut and bolt to further reduce weight and secured with a small dot of superglue on the exposed bolt thread. This resulted in a total of six male and two female reintroduced warru fitted with GPS collars. As for wild warru, signals were monitored weekly by Warru Rangers to retrieve any collars found in mortality mode. Collars were then removed during two trapping trips in 2012 (February and October).

3.5.4 *Collar data screening*

For each fix, both GPS collar types recorded the following data; date and time of fix in UTC time (Coordinated Universal Time), latitude and longitude of the fix, a speed and heading (calculated as a vector of the previous location), the number of satellites used in the location calculation and a Horizontal Dilution Of Precision (HDOP) value. GPS fixes were downloaded from the collars in decimal degrees and transformed into UTM (Universal Transverse Mercator) coordinates. The date and time of each fix was converted into Australian Central Standard Time (UTC+9.5). The HDOP is a value between 0 and 99 which estimates the precision of the location based on the satellite geometry (Sirtrack 2010). Higher HDOP values have been associated with large location errors, therefore any locations with a HDOP>10 were removed from subsequent analyses (D'Eon and Delparte 2005). As collars were added opportunistically across multiple nights for each trapping session, any fixes collected during the trapping session were also discarded from the dataset as these points were unlikely to reflect normal habitat use.

3.5.5 *Data analysis*

3.5.5.1 Habitat selection

An aerial image of each site was projected in ArcMap10 (ESRI 2011) and a polygon created to delineate the perimeter of the hill. Location fixes were projected over the aerial image and the proportion of fixes occurring on and off the hill calculated and expressed as a percentage of the total number of fixes at each site. A Digital Elevation Model (DEM) was used to create a 3D projection of the hill at each site using the 3D Analyst Toolbox in ArcMap10. The elevation of each fix was then extracted from this layer and a mean and range of fix elevations calculated for each site.

3.5.5.2 Home Ranges

Calculation of home ranges

GPS collars from wild warru produced data in four discrete 30 day periods of fixes and thus four monthly home range estimates were calculated for each wild warru. For reintroduced warru, the 180 day data period was divided into 30 day blocks, enabling six ‘monthly’ home range estimates to be calculated for each reintroduced warru.

Kernel Density Estimate (KDE) methods were used to calculate monthly home range sizes as they are considered the most efficient, robust and unbiased estimates of home range (Borger *et al.* 2006; Hemson *et al.* 2005; Nilsen *et al.* 2008). Gaussian fixed kernels were generated using the Geospatial Modelling Environment (GME) ArcMap10 extension software (Beyer 2012) using the smooth-cross validation (SCV) algorithm of the *R* package '*ks*' (Duong 2013). Bandwidth smoothing was conducted using the SCV method as it produced the most appropriate kernels in comparison to the other available algorithms. The commonly used least-squares cross-validation (LSCV) method was rejected as it has been reported to fail with large data sets that display temporal and spatial autocorrelation (inherent features of GPS collar datasets) (Hemson *et al.* 2005). Percent isopleths were calculated for each kernel, also using GME, to conduct incremental area analyses (Kenward *et al.* 2001) for each 'monthly' dataset. Asymptotes were examined to assess the mean number of fixes required to determine a stable home range estimate. A 90% isopleth was selected as the indicator of home range area. A 95% area produced discontinuous isopleths and, although once commonly used as the indicator of home range, is not recommended due to increased bias in outlying points (Borger *et al.* 2006). Five percent incremental utilisation plots for each kernel for wild warru showed discontinuity in the line at 50% of fixes, suggesting this was an appropriate level at which to examine a core area (Kenward *et al.* 2001). This same core area was therefore also calculated within each monthly home range for reintroduced warru. Due to variation in the number of fixes collected by each collar in each monthly period, a Pearson's product-moment correlation in the program *R* (R Core Team 2013) was conducted to test for a relationship between the number of fixes collected per month and the home range size. Mean home range and core area sizes were then calculated for wild warru and for reintroduced warru, and sex differences in home range size determined.

Temporal home range characteristics

To examine whether wild warru exhibited seasonal variation in the size of the home range, a linear mixed effects model was fitted to the wild dataset using the package '*nlme*' (Pinheiro *et al.* 2013) in the program *R* (R Core Team 2013). In the model, SEASON (winter, summer), SEX and an interaction between SEASON*SEX were fitted as fixed effects. ID was fitted as a random effect to account for the repeated measures made on the same warru across multiple months. To determine if reintroduced warru demonstrated adjustment in home range size following release, a linear mixed effects model was fitted to the reintroduced warru dataset. MONTH, SEX and an interaction between MONTH*SEX were fitted as fixed effects, with a random effect of ID, to again account for repeated measures on each warru. Residual plots for each fitted model were examined for linearity, homoscedasticity and normality.

Spatial home range characteristics

To determine home range fidelity, the 'common area' of a home range and core area for each individual was calculated using the 'Intercept' tool within ArcToolbox. This 'common area' was the area consistently used by an individual in each monthly home range and was expressed as a percentage of their mean core or home range area across all months. Large changes in home range location would be expected to show lower percentage common areas. A one-way ANOVA in *R* was used to examine differences in fidelity to home range and core areas within each population and also between reintroduced and wild warru. To examine sociality, the degree to which each wild and reintroduced warru shared their home range with conspecifics was expressed as a percentage of the monthly 90% area that was overlapped by the 90% home ranges of all other warru. The same calculation was conducted to examine the degree of 50% core area sharing. Linear mixed effects models were then fitted to each dataset to examine the influence of SEASON (wild) or MONTH (reintroduced), SEX and an interaction between SEASON*SEX or MONTH*SEX as fixed effects. Again, ID was included as a random effect to account for the repeated monthly observations on each individual and residual plots were examined.

3.6 Results

3.6.1 *Collar retrieval and data screening*

For wild warru, eight out of the ten deployed collars were retrieved (four males, four females), five of which were retrieved through trapping in July 2012 and the remaining three through mortality of the individual. Of the retrieved collars, seven had successfully logged fixes for the duration of their deployment and one failed to record any fixes. This resulted in full data sets for four females and two males; with an additional male dataset for the August 2011 session (this male died in October 2011). On average, collars collected 59% of the programmed fixes across the tracking period, with 3.4% of the fixes having a HDOP value greater than 10. Missing fixes were predominantly at the programmed times of 7am and 10am and as a result, the data collected are considered to reflect nocturnal habitat selection and home range behaviours of warru. Data screening produced a mean of 100 location fixes for each wild warru per month. Incremental area analysis for each monthly kernel indicated range stabilisation at an average at 63 fixes (±16 SD) which equated to an average of 16 nights of data for each wild warru.

For reintroduced warru, seven out of eight collars were retrieved. Six collars were retrieved through trapping and one as a result of mortality. This resulted in full datasets for two females and four males with an additional male dataset for 40 days. None of the collars fitted to reintroduced warru ran for the programmed six months (average 168 days out of 180 days) and so to avoid underestimates of home range in the final 30 days, only the first 150 days, divided into five ‘monthly’ (30 day) blocks were used in analysis. Mean fix acquisition was 55% with 4% of these fixes having a HDOP value >10. As for wild warru, the last fix of the night (8am) was most likely to be missed and thus home ranges and habitat selection of reintroduced warru is also considered nocturnal. Following data screening, a mean (±SD) of 47± 9 fixes were available for analysis within each 30 day period. Incremental area analysis found a stable estimate of home range at 40 ± 10 fixes, although no range asymptote was found for three individuals in the first month. However, it was expected that reintroduced warru would make adjustments to the area used as they explored a novel environment and were therefore unlikely to produce stable home range estimates. As a result, despite a lack of asymptote for these three individuals, a monthly home range estimate (90% kernel) was calculated for each of the 30 day periods (n = 5) for all reintroduced warru.

3.6.2 *Habitat selection*

For wild warru, 91.8% of fixes were located within the delineated hill boundary. Of the fixes outside of the hill boundary, the majority (73%) were within 50m of the hill boundary. Wild warru GPS fixes were collected at a mean elevation (±SD) of 701 ± 38m. For reintroduced warru, 86% of GPS fixes were within the delineated hill boundary. The percentage of locations found on the hill did not increase considerably with time since release for reintroduced warru (month 1, 85%; month 5, 88%). Only 5 reintroduced warru fixes were located >50m from the hill base. Of these fixes, three were within 100m of the fence line, and all were collected from reintroduced male warru. The mean elevation (±SD) of the GPS fixes collected for reintroduced warru was 677 ± 8m.

3.6.3 *Home range areas and temporal variation*

Wild warru utilised mean (±SD) home range areas of 22.9 ± 11.8ha estimated with a 90% kernel and mean core areas (50% kernel) of 6.9 ± 4.3ha. There was no correlation between the number of fixes obtained per month and the overall home range size (90% kernel: $r = 0.010$, $P = 0.634$). There was no significant difference in the size of winter and summer home ranges ($F_{1,16} = 0.28$, $P = 0.60$) or core areas ($F_{1,16} = 0.05$, $P = 0.83$) for wild warru. Mean wild male home ranges of 34.5 ± 10.8ha were significantly larger than female home ranges of 16.2 ± 5.9ha ($F_{1,5} = 8.06$, $P = 0.03$) (Figure 3.1, Figure 3.2). Male core areas of 10.8 ± 4.6ha were also generally larger than female core areas of 4.7 ± 1.9ha but the difference between the means was not significant ($F_{1,5} = 5.1$, $P = 0.07$) (Figure 3.2, Figure 3.1).

Figure 3.1. Winter and summer home range (90% kernel) and core area (50% kernel) size for male and female wild warru (*Petrogale lateralis* MacDonnell Ranges race) at 'New Well' on the APY Lands, South Australia.

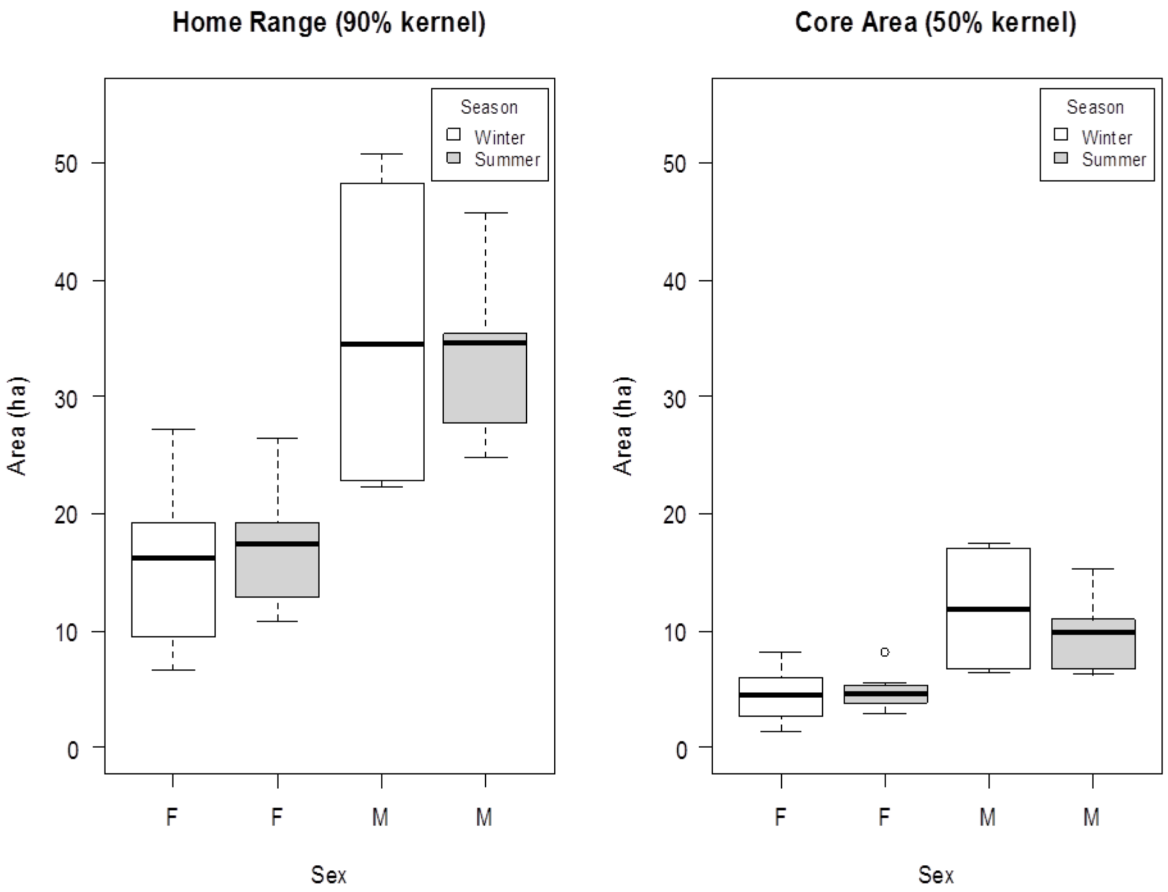
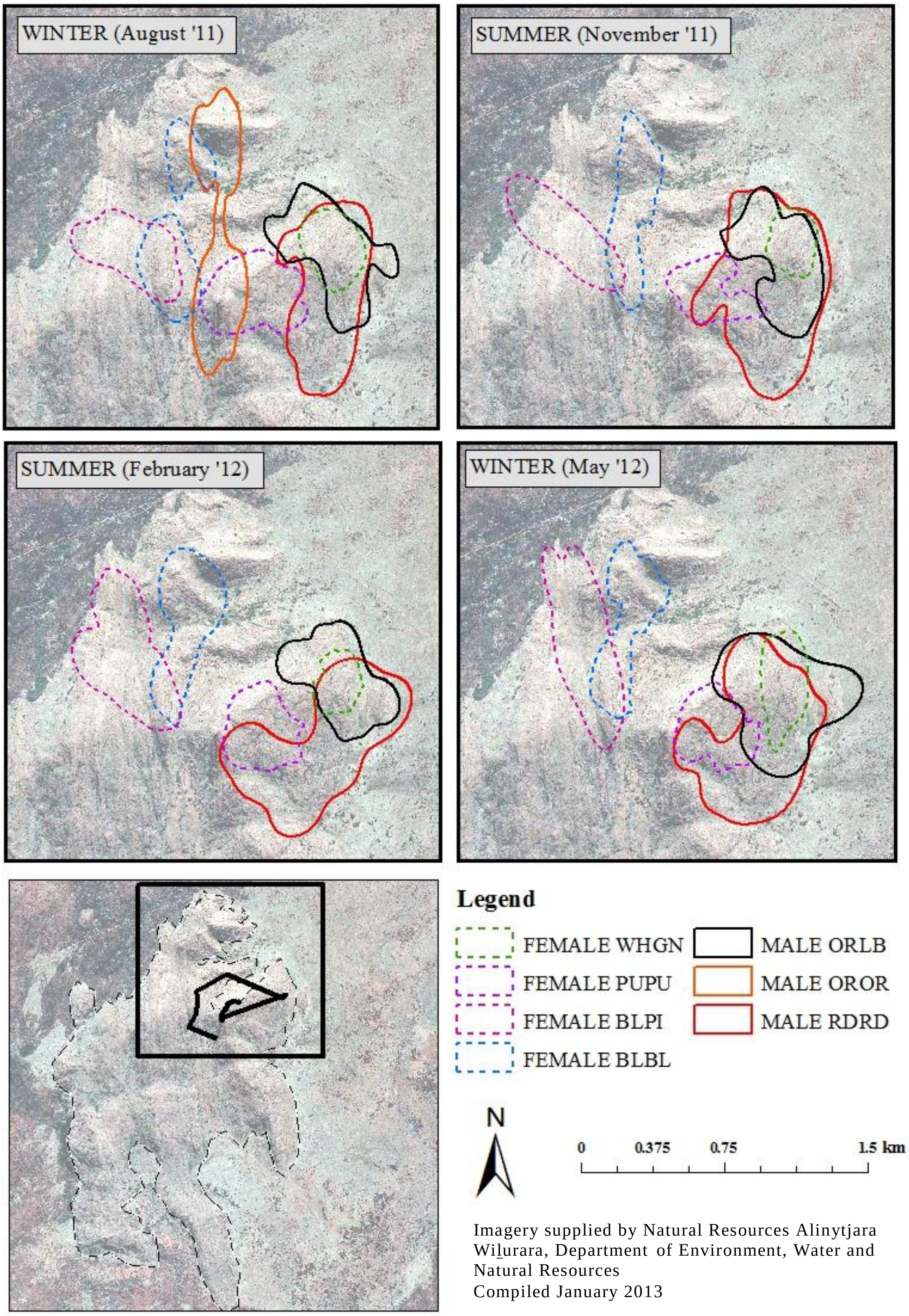


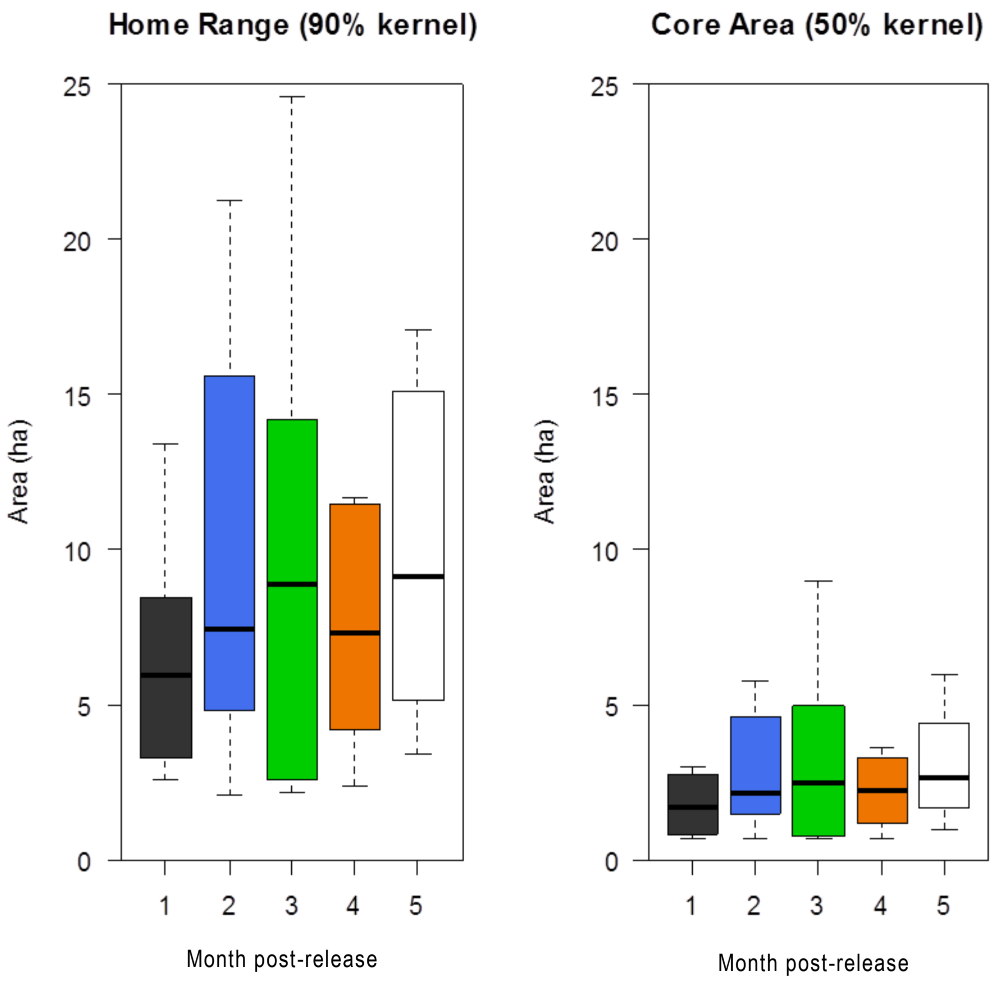
Figure 3.2. Seasonal home ranges (90% kernel), of wild male and female warru at ‘New Well’ on the APY Lands, SA. Bottom left map indicates broad location on the New Well hill with dashed line outlining the hill boundary and solid black line showing the annual trap line. Note: Male OROR died Oct’11.



Imagery supplied by Natural Resources Alinytjara Wilurara, Department of Environment, Water and Natural Resources
Compiled January 2013

Reintroduced warru utilised mean (±SD) home range areas of 8.9 ± 5.9ha and core areas of 2.6 ± 2.0ha. There was no relationship between the number of fixes per month and home range area for reintroduced warru (r = 0.196, P = 0.291). There was no effect of month on the size of home ranges ($F_{4,16}$ = 1.47, P = 0.26) or core areas ($F_{4,16}$ = 1.47, P = 0.26) utilised by reintroduced warru (Figure 3.3). No significant sex differences were found in home range size ($F_{1,5}$ = 4.4, P = 0.09) or core area ($F_{1,5}$ = 4.5, P = 0.09) for reintroduced warru, despite a tendency for male areas to be larger (male home range 11.2 ± 5.7ha cf. female 3.4 ± 1.3ha; male core area 3.4 ± 2.0ha cf. female core area 1.0 ± 0.4ha).

Figure 3.3. Monthly home range (90% kernel) and core area (50% kernel) areas for captive warru (n = 7 month 1 and n = 6 months 2-5) reintroduced into a 97-ha predator exclosure on the APY Lands, SA.

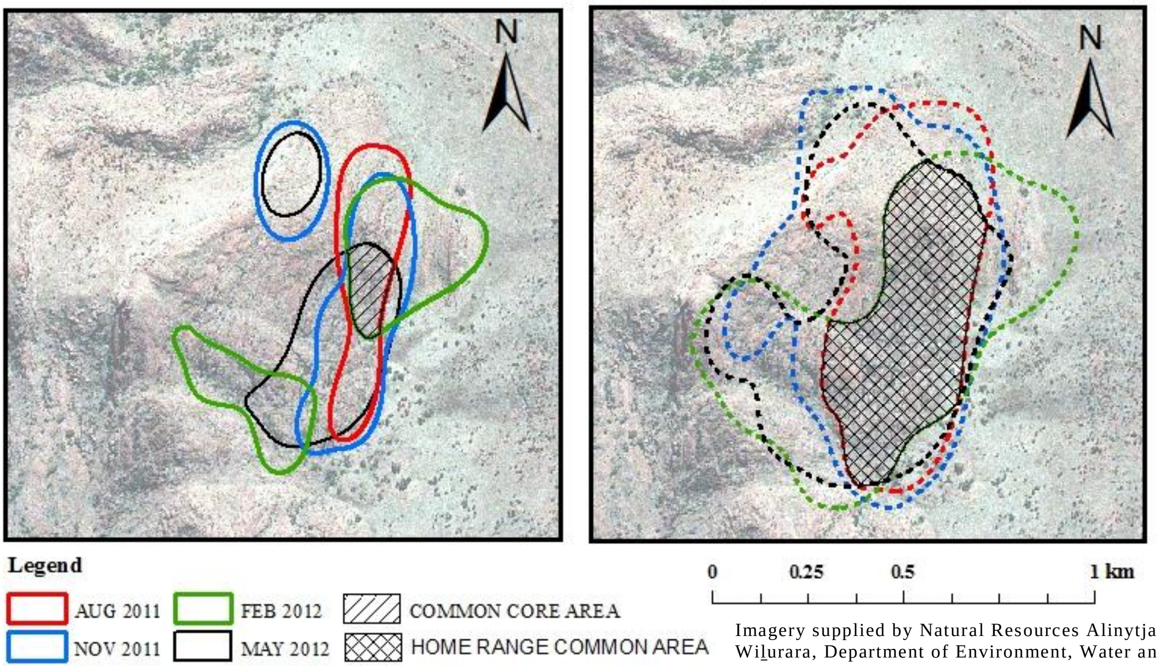


3.6.4 *Spatial variation in home range*

Wild warru utilised, on average, 53.8% of the same home range area in each tracking session. Fidelity to core areas was significantly less than home range areas for wild warru (Figure 3.4), with a mean common core area of 24.16% ($F_{4,1}$ = 14.74, P = 0.02). However, the degree of core area fidelity also varied between individuals (Figure 3.5). All wild warru home ranges were overlapped by conspecifics to some extent in each month (Figure 3.2) (mean percentage overlap 54%). Wild warru core areas also overlapped although individuals shared less of their core area (mean 35%) with conspecifics than their overall home range. No significant sex differences in core ($F_{1,5}$ = 0.03, P = 0.88) or home range sharing ($F_{1,5}$ = 0.11, P = 0.75) were observed.

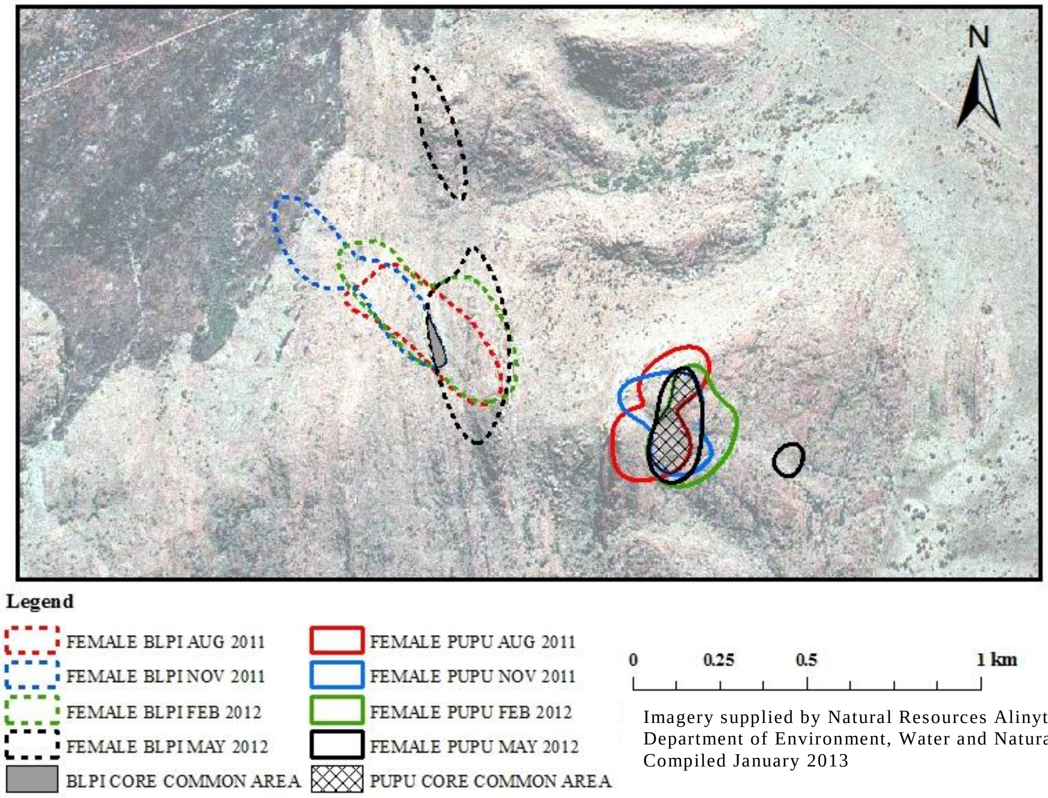
Reintroduced warru used, on average only 31.7% of the same home range area in each month and 10.9% of their core area. In particular, the core area locations of some individuals showed significant adjustments between month 1 and month 5 (Figure 3.6) but this was no different to the core area fidelity displayed in wild warru ($F_{10,1}$ = 2.75, P = 0.13). Reintroduced warru showed significantly less fidelity to their home range location than wild warru ($F_{10,1}$ = 8.36, P = 0.02). Reintroduced warru shared on average 88% of their home range with conspecifics and 78% of their core area. There was no significant difference in the degree of sharing between the sexes in reintroduced warru home ranges (Females =96.9%, Males 83.9%; $F_{1,4}$ = 1.24, P = 0.33) or core areas (Females=88.5%, Males=73.1%; $F_{1,4}$ = 1.15, P = 0.34).

Figure 3.4. Core areas (left) and home ranges (right) of one wild male warru at New Well, APY Lands, SA, between August 2011 and May 2012. Fidelity to a particular core area between months was lower than fidelity to overall home range.



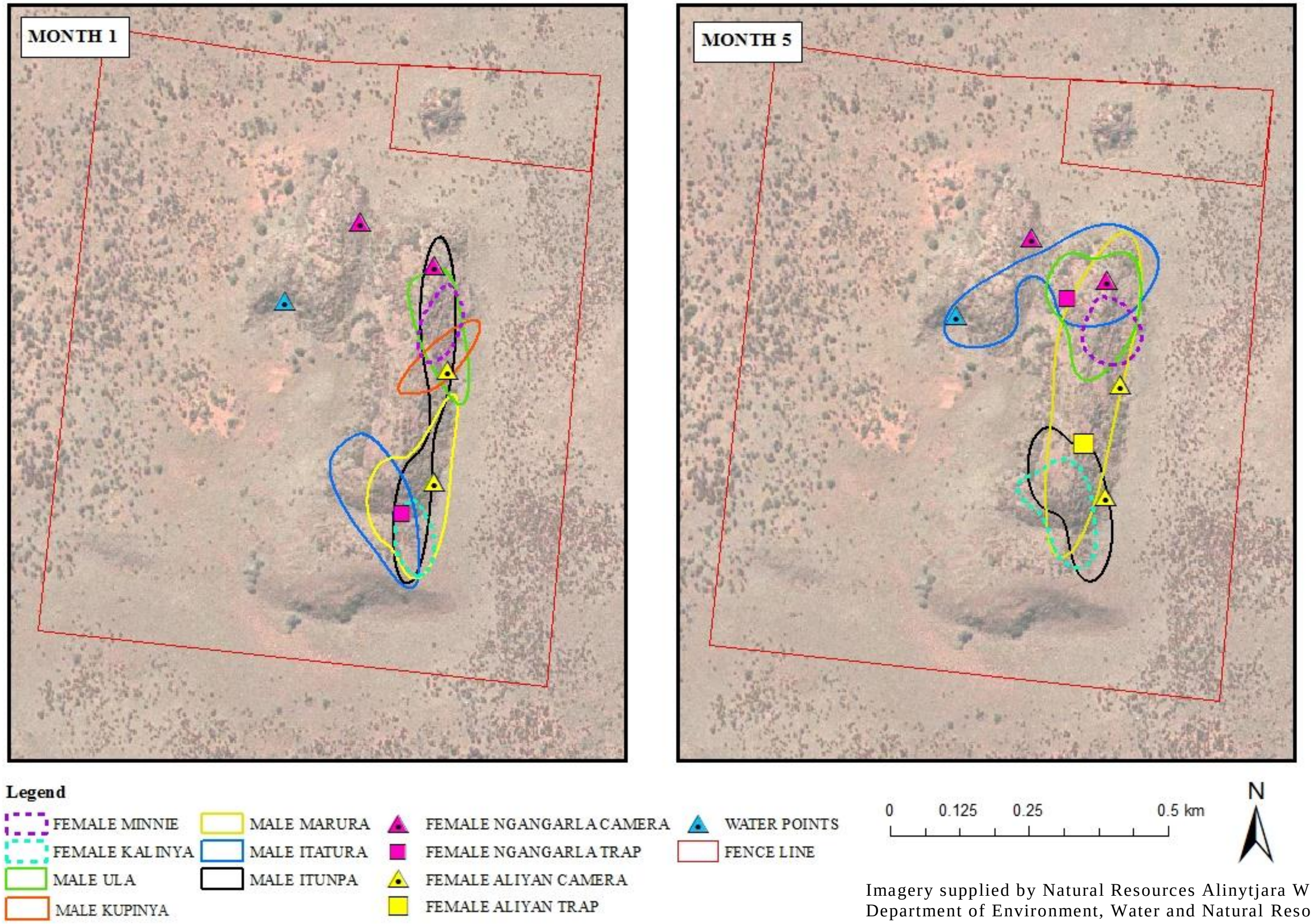
Imagery supplied by Natural Resources Alinytjara Wilurara, Department of Environment, Water and Natural Resources. Compiled January 2013

Figure 3.5. Core areas of two wild female warru at New Well, APY Lands, SA between August 2011 and May 2012, displaying individual variation in fidelity (female on west: low fidelity, female on east: high fidelity).



Imagery supplied by Natural Resources Alinytjara Wilurara, Department of Environment, Water and Natural Resources Compiled January 2013

Figure 3.6. Adjustments in the core area location (50% kernel), of male and female warru following soft-reintroduction to the APY Lands, SA, between August 2011 (Month 1) and December 2011(Month 5). 'Month 1' was calculated from fixes recorded in the first 30 days post supplementation of 6 warru (5 displayed - Minnie, Kalinya, Aliyan, Ula, Kupinya). Individuals Itatura, Marura, Itunpa and Ngangala were previously released in March 2011. The positive remote camera and trapping records for the two known-to-be-alive females not fitted with GPS collars (Ngangala and Aliyan) are provided to give an indication of space use. Water point locations are indicated. Note: male Kupinya died Sept 2011.



Imagery supplied by Natural Resources Alinytjara Wil̲urara,
Department of Environment, Water and Natural Resources
Compiled December 2012.

3.7 <u>Discussion</u>

3.7.1 *<u>Habitat selection</u>*

Wild *Petrogale* species are well documented to demonstrate preference for rocky outcrops in which they seek diurnal refuge, emerging to forage on hill slopes and surrounding grasslands at dusk (Laws and Goldizen 2003; Sharp 2009; Short 1982; Telfer and Griffiths 2006). These habitat selection behaviours were further evidenced in the current study. Only 8% of all GPS locations for wild warru were situated on the flats around the hill, indicating that the majority of warru nocturnal activity was restricted to the New Well outcrop. Of the fixes located off the hill, only 27% of these were >50m from the hill, with the furthest distance observed for a male warru location, 140m from the base of the hill. The majority of the fixes that were not collected on the main hill were located on a separate outcrop to the east of New Well (50m from the base of the main hill). This outcrop is ~3ha in area, ~40m in elevation, has an abundance of fig (*Ficus brachypoda*) and spearbush (*Pandorea doratoxylon*) (R West, pers. obs.) and thus may be providing a high quality foraging area for warru that also provides predator protection within the rocky boulders. Studies of other *Petrogale* species have found higher degrees of off-hill movement for foraging (Batchelor 1980; Telfer and Griffiths 2006) and drinking purposes (Lim and Giles 1987; Sharp 2011). The rarity of long distance movements away from the hill displayed in the current study was also documented for a female warru at Alalka in the Musgrave Ranges (Ruykys *et al.* 2011), suggesting that warru at both sites rarely move off the hills to forage and seek water. Whether these behaviours are the result of density of food resources on the hill, warru population density, increased predation risk on the flats, or a combination of these factors, requires further investigation.

Selection of poor quality habitat and high rates of dispersal from the release site are common in species reintroduction programs (Biggins et al. 1999; Clarke and Schedvin 1997; Tweed *et al.* 2003;) particularly those involving the release of captive born founders, a factor which is partially attributed to the creation of artificial habitat preferences in captive environments. This leads individuals to seek habitat similar to their ‘natal’ habitat upon release, known as natal habitat preference induction (NHPI) (Stamps and Swaisgood 2007). For example, captive reared Guam rails (*Gallirallus owstoni*) rejected dense native forest habitat at the release site and instead selected open habitats similar to their zoo enclosures which led to a strong preference for travelling along roads (Witteman Jr *et al.* 1991). Previous habitat experience for captive warru was that of low scrub enclosures with small shelter structures at Monarto Zoo and thus it was expected that captive individuals may spend more time in the grasslands of the warru pintji as opposed to the hill slopes and rocky areas. However, despite this prior experience, the majority of fixes for reintroduced warru (86%) were located on the outcrop. A low proportion of fixes on the flats even in the first month (15%), suggests that captive warru quickly adapted to seek refuge within the caves and crevices of the hill, and did not display NHPI, but rather habitat selection behaviours similar to their wild counterparts. Supplementary water points were present within five caves on the hill. Although the exact role these water points may have played in the habitat selection of captive warru post-release cannot be determined from the current study, visits to these water points by all warru (those released in March and those released at the start of this study) did not occur until 8-10 days following the supplementation and thus similar habitat selection behaviours would likely have occurred in the absence of water points.

There was no evidence for attempted dispersal of reintroduced individuals from the site in the first five months post-release with all but five fixes located on, or within 50m of, the base of the hill. Dispersal away from the site was therefore apparently not restricted by the fence line. These results suggest that captive conditioning did not decrease the ability for captive warru to identify suitable habitat for refuge and foraging. This shows promise for future releases, as it will be essential that released individuals seek refuge in complex rocky outcrops to reduce predation risk. Captive brush-tailed rock-wallabies (*Petrogale penicillata*) that selected poor refuges (small bushes and boulder piles) following reintroduction were quickly lost to predation (Soderquist 2011).

Although there is evidence to suggest that some soft-release protocols do not improve habitat selection and survival post-release (Short 2009; Wolf *et al.* 1996), the soft-release protocol used in this project is different in that it will take place over a number of years and provides natural warru habitat. Warru are exposed to aerial predation, feed on wild plant species and have to adapt to the fluctuating conditions of the arid zone. In addition, warru will spend an extended period of time within this facility prior to hard release (at least 3 years for initial founders). Together these factors should assist future reintroduction success as individuals will already have developed a suite of wild habitat selection behaviours, improved fitness and survival strategies. However, monitoring site fidelity, when warru are released in alternate group sizes and structures, will be required. Release group structure was found to affect habitat selection in reintroduced grey partridges (*Perdix perdix*) (Rantanen *et al.* 2010) and dispersal from the release site has been documented to be male biased for larger reintroduction groups of hare-wallabies (*Lagorchestes sp.)* (Hardman and Moro 2006). In the current study, releases into the warru pintji occurred across three sessions with a maximum group size of six. As group sizes >6 are recommended to improve the establishment probability of the hard release group (ref Ch. 6), monitoring to assess the impacts of larger group sizes on site fidelity and thus survival and reproduction will be important to determine the most appropriate release protocols.

3.7.2 Home range behaviours

Wild warru utilised mean home range areas of 22.8ha (90% kernel) and core areas of 6.9ha (50% kernel). These are most similar to home range estimates for semi-arid *Petrogale xanthopus xanthopus* (23.5ha; 95% kernel) (Sharp 2009). The only other documented home range for an arid-zone *Petrogale* species is that of a single female warru at Alalka who had a considerably larger home range, 57.9ha, but similar core area of 9.3ha (Ruykys *et al.* 2011). The tropical allied rock-wallaby (*P. assimilis*) has been shown to expand home range size during the dry season when good quality resources are in short supply (Horsup 1994). However, semi-arid *P. xanthopus* did not display temporal variation in home range size (Sharp 2009) and a lack of seasonal variation in home range size was also evidenced in the current study for warru at New Well. It is possible that this lack of strong seasonal variation in arid and semi-arid *Petrogale* home ranges is related to the unpredictable nature of rainfall in these areas, which would result in stochastic fluctuations in home range size rather than discrete seasonality (Sharp 2009). The large home range for the female warru (Ruykys *et al.* 2011) was recorded during winter in July 2008. The monthly rainfall total (1.6mm) in July 2008 was significantly less than both July 2011 (32.8mm) which preceded the first ‘winter’ observation period in the current study (August 2011) and April 2012 (24mm) prior to the second winter observation session (May 2012) (Bureau of Meteorology, Pukatja station records). It is therefore possible that the larger home range of the Alalka female represents a stochastic expansion in response to low habitat productivity that year. However, the larger area may also represent variation in resource density at the two sites or a lower warru population density at Alalka, and thus an increase in sample size for Alalka warru home ranges is required before drawing further conclusions.

Home range and core area sizes were smaller in reintroduced warru (home range: 8.7ha, core area: 2.6ha) than the wild warru at New Well and very similar to those of reintroduced *Petrogale xanthopus* 6 months post release (9.8ha home range, 2.9ha core area) (Lapidge 2001). Reintroduced *Petrogale penicillata* used similar core areas (2.5ha) but occupied larger home range areas (26ha) (Molyneux *et al.* 2011). Smaller home ranges of reintroduced captive-bred cotton-top tamarins (*Saguinus oedipus*) were suggested to be reflective of the small pens in which they were previously housed and thus individuals were not accustomed to navigating around a larger area (Price 1992). Whilst it is possible that this led to smaller home ranges in captive warru, it must also be noted that there was a significantly smaller area of available habitat within the pintji (~17ha) in comparison to New Well (~590ha). The incorporation of the majority of the hill within the monthly home ranges of the reintroduced males indicates that larger home range sizes may be displayed if reintroduced warru were provided with a greater area of hill habitat. However, home range sizes have also been documented to decrease significantly in *Petrogale xanthopus* following several years of fox (*Vulpes vulpes*) and goat (*Capra hircus*) control, an adjustment thought to have occurred because wallabies could meet their resource requirements within a smaller area in the absence of competition (Hayward *et al.* 2011). Euros (*Macropus robustus*) are known to exhibit dietary overlap with warru (Creese 2007) and compete for shelter sites (Ruykys 2011) and have been increasing in density at New Well (Read and Ward 2011). The removal of euros and absence of introduced predators within the warru pintji may be driving the smaller home range patterns observed in reintroduced warru. This question could be addressed, in part, through monitoring of warru home range sizes following hard-release to areas of larger habitat in the presence of introduced predators and competitors.

Wild warru displayed similar home range fidelity to other studied rock-wallabies with a mean overlap of 53.8% between the four monthly ranges recorded. This sedentary behaviour has been documented in *P. xanthopus* who displayed 63.6% overlap of nightly foraging ranges (Sharp 2009) and in *P. assimilis* who consistently used 68% of their home range area (Horsup 1994). However, wild warru demonstrated much lower fidelity to their core areas (24.2%) in comparison to those reported for *P. assimilis* (52%). A possible explanation for this could be the strong pair bonds that have been documented for *P. assimilis*, leading to stable overlapping home ranges between a pair (Horsup 1994), in comparison to the polygamous mating system documented in warru (Ruykys 2011). In addition, these adjustments in core area location by wild warru could reflect seasonal alteration in resources within the vicinity of their shelters (Stirrat 2003) and changes in shelter sites, which has been suggested by indigenous consultants to be a predator avoidance strategy in rock-wallabies (Telfer and Garde 2006). Use of multiple den sites by warru has been found at New Well, with certain males using den sites on the opposite sides of a 100m gully on consecutive nights (Read and Ward 2007). Although the current study cannot conclude the exact reasons for these alterations in core location, it suggests that the exhibition of a stable core area is not necessarily an innate behaviour of warru and, as such, should not be used as a sole criterion to assess adaptation to wild type behaviours by reintroduced warru.

Reintroduced warru displayed less fidelity to their home range location than wild warru (31.7% cf. 53.8%) and similar to wild warru, lacked consistency in their core area location (10% fidelity between months). This difference in home range fidelity likely reflects the exploratory behaviours of captive warru as they located resources within a novel environment and is not unexpected. The exceptionally low fidelity to a particular core location in reintroduced warru may be further explained by maturity and dominance status, which has been shown to influence core area selection in other *Petrogale* species (Batchelor 1980; Horsup 1994). Itatura, the male who was smallest in body mass and scrotal diameter, which are indicators of male maturity and dominance in *Petrogale* (Jones 2001), displayed a complete change in core area from one end of the hill to the other across four months (Figure 3.6), whilst the adult male (Ula) showed a relatively stable core location following release Figure 3.6. A similar change in core location was also observed for one of the younger females (Ngangala). Although not GPS collared, remote camera and trapping locations had consistently recorded this female at the south of the hill since release in March 2011 (Figure 3.6). Following the supplementation of six older individuals in July 2011 (including three adult females), remote camera and trapping records documented a complete shift of area use to the north of the hill (Figure 3.5). Whilst neither of these adjustments resulted in these individuals moving off the hill into the flat grasslands, or dispersal away from the hill, they suggest that monitoring of habitat selection following supplementation, particularly as density increases, will be warranted. It is possible that the period of this study did not document home range stabilisation. Reintroduced *Petrogale penicillata* stabilised core and overall home ranges 4 months after release (Molyneux *et al.* 2011) but *Petrogale xanthopus* were found to make continual adjustments to home range size up to 12 months post- release and core areas continued to adjust even 24 months later (Lapidge 2001). It is therefore expected that the home range patterns for reintroduced warru will continue to change as a result of systematic adaptation to wild habitat.

Wild male warru had significantly larger home ranges (34.5ha) than female warru (16.2ha). Sex differences in home range size have been documented in other studies of *Petrogale* and have been attributed to the mating system of the species and/or greater resource requirements of larger males (Batchelor 1980; Hazlitt *et al.* 2006; Laws and Goldizen 2003; Sharp 2009). Genetic data indicates a polygamous mating system within wild warru (Ruykys 2011) and the home range overlap patterns observed in the current study indicate the behavioural system that may facilitate this, with female home ranges overlapped by multiple males and males overlapping multiple females (Figure 3.2, Figure 3.6). It must be noted that only 21% of the known wild warru population were fitted with radio collars during this study and hence any inferences of the extent of home range overlap will be underestimated. The home range overlap patterns observed in reintroduced warru are consistent with those of wild warru and, combined with the high reproductive rates observed in reintroduced warru females post-release (ref Ch. 2), suggest the establishment of a functioning social system. Home ranges that facilitate a mating system are important drivers of initial population growth and reintroduction success (Sigg *et al.* 2005). A polygamous mating system in reintroduced warru should be confirmed by future genotyping of pintji born offspring.

3.7.3 Management implications and future research

The initial pre-requisite for population establishment post- release is that individuals remain at the site of release and are able to survive in the habitat chosen for them (Le Gouar *et al.* 2011). The home range behaviours of reintroduced warru corroborate the site selection of the pintji hill which was deemed to have abundant food resources and a large variety of suitable shelter sites (Ward *et al.* 2010). This also suggests that a similar attribute ranking system will be able to identify quality areas for future release sites. However, given the high hill fidelity in wild warru and confinement of core areas to the upper elevations, it is likely that the sites will also need to be assessed for elevation of structurally complex shelter sites, potentially decreasing introduced predator accessibility.

New naive individuals can disrupt settled home range patterns, which can not only increase their probability of mortality post release (Kleiman 1989) but also induce dispersal from the release site. For example, reintroduced European otters (*Lutra lutra*) displayed greater movements away from the release site if these areas were already occupied by resident otters (Sjoasen 1997). Density estimates of the warru pintji population should be combined with continued monitoring of habitat use via radiotracking and remote cameras to determine potential impacts on habitat selection, particularly the use of areas closer to the fence line. Such analyses could then be used to indicate a carrying capacity (Finlayson and Moseby 2004), which is a key question for the future management of the warru pintji (Read and Ward 2011). These analyses will also provide guidance on the extent of the habitat area over which multiple releases of larger group sizes should take place.

The display of wild type habitat selection and home range behaviours by reintroduced warru demonstrates that captive warru are capable of adapting quickly to new wild habitat. Whilst future hard releases of individuals from within the warru pintji are likely to display appropriate habitat selection and refuge choice it will be essential that they also develop introduced predator avoidance behaviours and the capability to outcompete or persist with other competitor species. However, given effective introduced predator control at future release sites, the current study suggests that, in habitat of comparative quality to the warru pintji, reintroduced warru will be capable of recolonising the hill habitats of their former range across the APY Lands.

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CHAPTER 4: THE USE OF SUPPLEMENTARY WATER BY WARRU: A POTENTIAL TOOL FOR INCREASING PERSISTENCE OF WILD AND REINTRODUCED POPULATIONS.

A female warru carrying a large pouch young visits a supplementary water point at New Well. Photo: R West.

4.1 **Preamble**

Supplementary water points monitored by remote cameras were established within a remnant warru colony at New Well and the reintroduced population in the warru pintji. This chapter presents a multivariate modelling approach to understand the influence of changes in natural water availability on the observed drinking rates of warru. The results were used to determine whether supplementary water points could be used as a management tool to relieve resource pressure when natural water is limiting and thus potentially increase the survival of *in situ* and reintroduced warru populations to facilitate long-term persistence.

4.2 Statement of Authorship

Title of paper	**The use of supplementary water by warru; a potential tool for increasing persistence of wild and reintroduced populations.**							
Publication status	☐	Published	☐	Accepted for Publication	☐	Submitted for Publication	☒	Publication style

Author contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author	**Rebecca West**		
Contribution to the paper	Designed and developed the study. Conducted all data collection, analysis and interpretation. Wrote manuscript.		
Signature		Date	28/10/2013

Name of Co-Author	**Matthew Ward**		
Contribution to the paper	Supervised design and development of work.		
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Contribution to the paper	Supervised development of work and commented on a draft manuscript.		
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Contribution to the paper	Supervised development of work and commented on a draft manuscript		
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Contribution to the paper	Commented on a draft manuscript.		
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4.3 Abstract

Supplementary resource provision is increasingly used by conservation managers to manipulate habitat conditions that are identified to be limiting population growth of threatened species. These methods are also popular tools in reintroduction programmes as they can reverse conditions associated with previous decline and assist released individuals to adapt to novel environments. *In situ* management and reintroductions are being used to recover warru (black-footed rock-wallaby, *Petrogale lateralis* MacDonnell Ranges race) on the arid Anangu Pitjantjatjara Yankunytjatjara (APY) Lands of South Australia. Introduced predators are thought to be primarily responsible for the decline in warru populations, and in addition to direct predation, have limited warru access to suitable habitat conditions by forcing populations to reside in predator refugia rather than optimal habitat. One impact of this has been to limit access to permanent water resources. Reduced access to more permanent water sources, particularly during the drier winter period on the APY Lands may have decreased warru population persistence. The current study investigated whether supplementary water could be used to alleviate resource pressure during the dry winter and hence become a tool to potentially increase survival of warru during dry and drought periods. The current study provided supplementary water to a remnant and reintroduced warru population across a 12 month period. Drinking rates were calculated through monitoring water points using remote cameras and compared to both plant moisture content and available free water (total rainfall) during each water supplementation session. Multivariate modelling indicated that natural free water and plant moisture content had a significant influence on the drinking rates of wild warru, with drinking rates significantly higher during dry winter months. It is recommended that supplementary water be provided to warru populations with limited access to free water during winter (March – October) and periods of drought, when food resources will be of lower quality and/or less abundant. This should aid in increasing the chances of survival for remnant and reintroduced warru, improve the probability of persistence for populations and thus contribute to the recovery of warru on the APY Lands.

Keywords: supplementary water, *Petrogale*, reintroduction, drinking frequency, water use.

4.4 Introduction

The term ‘habitat quality’ refers to the environmental features that provide the conditions necessary for survival, reproduction and hence persistence of a species (Hall *et al.* 1997). These ‘conditions’ include: vegetation characteristics, environmental variables such as temperature and altitude, food and water resources, abundance thresholds of competitor/predator species, disease, and human disturbances (Hall *et al.* 1997). Managers of threatened species therefore seek to understand the exact habitat condition requirements of a species because this knowledge is fundamental to designing effective practical management strategies to aid population persistence (Norris 2004).

Resource limitation can contribute to population decline, particularly if the threatened species has experienced significant habitat loss, reducing resources to quantities insufficient to support the population and intensifying resource competition (Norris 2004). As a result, supplementary resource provision is widely and increasingly used by conservation managers to manipulate this aspect of habitat quality for the recovery of threatened species (Blanco *et al.* 2011). Resource provision can directly improve the nutritional condition, growth rates, and reproductive success of a species (Elliott *et al.* 2001; Robb *et al.* 2008). For example, supplementary feeding programmes using farmed rabbits led to an improvement in breeding success for pairs of the endangered Spanish imperial eagle (*Aquila adalberti*) (González *et al.* 2006) and helped to maintain a population of the threatened Iberian lynx (*Lynx pardinus*) during a period of extremely low prey abundance (López-Bao *et al.* 2008). Supplementary water programmes have also been implemented for populations of the herbivorous northern hairy-nosed wombat (*Lasiorhinus krefftii*) in Australia, thought to be limited by poor quality and low water content of pasture during drought periods (Treby *et al.* 2007).

Resource supplementation techniques have also become popular in conservation reintroduction programmes where managers need to ensure that the threats identified to have caused the initial decline of a species are removed or reversed prior to commencing a translocation (IUCN/SSC 2013). Successful reintroductions of the Arabian oryx (*Oryx leucoryx*) were partially attributed to the provision of supplementary feed and water during a three year drought, which would undoubtedly have otherwise decreased survival of the reintroduced individuals (Spalton *et al.* 1999).

Experimental approaches to resource supplementation during reintroductions have further investigated the habitat conditions required by a species. Provision of sugar water to reintroduced populations of the hihi (stitch bird, *Notiomystis cincta*) was found by Armstrong and Perrott (2000) to have no significant effect on survival rates and body condition, and they concluded that adult hihi were unlikely to be limited by food supply. In addition, supplementary resources have been used to encourage the establishment of reintroduced individuals (fidelity and survival) at the release site (Armstrong and Perrott 2000; Friend and Beecham 2004; Moseby and Bice 2004). This can be particularly important for maintenance of body condition and survival of captive individuals that experience additional stresses associated with adapting to the use and location of food resources with which they may not be familiar (Bright and Morris 1994; Mathews *et al.* 2005).

In situ management and captive reintroduction strategies are being used to conserve threatened populations of warru (black-footed rock-wallaby, *Petrogale lateralis* MacDonnell Ranges race) on the arid A<u>n</u>angu Pitjantjatjara Yankunytjatjara (APY) Lands of South Australia (SA) (Read and Ward 2011b). Predation by the introduced red fox (*Vulpes vulpes)* and domestic cat (*Felis catus)* are thought to be the primary drivers of warru decline in SA (Ward *et al.* 2011b), with additional threats of buffel grass (*Cenchrus ciliaris*) invasion, inappropriate fire regimes and introduced herbivores (Read and Ward 2011a). Predator baiting (1080 poisoning) is being conducted at *in situ* colonies and initial soft reintroductions have occurred inside a 97-ha enclosure on the APY Lands (Muhic *et al.* 2012) to remove the threats of introduced predators and herbivores. Controlled burning is used to create fire breaks to protect both reintroduced and *in situ* populations from wild fires and small scale buffel grass control has been conducted at each site (WRT 2012).

Changes in access to water have been proposed as an additional cause of warru population decline (Read and Ward 2011a). Although water turnover experiments have shown that *Petrogale* can survive in the absence of water (Hume *et al.* 2004; Lapidge and Munn 2012), fluctuations in *Petrogale xanthopus* population size are significantly correlated with rainfall events (Lethbridge and Alexander 2008; Sharp and Norton 2000). This is likely due to direct impacts on adult survival through improvements in the quality of forage (Lethbridge and Alexander 2008) in addition to increasing population recruitment, through improved lactational output of dams (Kennedy and Heinsohn 1974), which positively influences *Petrogale* pouch young survival (Delaney 1997). It is therefore unsurprising that drinking rates of semi-arid yellow-footed rock-wallabies (*Petrogale xanthopus celeris*) at an artificial water source were negatively correlated to prior rainfall and moisture content of dietary items (Sharp 2011). Habitat selection modelling indicated that historical warru distribution on the APY Lands was strongly influenced by the presence of drainage features, natural soaks and springs (Ruykys 2011), with rockholes common features of previously occupied sites (Read and Ward 2011a). However, most extant warru populations do not have access to permanent or reliable free water (Read and Ward 2011a). In the arid zone, rockholes and water points are known to act as focal areas of predator activity (Hume *et al.* 2004) and hence it is proposed that increases in predation pressure may have forced remnant warru populations into 'predator refugia' rather than optimal habitat (Ruykys 2011), reducing their access to more permanent water sources. This would have been likely to impact warru population persistence, particularly during drought and winter, which correlates with the driest period on the APY Lands (Robinson *et al.* 2003). Indeed, annual juvenile survival rates for warru were recently correlated with winter rainfall in that year (Ward *et al.* 2011a).

Traditional owners of the APY Lands highlight the importance of 'mina' or 'kapi' (Pitjantjatjara for 'water' and 'rain') when discussing warru management (R West, pers. obs.) yet specific options for addressing this possible limiting factor have not yet been tested or implemented. The current study aimed to answer the following specific questions using supplementary water points:

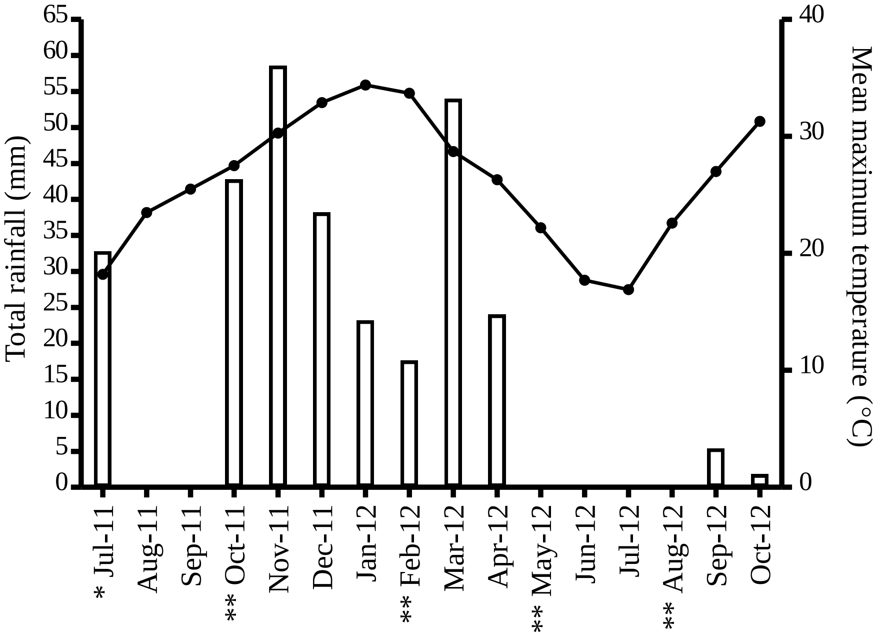
1. Do wild and reintroduced warru use supplementary water points?
2. What is the frequency of drinking at supplementary water points and does this vary with changes in rainfall driven resource availability or demonstrate habituation to supplementary resources?
3. Does the presence of supplementary water points influence visitation rates of other species and potentially increase competition or predation for warru?

4.5 Methods

4.5.1 *Study sites*

The study was conducted at two warru populations within the APY Lands of SA. ‘Wild’ population studies were conducted at New Well (26.080^{0}S, 132.215^{0}E), an extant warru colony which has been managed by the Warru Recovery Team since 2007. New Well is a ~590ha granitic inselberg (Robinson *et al.* 2003) with an elevation range of 624 – 830m. There are no permanent water sources on the hill but water is known to collect in depressions on rock surfaces post-rain and in gully catchments at higher elevations which causes it to run down the hill and seep out from under some of the larger granitic slabs after heavy rain (R West, pers. obs.). ‘Reintroduced’ population studies were conducted at the warru ‘pintji’ site (26.184^{0}S, 132.345^{0}E), a 97-ha predator free exclosure constructed on the APY Lands (Muhic *et al.* 2012). Captive warru were released at this site between March 2011 and July 2012 (n = 16) (ref. Ch. 2). The pintji hill is ~17ha in size. There are also no permanent water sources within the warru pintji but water collects readily in depressions on the surface of the boulders at the summit of the hill and runs along the rocky creek bed after heavy rain (R West, pers. obs.). These site characteristics lead to an unreliable presence of free water at both sites post-rain. Bureau of Meteorology records from Pukatja (Station #016097: located 23km south west of New Well and 24km west of the warru pintji) indicate a mean annual rainfall of 278.6mm (1997-2012) with 73% of this rainfall occurring in summer, between October and March, which are also the months of highest maximum temperature (Robinson *et al.* 2003). Monthly mean maximum temperature and total rainfall volumes for the duration of the study are displayed in Figure 4.1.

Figure 4.1. Monthly total rainfall and mean maximum temperature records for Pukatja Station (#016097, Bureau of Meteorology) during the study period. ** denotes month in which supplementary water was provided. * denotes additional supplementary water session for reintroduced warru at the warru pintji site.



4.5.2 Selection of water point locations and monitoring

Water was provided in 8-litre chicken water feeders (Horsley Wholesale, Australia) which were placed into caves (Figure 4.2). Caves were selected on three attributes a) sheltered from high sun exposure to reduce evaporation rates; b) not easily accessible to euros (*Macropus robustus*) known shelter and dietary competitors (Capararo 1994; Geelen 1999; Ruykys 2011) and c) enabled easy placement of a remote camera to monitor activity. Remote cameras (Scoutguard KG680V, Faunatech Austbat, Australia) were mounted to fence droppers and positioned so as not to obstruct the entrance to each cave but with a clear view of the water point/cave interior (Figure 4.2). Remote cameras ran continuously across the study period, with 16GB SD cards and AA professional alkaline batteries replaced when water points were filled. Cameras were set to take a sequence of 3 photographs upon trigger with a 10 second interval after an animal was first detected before response to additional triggers. Sensor level was set to high and a date and time stamp imprinted on every photograph.

4.5.3 Supplementary water schedules

4.5.3.1 *Wild population*

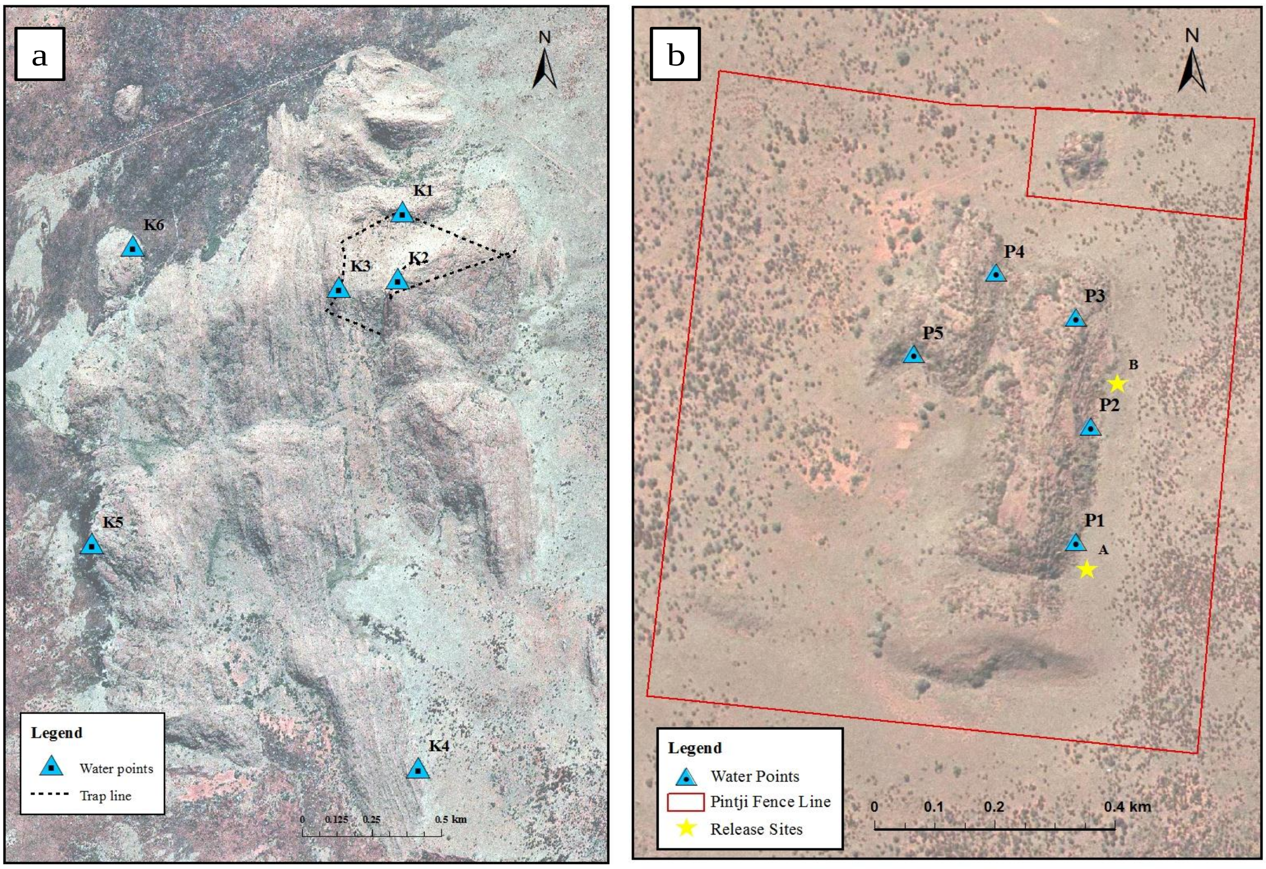
In October 2011, six water points were established at the New Well site (Figure 4.3). Three water points were established central to the known warru colony (referred to as ‘central’) along the rocky outcrops on which the annual trap line is situated and three water points were established at locations around the perimeter of the New Well hill, referred to as ‘outer’ (Figure 4.3). Spotlighting had suggested the presence of warru at one of these locations (K5) (J Read, pers. comm.). The other two sites were selected in part to determine if warru were occupying these locations but also to observe whether marked warru (i.e. those trapped and marked with ear-tags in the central colony) would visit the water points around the perimeter of the hill. In order to determine whether the presence of water points affected cave visitation rates of other species (predators, competitors and sympatric), a cave of similar size and shape was selected within 100m of each water point to act as a control (no water point) (n = 6). Water points were filled at four time intervals across the year (Figure 4.1), equating to four supplementary ‘sessions’.

4.5.3.2 *Reintroduced population*

Prior to the supplementation of captive warru into the pintji in July 2011, five water points were established in caves across the pintji hill (Figure 4.3b). Supplementary water was provided at three water points for the March 2011 release but the results are not incorporated into the current study as these water points were clustered at the first release site and so locations were adjusted and number of water points increased for the second release in July 2011. The water points were placed across the hill in attempt to avoid biasing habitat selection by reintroduced warru and to avoid potentially increasing competition between individuals (Figure 4.3b). Control caves were not established within the pintji as introduced predators and competitors were either excluded by the fence/had been removed/reduced prior to release (ref. Ch 2). Due to the release schedule, there was an additional session of water supplementation for reintroduced warru in July 2011 (Figure 4.1). Other supplementation sessions were as per the wild experiment, with cameras running continuously across the study period (Figure 4.1).

Figure 4.2. Example of the experimental set up used to monitor drinking rates at supplementary water points for wild and reintroduced warru and an exemplar photograph at this point.

Figure 4.3. Location of supplementary water points at the wild population (a) and the reintroduced site (b) on the APY Lands, SA. Dashed line in (a) denotes annual trapping line, hence K1-K3 were 'central' (to known colony) and K4-K6 were 'outer'.



4.5.4 Natural water availability during supplementation

Natural water availability (dietary and free water) at each site during each supplementary water session was calculated in two ways.

4.5.4.1 *(1) Dietary water availability*

For each supplementary session, an analysis of plant moisture content was used to represent the potential dietary water available to warru. Ten species were selected for monitoring at the wild/New Well site and eleven at the reintroduced/Pintji site (Table 4.1). As a comprehensive study of warru diet composition on the APY Lands was lacking, selected species constituted a mix of presumed dietary items based on a preliminary study (Geelen 1999) and studies of the diet of a population of *P. lateralis* MacDonnell Ranges Race in Alice Springs (Blackbourn 1991; Capararo 1994) and *P. l. lateralis* in Western Australia (Creese 2007). The remaining species were not known to be dietary items but were in high abundance at either site enabling guaranteed sampling at each session (Table 4.1).

Table 4.1. List of plant species collected for moisture content analysis at the site of the wild and reintroduced study populations on the APY Lands, SA. Species known to be food items for *P. lateralis* (Y) and those not known to be eaten (UK) are indicated.

Genus	Species	Common name	Presumed dietary item?	Study
Aristida	*contorta*	Kerosene grass	Y	Geelen 1999
Cenchrus	*ciliaris* ^	Buffel grass	Y	Creese 2007; Blackbourn 1991
Cymbopogon	*ambiguus* ^	Lemon scented grass	Y	Capararo 1994; Blackbourn 1991; Geelen 1999
Dodonaea	*viscosa*	Sticky hopbush	Y	Capararo 1994; Geelen 1999
Enchylaena	*tomentosa**	Ruby saltbush	Y	Geelen 1999
Ficus	*brachypoda*	Fig	Y	Creese 2007; Geelen 1999
Pandorea	*doratoxylon*	Spearbush	Y	Capararo 1994; Geelen 1999
Ptilotus	*obovatus*	Silvertails	Y	Creese 2007; Geelen 1999
Aristida	*?holathera*	Tall kerosene grass	UK	
Rhagodia	*parabolica**	Mealy saltbush	UK	
Rhagodia	*spinescens**	Creeping saltbush	UK	
Sida	*petrophila*	Rock sida	UK	
Themeda	*triandra*	Kangaroo grass	UK	

^ only collected at New Well
* only collected in the pintji

Plant species were sampled in each session at both sites except for the additional early session (July 2011) for the reintroduced population. For each session (n = 4), ten samples of each species were collected opportunistically across the hill at each site. Samples from a plant were collected at random with no bias to healthier or younger parts of the specimen and then sealed within a large zip-lock bag. Mass within the zip-lock bag was recorded for each specimen before transfer to a paper bag. A mean zip-lock bag mass (n = 10) was calculated and deducted to give a 'wet mass' for each sample. Samples were partially air dried in their paper bags whilst on the APY Lands and the drying process finished upon return to Adelaide, SA. To complete the drying process each specimen was oven-dried at 70°C within its paper bag for four days. A mean dried paper bag mass (n = 10) was calculated and deducted to provide a 'dry mass'. The moisture content for each sample was then calculated as mass lost as a percentage of the wet mass. A value for the mean percentage moisture content was then calculated for all samples for each session. No distinction was made between presumed and unknown dietary items.

4.5.4.2 *(2) Free water availability*

In each supplementary session, the days during which water was available at each water point (n = 6 wild population; n = 5 pintji population) was determined through the remote camera photographs showing when water was no longer visible in the lip of the water point or it was pulled or knocked down by a warru. Bureau of Meteorology records were used to calculate the total rainfall (mm) that fell during the days that water was present in each water point. This was used as a measure of the free water available in the habitat during each supplementary session.

4.5.5 Analysis of remote camera data

4.5.5.1 *Wild population*

Photographs from each water point camera were analysed as follows: Date, Time, Species, Drinking/Visit and Marked/Unmarked (presence of ear-tag/collar - warru only). A 'drink' was recorded when the animals snout was pictured either on or in close proximity to the lip of the water point (Figure 4.2). Presence in the cave but not in association with the water point was logged as a 'visit'. Sequences of multiple photographs of the same animal 'drinking' or 'visiting' a cave were recorded as a single event which ended when the animal left the cave. The total number of drinks and visits by each species during the days of water provision were calculated for each water point. Photographs from each control cave camera were analysed for: Date, Time, Species and Marked/Unmarked (warru only). Control cave cameras were analysed for the entire period between water top ups (~3 months). For each control point, the total number of visits by each species was calculated.

4.5.5.2 *Reintroduced population*

All released warru were fitted with a radio collar (ref Ch. 3) with a unique black and white antenna marking (placed using heat shrink tubing) and black/white ear-tags for some individuals. This enabled individual warru to be identified on the infra-red night-time photographs, enabling sex differences in drinking rate to be examined for this population. Photographs from the reintroduced population water points were analysed by water point as follows: Date, Time, Warru ID, Sex, and Drink or Visit.

4.5.6 *Statistical analyses*

For the wild population, analyses were conducted on a ‘point’ basis i.e. water points/control caves were independent repeated measures with individual session lengths (number of days water available at water points/ total time between supplementation periods for control points). Observations of drinking/visitation rates and measurements of available free water (total rainfall) were determined for each ‘point’ and repeated on four occasions across the study period.

As individual warru could be identified within the reintroduced population, sex differences in drinking rates could be examined. It was therefore necessary to conduct analyses on an ‘individual’ basis i.e. each marked warru was an independent repeated measure. As each individual did not have equal access to each water point (due to home range location) the number of days for each session was truncated to equate to the shortest number of days that water lasted in any water point. This standard observation period therefore reduced bias due to differential access to water points and enabled comparison of individual warru drinking rates when water was available at all water points. As a result, available free water during each supplementation session was re-calculated for reintroduced water points to reflect this standard observation period.

All statistical analyses were conducted using SAS (ver. 9.3; SAS Institute Inc., Cary, NC, USA). To examine the role of supplementary water points in improving habitat conditions for warru, three categories of analysis were implemented.

4.5.6.1 *Use of supplementary water points by warru*

The number of days from water point installation to the first recorded warru visit was calculated for each water point location in each population (wild/reintroduced). Locations across each hill were compared to examine differential use. To determine whether warru displayed differential use of caves with water and without water (i.e. were they actively selecting caves with water points?) the wild population dataset was used. For each water point, drinks and visits were combined to give a daily 'use' rate. The daily use rate between water points and control caves was then compared using a negative binomial generalised estimating equation (GEE). A GEE approach, clustering on the variable 'water point' was used to take into account the repeated measures on each water point over the four different supplementary sessions. An offset of natural logarithm of number of days was used to account for the differing lengths of time that water lasted at each water point and the varying number of days of observation at control caves within each session.

To establish whether visits to caves with water points were actually to drink, the number of visits where drinking occurred, 'drinks', at water point cave was compared to the number of visits where no drinking occurred, 'visits', using a negative binomial GEE. For the wild population dataset, the GEE approach clustering on 'water point' was used to account for the repeated measures on each water point and an offset of natural logarithm of number of days was used to account for differing lengths of time that each water point was observed (i.e. water lasted). For the reintroduced population, a negative binomial GEE was used to compare the rate of drinking visits to non-drinking visits for each warru across all of the water points. A GEE approach, clustering on 'warru ID' was used to account for the repeated measures of each warru across the five different sessions. An offset of natural logarithm of number of observation days was used to account for the differing length of time that water lasted and hence water points were observed in each session.

4.5.6.2 *Response of other species to installation of supplementary water points*

Using the wild data set, the number of visits that other species made to the water point and control caves was compared using logistic GEEs. As drinking events were very rare for other species, drinking and non-drinking visits were combined to form the outcome variable ‘visit’. Visits were dichotomised into ‘no visits’ or ‘at least one visit’ for all water points and all control caves (K1-6 and C1-6, ref Figure 4.3a), because there were a large number of zero values for some species at certain points. A GEE approach was used, clustering on the variable water point/control cave to account for the repeated measures at each water point/control cave and a natural logarithm of number of days was used to account for the different number of days that each water point/control cave was observed.

4.5.6.3 *Predicting drinking frequency at supplementary water points*

To examine whether drinking rates correlated with changes in natural water availability a number of predictor variables were identified which could influence this availability (Table 4.2). In addition, variation in drinking rates with respect to location of the water point (‘central’ or ‘outer’) was included as a predictor variable for the wild population and sex as a predictor for the reintroduced population (Table 4.2).

Table 4.2. Predictor variables entered into initial univariate models of the rate of wild and reintroduced warru drinking at supplementary water points.

Predictor variable
Mean minimum temperature (5^{0}C increments)
Mean maximum temperature (5^{0}C increments)
Total rainfall (10mm increments)
Plant moisture content (5% increments)
Mean 0900 hours humidity (%)
Mean 1500 hours humidity (%)
Rainfall 4 weeks prior to session (mm)
Location (inner or outer) *
Sex (male or female) ^

* modelled for wild only
^ modelled for reintroduced only

Modelling of the influence of predictor variables on the observed warru drinking rate was conducted in a two stage process. Firstly, univariate modelling was undertaken to determine which of the identified variables (Table 4.2), were significant predictors of warru drinking rate. A negative binomial GEE was fitted to each of the datasets (wild and reintroduced) to explore the association between rate of warru drinking and each of the predictor variables (Table 4.2). A GEE was used to account for the repeated measures at each water point across the year (wild dataset) or multiple observations on the same individual (reintroduced dataset). Temperature was examined in 5°C increments and rainfall in 10mm increments to represent monthly variations in these variables (Bureau of Meteorology, Pukatja). Plant moisture content was examined in 5% increments, again representative of within session variation.

Secondly, multivariate modelling was conducted to assess the influence of each predictor on warru drinking rate whilst controlling for all other predictor variables in the model. Predictor variables had to satisfy a P-value of <0.05 in the univariate models for each dataset before consideration in the multivariate models. In addition, Pearson's rank correlations between predictor variables were examined. Predictor variables that satisfied a P-value of <0.05 in the univariate model but displayed high co-linearity with other predictor variables ($P > 0.05$) were not included in the same multivariate models. Multivariate modelling used a negative binomial GEE model, clustering on 'water point' (wild dataset) or 'warru ID' (reintroduced dataset) and using the offset of log of number of days to account for differences in the number of days each water point was observed (wild dataset) or the length of each observation session (reintroduced dataset).

4.6 Results

4.6.1 *Use of supplementary water points by warru*

4.6.1.1 *Wild population*

At New Well, the first warru visits to all central water points were recorded within 0-2 days of installation. The outer water point known to be close to a warru group (K5) recorded a visit within one week of installation whilst the remaining outer water points took 17 and 42 days to record a visit. Marked warru were confirmed at K1 – K3, K4 and K6. There did not appear to be any marked warru visits at K5 even though ten warru had been marked at a trap site just 800m from the K5 water point. However, as both ears could not be seen in all pictures conclusive ‘marked’ or ‘unmarked’ identification could not be made.

Following top up, water was available at each water point for an average of 27 days (± 13 SD). The mean daily use rate (visits and drinks combined) for water point caves was 2.20 (95% confidence interval [CI] 1.30, 3.73) in comparison to a mean daily use rate of 0.19 at control caves (no water point present) (95% CI: 0.08, 0.45). Therefore, the rate of cave use was 11.5 times higher in caves with water points than caves without water points (rate ratio [RR] = 11.52, 95% CI 4.20, 31.61, $P < 0.0001$). In addition, 83.6% of the visits to caves with water points resulted in a drink. Mean daily warru drinking rate was 1.8 (95% confidence interval [CI] 1.08, 3.15) in comparison to a daily visit rate of 0.3 (95% CI 0.18, 0.65). Warru were 5.4 times more likely to make a visit for a drink than simply a visit (RR = 5.39, 95% CI 3.52, 8.26).

4.6.1.2 *Reintroduced population*

In the warru pintji, the three points on the eastern hill, closest to the release sites were visited first (8 – 11 days after installation). Warru were not seen using the water points on the western hill, P4 and P5, until 1 and 3 months, respectively, after installation. Points on the eastern hill also received on average, a higher number of visits per session (44.7 visits ± 28.4 SD) than either of the points on the western hill (10.5 visits ± 3.5 SD). For reintroduced warru, 76.6% of visits to caves with water points resulted in a drink. The mean daily visit rate to water points by reintroduced warru was 0.7 (95% CI 0.4, 1.17), with a mean daily drinking rate of 0.56 (95% CI 0.35, 0.92).

4.6.2 *Response of other species to installation of supplementary water points*

Several bird species were captured on camera but due to low samples sizes for each species these were grouped as 'bird' for analysis. There were eight other species in addition to 'bird' captured at the water points and control caves (Table 4.3). The odds ratio of visits to water point caves for each species in comparison to control caves is shown in Table 4.3.

The visit odds for fat-tailed false antechinus (*Pseudantechinus macdonnellensis*) and perentie (*Varanus giganteus*) were significantly higher at water points than in control caves (Table 4.3). The probability of a fat - tailed false antechinus visiting a water point were 47 times greater than a control cave (odds ratio [OR] = 46.57, CI 2.6, 843, P= 0.0093) (Table 4.3). The odds of a perentie visiting a water point were 30 times more likely than a control cave (OR = 29.62, CI 2.8, 309, P = 0.0046) (Table 4.3). There was no significant difference between visit odds for cat (*Felis catus*), euro (*Macropus robustus*), short-beaked echidna (*Tachyglossus aculeatus*) or the birds (Table 4.3).

Only single records of a pair of foxes (*Vulpes vulpes*) and a lone dingo (*Canis lupus*) were recorded during the study period, both at control caves. European rabbit (*Oryctolagus cuniculus*) visits were only recorded during the first session (once at a water point and 20 visits to a control cave). As the total visit outcome variable was dichotomised into 'no visits' and 'at least one visit' for each water point/control cave in each session, fox, dingo and rabbit could not be modelled as they did not converge due to an insufficient number of observations.

Table 4.3. Results of logistic regression of number of visits by species other than warru against group (water point or control cave), clustering on point and log adjusted for number of days observed. NM = not modelled due to insufficient data. Significant odds are highlighted in bold.

Species	**Odds Ratio**	**CI (lower, upper)**	***P* value**
Cat	3.73	0.4, 34.41	0.246
Euro	0.35	0.03, 4.07	0.399
Fat-tailed false antechinus	**46.57**	**2.57, 843.06**	**0.009**
Short-beaked echidna	1.83	0.06, 52.87	0.725
Bird	3.91	0.41, 37.69	0.238
Perentie	**29.62**	**2.84, 309**	**0.005**
Fox	NM		
Dingo	NM		
European rabbit	NM		

4.6.3 Predicting drinking frequency at supplementary water points

4.6.3.1 *Wild population*

Univariate modelling indicated that four of the predictors had a significant association with wild warru drinking rate and thus satisfied the criterion ($P<0.05$) for entry into a multivariate model (Table 4.4). However, due to the number of observations (n = 24), multivariate models were limited to the inclusion of three variables to avoid over-factoring. As rainfall in the four weeks prior to the supplementation session had a high correlation with plant moisture content during the session ($r = 0.84$, $P < 0.0001$), rainfall in the four weeks prior was excluded from the multivariate model.

Table 4.4. Results of univariate modelling (negative binomial regression) to examine rate of wild warru drinking rate against each predictor variable. Significant *P* values are highlighted in bold.

Predictor	**Rate ratio**	**Lower CI**	**Upper CI**	***P* value**
Location: Inner vs outer	1.99	0.65	6.04	0.23
Mean minimum temperature (5^0C increments)	0.67	0.42	1.07	0.09
Mean maximum temperature (5^0C increments)	0.56	0.37	0.87	**0.01**
Total rainfall (10mm increments)	0.82	0.75	0.90	**<0.001**
Plant moisture content (5% increments)	0.60	0.54	0.66	**<0.001**
Mean 0900 hours humidity (%)	1.03	0.86	1.24	0.71
Mean 1500 hours humidity (%)	0.98	0.75	1.28	0.89
Rainfall 4 weeks prior (mm)	0.92	0.89	0.95	**<0.001**

Due to the high correlation between the values for ‘rainfall’ and ‘mean maximum temperature’ ($r = 0.93$, $P<0.0001$), two separate multivariate models were used to assess the influence of each predictor variable on warru drinking rate with two of the three predictor variables in each (Table 4.5).

Table 4.5. Multivariate modelling results for the influence of three predictor variables on the drinking rate of wild warru. Significant *P* values are highlighted in bold.

Model	Rate Ratio	Mean estimate	Lower CI	Upper CI	*P* value
1	**Mean max temp (5^{o}C increments)**	0.63	0.40	0.99	**0.05**
	Plant moisture content (5% increments)	0.74	0.59	0.91	**0.01**
2	**Rainfall (10mm increments)**	0.85	0.78	0.93	**<0.001**
	Plant moisture content (5% increments)	0.79	0.68	0.92	**<0.001**

Plant moisture content was negatively associated with warru drinking rate in both models (Table 4.5). A rate ratio [RR] of 0.74 in model 1 indicates that for every 5% increase in plant moisture content, warru drinking rate decreased by 26% (CI 0.59, 0.91; $P = 0.0055$). A similar result was found in model 2 (RR = 0.79; CI 0.68, 0.91; $P = 0.0024$), with warru drinking rate decreasing by 21% with every 5% increase in plant moisture content.

Warru drinking rate decreased by 37% with every 5^{o}C increase in maximum temperature (model 1 RR = 0.63; CI 0.4, 1.0; $P = 0.046$) and decreased by 15% with every 10mm increase in rainfall (model 2 RR = 0.85; CI 0.78, 0.93; $P = 0.0002$).

4.6.3.2 *Reintroduced population*

For reintroduced warru, five predictor variables were tested (Table 4.6). The univariate model indicated that the same predictor variables as those for the wild dataset had a significant association with the drinking rate of reintroduced warru (Table 4.6). However, no significant difference was found between drinking rates of each sex (RR= 1.16, CI: 0.56, 2.43, $P = 0.71$).

Table 4.6. Results of univariate modelling (negative binomial regression) to examine rate of reintroduced warru drinking rate against each predictor variable. Significant *P* values are highlighted in bold

Predictor	Rate ratio	Lower CI	Upper CI	*P* value
Sex: male vs. female	1.16	0.56	2.43	0.69
Mean minimum temperature (5^{0}C increments)	0.82	0.66	1.02	0.07
Mean maximum temperature (5^{0}C increments)	0.76	0.59	0.99	**0.04**
Total rainfall (10mm increments)	0.85	0.80	0.91	**<0.0001**
Plant moisture content (5% increments)	0.43	0.34	0.55	**<0.0001**

As per the wild analysis, a high correlation between the variables of maximum temperature and rainfall was found for the reintroduced dataset (r = 0.82, *P*=0.0001), and so two multivariate models were built to examine the effect of each predictor variable on warru drinking rate (Table 4.7).

Table 4.7. Multivariate model results for the influence of three predictor variables on the drinking rate of reintroduced warru. Significant *P* values are highlighted in bold.

Model	Rate Ratio	Mean estimate	Lower CI	Upper CI	*P* value
1	**Mean max temp (5^{0}C increments)**	0.92	0.73	1.16	0.49
	Plant moisture content (5% increments)	0.45	0.32	0.63	**<0.001**
2	**Rainfall (10mm increments)**	0.93	0.83	1.04	0.21
	Plant moisture content (5% increments)	0.50	0.33	0.76	**<0.001**

A 5% increase in the plant moisture content of the pintji hill decreased reintroduced warru drinking rate by 55% (model 1 RR = 0.45; CI 0.32, 0.62; *P* = 0.001) and 50% (model 2 RR = 0.5; CI 0.33, 0.76; *P* = 0.0013) (Table 4.7). Maximum temperature and rainfall did not have a significant influence on reintroduced warru drinking rate (Table 4.7).

4.7 Discussion

4.7.1 *The use of supplementary water points by warru*

The current study represents the first examination of the impacts of the provision of supplementary water points for remnant ('wild') and reintroduced rock-wallaby populations. Both populations rapidly habituated to the presence of supplementary water points, with water points at both sites being used for drinking within 0–10 days post-installation. The presence of a small stable warru group on the western side of the New Well hill was confirmed through the rapid use (six days after installation) of the water point placed there and a consistent visitation rate across the year. The other points situated outside of the main colony area (n = 2) were not used so readily, most likely due to very few warru residing at these locations.

Although the current study did not deliberately assess the distance travelled to water by warru, one marked male at the wild colony was captured at a remote camera drinking from a water point 2.7km from where he had been trapped four months prior, and a marked female warru was recorded twice in one night at two different water points (1.1km apart). This female was carrying a large pouch young and, as lactating *Petrogale* are known to have higher water turnover rates (Kennedy and Heinsohn 1974), it is possible that she was searching for additional free water, given that the first point she visited was almost empty. Due to an absence of home range data for either individual it is not known whether these outer water points fell within the individual's home range or whether they were making specific forays to visit the water points. However, mean home range area and movement patterns of other warru at this site (ref. Ch. 3) would suggest that either location was outside of the normal home range area for each individual based on their trapping location that year. In addition, the relatively few visits recorded for the individuals suggest they were not regularly using the area. Movements across similar distances in search of water have been observed previously in *Petrogale*, with yellow-footed rock-wallabies (*Petrogale xanthopus*) travelling between 1.5 and 2.5km to access water points (Sharp 2011) and 5km to access a water tank during a drought period (Lim and Giles 1987). Future research using colour photograph remote cameras may be able to increase individual identification rates, and combined with individual home range data, be used to calculate specific distances travelled to access free water.

Modelling indicated that natural free water and plant moisture content had a significant influence on the drinking rate of wild warru. The APY Lands experiences two main seasons during the year; a hot, wet summer and a cool, dry winter (Robinson *et al.* 2003). Warru drinking rate was significantly higher during drier winter observation periods. This is consistent with drinking behaviour being primarily driven by a reduction in dietary moisture as seen in other arid and semi-arid zone macropodid species (Sharp 2011; Underhill *et al.* 2007). Semi-arid *Petrogale xanthopus celeris* displayed a rapid decline in drinking frequency at an artificial water source after the first summer rainfall with drinking frequency increasing dramatically again once pasture moisture content became limiting at 20% (Sharp 2011). Indigenous consultants have also reported that rock-wallabies in Arnhem Land will drink from sandy billabongs when ‘desperate’ for water in the dry season (Telfer and Garde 2006). In the current study, plant moisture content was recorded at a low of 34% during the winter month of August 2011 with multivariate modelling predicting that drinking rate in wild warru would increase by 26% with every 5% decrease in plant moisture content. This therefore suggests that should plant moisture content reach the level of 20% recorded by Sharp (2011), drinking rates of wild warru will be expected to increase dramatically.

Seasonal changes in reintroduced warru drinking rate paralleled those patterns seen in wild warru. These findings provide evidence for the rapid adaptation of captive warru to resources in their new wild environment. The reduced drinking rates during the summer suggest that for the most part, reintroduced warru were obtaining the majority of their water requirements through dietary intake. This is further evidenced by the maintenance of excellent condition in individuals post-release (ref Ch. 2). This rapid transition to wild conditions was also documented in a study of water turnover rates for reintroduced *P. xanthopus* which found that individuals adapted relatively quickly to an absence of *ad libitum* free water as accustomed to in captivity (Lapidge and Munn 2012). In addition, these results allay concerns that the use of supplementary water in reintroduction programmes acts to discourage released individuals to adapt to wild resources (Bright and Morris 1994).

The results of the current study clearly indicate that both wild and reintroduced warru will seek free water to supplement dietary intake when low rainfall reduces dietary resources, as demonstrated in *P. xanthopus* (Horsup and Marsh 1992). Whilst water turnover rates clearly indicate that *Petrogale* can survive in the absence of rainfall (Hume *et al.* 2004; Lapidge and Munn 2012), these results provide further evidence that arid zone *Petrogale* may have a stronger reliance on free water than previously proposed (Sharp 2011). Restricted access to water during dry winters and drought periods may reduce the likelihood of wild and reintroduced warru population persistence.

4.7.2 *A potential tool for improving habitat quality and population persistence*

Arid and semi-arid zone macropodid population dynamics are tightly linked to environmental factors (Bayliss 1985; Cairns and Grigg 1993; Lethbridge and Alexander 2008; Underhill *et al.* 2007). Studies of kangaroo species have shown that population fluctuations in response to rainfall are mediated through juvenile recruitment and survival, with young-at-foot and weaned juveniles having the highest drought-related mortalities (Robertson 1986). These age classes have greater metabolic demands for growth which become problematic when only low quality, high-fibre forage, characteristic of arid regions during low rainfall and drought, is available (Munn and Dawson 2004). For example, weaned red kangaroos (*Macropus rufus*) were unable to sustain growth when fed forage with a fibre content above 40% (Munn and Dawson 2004). Additionally, in order to thermoregulate effectively, juvenile *M. rufus* were found to require 2.5 times more water than adults and thus may need to drink more regularly than adults to supplement dietary water intake (Munn and Dawson 2004).

The role of rainfall in boosting *Petrogale* survival is also well documented. In the allied rock-wallaby (*P. assimilis*), survival of young from birth to pouch emergence was strongly influenced by rainfall (Delean *et al.* 2009), likely due to lactating females being able to satisfy higher water turnover requirements when pasture was of high quality and water content (Kennedy and Heinsohn 1974). In addition, the survival rate of warru, particularly juveniles, is higher in years that experience winter rainfall (Ward *et al.* 2011a). This increase in juvenile survival is likely to contribute to positive population growth rates observed in *Petrogale* following a lag period ranging from 6 to 12 months after good rainfall (Lethbridge and Alexander 2008; Sharp and Norton 2000). However, rainfall is also likely to boost adult survival as low water turnover rates during winter for adult rock-wallabies (*P. x. celeris)* suggest that although they survived, adult individuals were also water limited during periods of low rainfall (Lapidge and Munn 2012).

It is therefore reasonable to assume that survival and recruitment is likely to decrease in warru populations that have restricted access to free water, particularly during dry periods and drought. Resource supplementation was found to reduce environmental and stochastic effects on the reproductive output of the Florida scrub-jay (*Aphelocoma coerulescens*) (Schoech *et al.* 2008) and the provision of sugar water significantly increased growth rates of reintroduced New Zealand hihi (stitchbird, *Notiomystis cincta*) populations (Armstrong *et al.* 2007). The provision of supplementary water during the drier months on the APY Lands (March to October) could therefore potentially improve habitat quality for warru, thus increasing the survival of adults and juveniles, which will have a positive impact on the growth rate of wild and reintroduced warru populations, thus assisting population persistence.

However, it is important to ensure that the implementation of one threat reduction strategy will not increase other known threats as a result. It was a concern of the Warru Recovery Team that the installation of water points would not only increase warru visitation rates to these caves but may also attract warru competitor species such as euros (Creese 2007; Ruykys 2011) and rabbits (Read and Bowen 2001; Read and Ward 2011a) or introduced predatory species such as cats, foxes and dingoes (Kinnear *et al.* 2010; Pearson 2012; Read and Ward 2011a). No significant difference was found between the visitation rates of competitor or introduced predator species at water points and control caves, suggesting these species were not actively selecting caves in which there was also high warru activity (at water points). However, perentie, thought to be occasional predators of warru (Read and Ward 2011a) had significantly higher visit odds to water points. The perentie visits were predominantly recorded at two of the water points (K3, n = 7 and K5, n = 14; cf. total perentie visits to all water points n = 38), no predation events were observed on the remote camera photographs and all but one of the perentie visits were recorded between 1000 and 1400 hours, when warru were not active. As individuals were not identified, these higher visit odds may have been the result of each water point being placed within the home range of a resident perentie. However, the impact of perentie predation on warru populations requires further investigation to evaluate whether this increased activity would pose a potential threat to warru recovery.

The few dingo and fox sightings suggest that presence around the hill is low and this is confirmed by track transects conducted by warru rangers (APY Land Management, unpub. data). However, cat numbers on the hill are suspected to be reasonably high with two 4-night spotlight shooting sessions during the study period removing 14 cats from the New Well hill (S Booth, pers. comm.). Euros are also regularly flushed whilst walking the hill slopes (R. West, pers obs.). The lack of cat and euro interest at the water points could have been due to the small volumes (8-L) of water provided, in addition to the fact that the points were placed within caves that restricted access to euros. Issues are more likely to occur when substantial artificial water sources are installed (such as rainwater tanks or drippers) which can act as focal points for introduced predator activity (Hume *et al.* 2004; James *et al.* 1999). Such artificial sources are also easily accessed by other herbivores, in particular large macropods, which can increase resource competition for the target species (Treby *et al.* 2007). The supplementary water point design used in this study may also be of benefit to other arid-zone rock dwelling species. Fat-tailed false antechinus were significantly more likely to visit a water point cave although a full analysis of drinking frequency was not conducted for this species.

4.7.3 Management implications and future research

The current study recommends the placement of small water points within the rocky habitat at *in situ* and reintroduced warru populations on the APY Lands which could be filled monthly with water during the drier winter months. The use of small water points (<10L) will enable the provision of this potentially limiting resource without problems such as increases in feral herbivores causing pasture damage or elevated predator presence that are incurred through the use of larger artificial water sources (Hume *et al.* 2004). The presence of the water points within the rocky habitat will also enable juveniles, who may need to drink more regularly, to access water without incurring additional predation risk, as found by Shepherd (1987) for juvenile *M rufus* that were targeted by dingoes when using a large watering hole. It would be wise to consider the use of water points following large scale wild fires which are known to place additional pressures on prey populations through acute resource depletion and removal of cover for predator protection (Letnic and Dickman 2006). In addition, it would be worth exploring the impact on warru population growth when rockholes and seeps are cleaned (as has traditionally occurred by traditional owners) so that water can collect naturally and which could be artificially filled during periods with little rainfall or severe drought.

The results of this study also have implications for future warru reintroduction protocols. Given that winter periods are clearly most challenging for warru in terms of water availability, it would be beneficial to time releases for reintroduction in the spring and autumn months. Although water availability is highest in the summer months on the APY Lands, temperature extremes may place additional stressors on newly released individuals who, like wild *Petrogale*, will have to adapt to using caves and crevices for shelter and thermoregulation (Lapidge 2001; Telfer and Griffiths 2006). Released warru will already be faced with the challenge of locating food in a novel environment, so releasing in months when food is likely to be of higher ‘quality’ should positively influence survival during the adaptation period. As the current study did not indicate habituation to the use of supplementary water points, it would also be worthwhile providing supplementary water in the first few weeks post-release, even when releases occur in spring and autumn, to further reduce physiological stress on released individuals and increase the chance of establishment (Dickens *et al.* 2010). However, placement of these water points should not be biased to particular habitat areas. The provision of one water point for the entire study period of the reintroduction of the greater stick-nest rat (*Leporillus conditor*) was proposed to have strongly influenced the behaviour and habitat use of the rats (Moseby and Bice 2004). Therefore, for future warru reintroductions water points should be distributed evenly across the selected hill and monitored by remote camera.

Further research could also repeat the methods of the current study to investigate the use of supplementary feeders in warru populations. Although supplementary food (kangaroo pellets) is currently being supplied to the Kalka colony, its use is not being formally assessed. When quokkas (*Setonix brachyurus*) were released into Jandakot reserve in Western Australia, grassed lawns were provided as additional food sources (Algar 1986). However, overgrazing by rabbits and other macropodid species was then attributed as the cause of failure for the reintroduction (Short *et al.* 1992) and hence highlights the importance in assessing the effective delivery of supplementary resources to the target species rather than inadvertently increasing threats to the population. One of the key questions in the warru recovery plan is ‘How important is water for warru, particularly during drought and for juvenile animals?’(Read and Ward 2011a). Although this question will be challenging to answer without long term monitoring of warru population trends, implementation of the methodology used in the current study could assist in addressing this knowledge gap. Future research could use standardised distances of cameras to water points so that relative sizes of visiting warru can be estimated. This could then be used to determine age-specific visitation rates.

Although conclusions on the specific requirements of warru for water and the effects on survival, recruitment and population growth cannot be made from the current study, the results clearly indicate that wild and reintroduced warru will be more reliant on free water during the dry winter months to supplement lower quality forage. Where access to free water is limited at *in situ* and reintroduced populations, supplementary water points can provide an effective substitute. Combined with a comprehensive monitoring programme, this tool should now be integrated within an adaptive management framework for the Warru Recovery Team to assist in increasing the probability of persistence for remnant and reintroduced warru populations.

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CHAPTER 5: MANAGING THE ADAPTIVE POTENTIAL OF CAPTIVE AND REINTRODUCED WARRU POPULATIONS.

Eric Abbott and Rebecca West taking an ear biopsy sample from a warru to unravel the secrets of historical connectivity and contemporary gene flow.
Photo: C Bailey

5.1 Preamble

This chapter presents genetic analyses using microsatellites and mitochondrial DNA to explore the historical and contemporary structure of the APY Lands warru populations and places this into the broader context of the MacDonnell Ranges race. This information is then used to assess the current genetic representation of the source populations within the captive groups at Monarto Zoo and the reintroduced population within the warru pintji. Recommendations are made for future management in order to maintain and increase genetic diversity within the captive populations and enhance the adaptive potential of reintroduced populations.

5.2 Statement of Authorship

Title of paper	**Managing the adaptive potential of captive and reintroduced warru populations.**						
Publication status	☐	Published	☐	Accepted for Publication	☐	Submitted for Publication	☒	Publication style

Author contributions
By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author	**Rebecca West**		
Contribution to the paper	Designed and developed work. Collated genetic samples, conducted all laboratory work/data analyses and wrote manuscript.		
Signature		Date	21/10/2013

Name of Co-Author	**Mark Eldridge**		
Contribution to the paper	Supervised design/development of work, laboratory work and data interpretation. Provided historical gDNA. Evaluated manuscript drafts.		
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Name of Co-Author	**Sally Potter**		
Contribution to the paper	Supervised design and development of work; laboratory work and data interpretation. Evaluated manuscript drafts.		
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Name of Co-Author	**David Taggart**		
Contribution to the paper	Commented on a final manuscript.		
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Contribution to the paper	Commented on a final manuscript.		
Signature		Date	21/10/2013

5.3 Abstract

The adaptive potential of a reintroduced population is, in part, driven by its genetic diversity and hence requires careful consideration in captive breeding and reintroduction programmes. Founders for a captive population of warru (black-footed rock-wallaby *Petrogale lateralis* MacDonnell Ranges Race) were collected in 2007 from three remnant populations on the Anangu Pitjantjatjara Yankunytjatjara (APY) Lands of South Australia. Using 14 microsatellite loci and a 672 base pair sequence of the mitochondrial DNA control region, this study examined the contemporary and historical genetic structure of warru populations on the Lands, more broadly within the MacDonnell Ranges race, and assessed the representation of this structure and diversity within the captive founders. Warru captive founders had significantly lower allelic diversity, heterozygosity and number of rare alleles than their wild source populations. The current management strategy to maintain founders from Kalka (n = 5) as a separate genetic group (a population 300km to the west of the two other source populations) limits pairing options and will rapidly result in inbreeding. Whilst microsatellite data present significant contemporary differentiation amongst the remnant warru populations, a lack of phylogeographic structure within the mitochondrial haplotypes suggests historical connectivity was higher. Based on these genetic data and ecological observations outbreeding depression is unlikely and hence the recommendation is made that captive populations be managed as one genetic group. This will increase the genetic diversity within the captive populations, and as result increase the adaptive potential of future reintroduced groups. A new genetically distinct population was also identified within the Musgrave Ranges, which could be used as a future source population for supplementing genetic diversity to the captive groups.

Keywords: *Petrogale lateralis*, mitochondrial DNA, microsatellites, genetic structure, genetic diversity, captive breeding management, reintroduction.

5.4 Introduction

Captive breeding for reintroduction is now widely used as a tool for threatened species conservation (Armstrong and Seddon 2008; Beck *et al.* 1994; Kleiman 1989; Seddon *et al.* 2012). Captive populations offer an *ex situ* insurance option for threatened species (Frankham *et al.* 2002; IUCN 1987) particularly important if the reasons for decline have not been identified and/or methods to remove threats are still being developed. The two key aims of the process are to (a) found a captive population that is genetically representative of the source population, displaying similar ecological characteristics and, (b) to provide individuals to establish a viable, free ranging reintroduced population that will require minimal long-term management to persist into the future (IUCN 1998). Success in producing self-sustaining populations has been variable among projects (Armstrong and Seddon 2008; Copley 1994; Fischer and Lindenmayer 2000; Griffith *et al.* 1989). Initial recruitment and population growth, often used to determine 'success', does not always equate to long-term persistence (Seddon 1999). Factors associated with the ability of a reintroduced population to establish (survive and recruit wild-born progeny) include the selection of appropriate habitat for resource provision (Armstrong and Perrott 2000; Cheyne 2006), removal of previous threats (Moseby *et al.* 2011; Short and Turner 2000) and demography and size of the release group (Le Gouar *et al.* 2008b; Maran *et al.* 2009). In order for reintroduced populations to persist into the future with minimal intervention they must also have the ability to adapt to future environmental change (Keller *et al.* 2012). In addition to ecological, environmental and demographic factors, the adaptive potential of a reintroduced population is driven by its genetic diversity and hence requires careful consideration in captive breeding and reintroduction programmes (Armstrong and Wittmer 2011; Robert 2009; Weeks *et al.* 2011).

The persistence of a population can be reduced by three genetic processes: genetic drift (changes in allele frequencies between generations), inbreeding depression and the accumulation of deleterious mutations. Each of these processes can increase extinction risk, either individually, through interactions with each other or interactions with demographic and environmental factors (Frankham *et al.* 2002; Gaggiotti 2003). These genetic issues are exacerbated in small populations (Armstrong and Wittmer 2011; Hedrick and Miller 1992; Nei *et al.* 1975) and thus are of particular importance in captive bred and reintroduced populations because the very nature of a threatened species means that from the outset, population sizes are usually small (Frankham *et al.* 2002). This often makes threatened species populations susceptible to multiple facets of the small population paradigm (Armstrong and Wittmer 2011; Caughley 1994). These genetic concerns are intensified by the fact that the threatened population is likely to be placed through a number of population bottlenecks in order to secure a captive population and initiate a reintroduction process (Keller *et al.* 2012).

Population bottlenecks occur when population size is suddenly reduced, resulting in decreased genetic diversity (Nei *et al.* 1975). In captive breeding for reintroduction programs, the first bottleneck invariably occurs during the process of 'genetic capture' (Weeks *et al.* 2011), when a usually small number of founders are harvested from a declining wild population (Keller *et al.* 2012). Whilst the process should aim to capture >95% of the genetic variation of the source population (Frankham *et al.* 2002; Weeks *et al.* 2011) studies have shown that captive and translocated populations typically show much reduced genetic diversity in comparison to their source populations (Sigg 2006; Stockwell *et al.* 1996) This is commonly linked to the initial number of founders harvested (Larson *et al.* 2002), poor breeding success following removal from the wild, and the number of generations spent in captivity (Frankham 2008; Robert 2009). An additional bottleneck often occurs during reintroduction when the captive population is sub-divided into individuals for release and individuals that remain as the *ex situ* insurance population (Keller *et al.* 2012).

In order to reduce the negative effects of small founding population size in both captive and reintroduced groups, the genetic diversity of the founding group needs to be maximised at each stage of the programme (Jamieson 2011). In the first instance this can be achieved through prior genetic monitoring to determine the source of and most appropriate number of founders to capture remnant population diversity (Earnhardt 1999; Jamieson 2011). Secondly, breeding management of founders should reflect the natural genetic structure and diversity of remnant populations (Groombridge *et al.* 2012; Le Gouar *et al.* 2008a). Thirdly, individuals for reintroduction can then be selected from known pedigrees to ensure that release groups are of diverse genetic composition to provide future populations with the best possible chance of persistence (Earnhardt 1999). Unfortunately genetic information to guide each of these stages is rarely available, or analyses are only conducted post-hoc, hence compromising long-term genetic management plans for reintroductions from captive animals (Weeks *et al.* 2011).

A captive breeding programme for reintroduction was initiated in 2007 for the warru (black-footed rock-wallaby *Petrogale lateralis* MacDonnell Ranges Race (MRR)) as part of the South Australian Warru Recovery Plan (Read and Ward 2011). Warru used to be widespread to the north and west of Lake Eyre in South Australia (SA), across the central eastern region of Western Australia (WA) and the southern Northern Territory (NT) (Pearson 2012). Dramatic population declines have occurred across the range in the last 80 years, with populations in SA currently found only on the Anangu Pitjantjatjara Yankunytjatjara (APY) Lands, where the extent of occurrence has decreased by 93% (Ward *et al.* 2011). The aim of the Warru Recovery Plan is to maintain genetic diversity and increase the distribution and abundance of warru in SA in order to achieve down-listing in conservation status from 'Endangered' to 'Vulnerable' (National Parks and Wildlife Act 1972) (Read and Ward 2011).

In order to sample the genetic diversity remaining in SA warru, pouch young were harvested from the largest known SA colonies (New Well, Alalka and Kalka) (Read and Ward 2011) and cross-foster reared (Taggart *et al.* 2010) at Monarto Zoo, SA between 2007 and 2009. A study using six microsatellite loci detected limited contemporary gene flow between the New Well and Alalka populations but not Kalka (Ruykys 2011). To avoid outbreeding depression, pouch young were managed as two genetic groups in captivity with Kalka individuals kept separate to New Well/Alalka warru (Ruykys 2011). Progress towards increasing the distribution of warru began in 2011 with the soft-release of 16 of the New Well/Alalka captive individuals into a predator exclosure (warru pintji) on the APY Lands (Muhic *et al.* 2012), leaving the Kalka group and a small number of New Well/Alalka individuals in captivity. The goal is that the warru pintji population will be used to provide groups for hard reintroductions on the APY Lands over the next five to ten years. As a result, careful consideration of the genetic management of the populations in captivity and the warru pintji was necessary to ensure that any reintroduced population has the greatest genetic diversity possible and thus adaptive potential. Knowledge of the *in situ* relationships between populations within the APY Lands and in the broader context of the MRR's range (which still extends into

WA and NT) was not fully understood and hence required a review in order to ensure historical and contemporary genetic structure could be reflected in future management decisions.

This study used microsatellite and mitochondrial markers to address the following aims:

1. Determine the natural genetic structure of the APY Lands populations in the broader context of the genetic structure of the MacDonnell Ranges race.
2. Examine the representation of source genetic diversity within the founding captive groups and the currently maintained captive and reintroduced individuals.
3. Make recommendations for future genetic management of captive and reintroduced groups to maintain/increase adaptive potential.

5.5 **Methods**

5.5.1 *Study sites and sample collection*

Warru have been trapped annually at three main sites (New Well - NW [26.080°S, 132.215°E], Alalka - AL [26.140°S, 132.115°S] and Kalka - KA [26.138°S, 129.160°E]) (Figure 5.1) on the APY Lands since 2005, as part of monitoring by the Warru Recovery Project (Read and Ward 2011). Between 2010 and 2012, following evidence of warru presence at Kaaṉka Mangka - KM [26.038°S, 132.231°E] and apparent recolonisation at New Well Far West - NWFW [26.087°S, 132.196°E], annual trapping was expanded to include these two sites. Serendipitously, one female was trapped on the south of the New Well hill - NWS [26.105°S, 132.213°E] during a one-off trapping event to retrieve missing GPS collars (ref. Ch. 3). At each trapping session, an ear biopsy (4mm diameter) was collected from previously unsampled independent warru and fully furred pouch young (PY) using a standard leather punch and stored in 90% ethanol. In total, 204 ear biopsies were collected across the six sites (Figure 5.1).

Between 2007 and 2009 a captive population of warru was established by cross-fostering (Taggart *et al.* 2010) 26 PY from wild dams to *P. xanthopus* (yellow-footed rock-wallaby) surrogates at Monarto Zoo, SA. Of those PY harvested, 22 were successfully raised and once fully furred, were sampled via an ear biopsy. Two PY thrown from their dam's pouches during trapping in 2010/11 were hand-reared at Adelaide Zoo and then added to the captive population. In total, 24 captive founders were taken from the wild populations (NW n = 10, AL n = 8, KA n = 6). Ear biopsies were also collected from offspring born at Monarto Zoo as a result of NW/AL pairings (n = 4). In total, 28 captive samples were analysed.

To place the APY Lands warru into a broader context, 22 *P. lateralis* MacDonnell Ranges race (MRR) samples from 7 sites throughout the taxon's range were obtained from the Australian Museum collection (extracted genomic DNA) (WA - Walter James Range [24.62795°S, 128.72286°E] and Bell Rock [26.20593, 128.71689], SA - Sentinel Hill [26.07947, 132.4502] and Davenport Ranges [28.40204, 136.01577], and NT - Arltunga Historical Reserve [23.449544, 134.719686], Telegraph Station Historical Reserve [23.652664, 133.867203] and Heavitree Gap [23.729071, 133.867203]) (Figure 5.4). A *P.*

penicillata sample (New South Wales) and a *P. l. lateralis* (WA) sample were obtained from the museum collection and used as outgroups in phylogenetic analyses.

5.5.2 *DNA extraction*

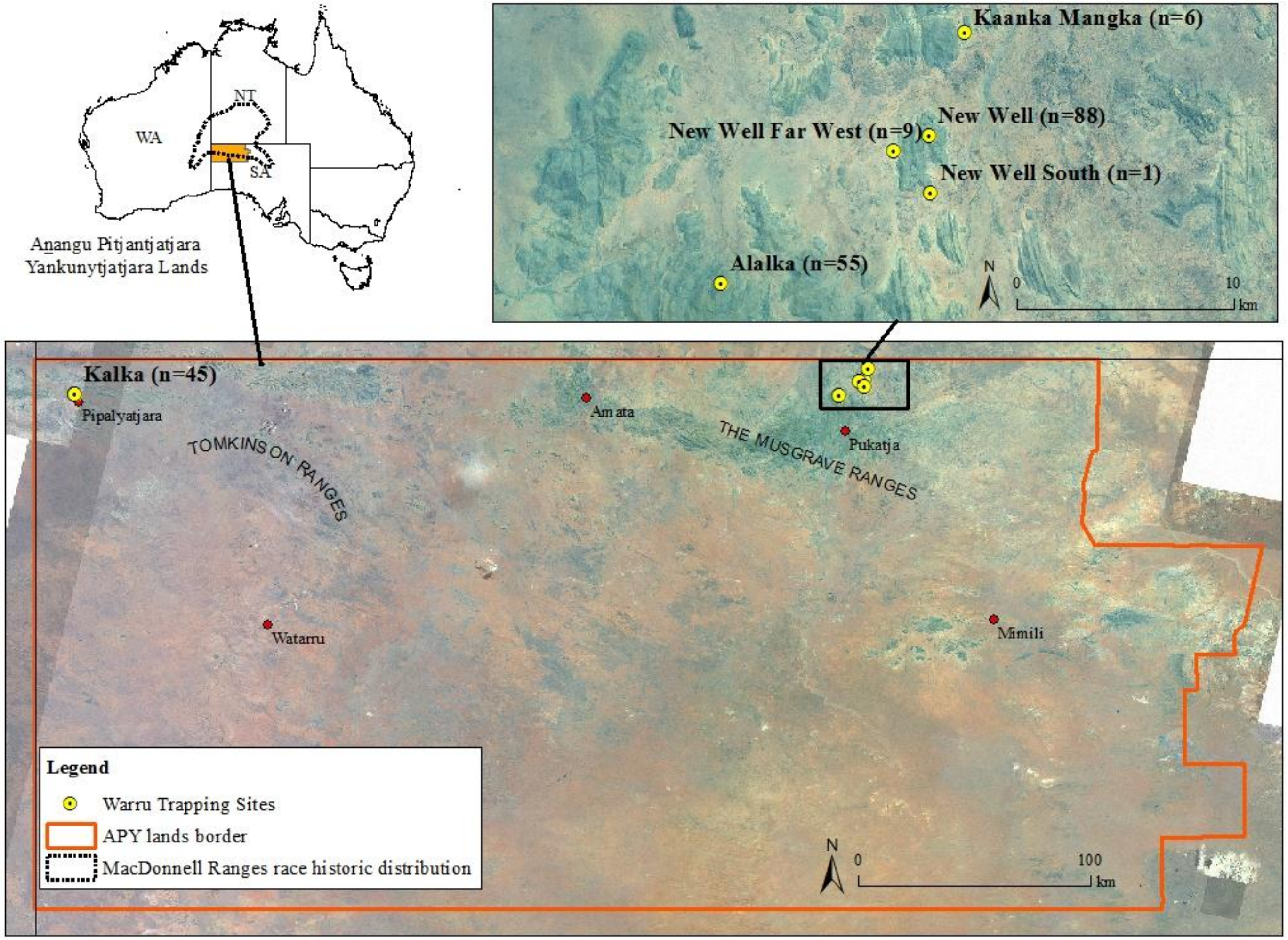
Genomic DNA (gDNA) from APY Lands warru sampled from 2005 to 2009 was already available in the Australian Museum collection, therefore extraction of gDNA was only conducted for samples collected from 2010 to 2012, using the 'salting out' method of Sunnucks and Hales (1996).

5.5.3 *Microsatellite genotyping*

A total of 189 wild (sampled PY were excluded) (NW n = 77; AL n = 54; KA n = 42; KM n = 6; NWFW n = 9; NWS n = 1) and 28 captive warru samples were screened with 15 microsatellite loci; five identified from *P. assimilis*, the allied rock-wallaby (Pa297, Pa385, Pa55, Pa593, Pa597) (Spencer *et al.* 1995); one from the eastern grey kangaroo *Macropus giganteus* (G26-4) (Zenger and Cooper 2001); four from the tammar wallaby *M. eugenii* (Me2, Me14, Me16 and Me17) (Taylor and Cooper 1998); and five from the yellow-footed rock-wallaby *P. xanthopus* (Y76, Y105, Y148, Y170 and Y175) (Pope *et al.* 1996). Samples were amplified, pooled and genotyped by the Australian Genome Research Facility (AGRF) in two panels; Panel 1: Pa597, Me16, Me14, Y175, Y170, Me2, Pa385 and Panel 2: Pa593, Y148, G26-4, Me17, Pa55, Y105, Pa297, Y76.

Each microsatellite locus was checked for conformance to Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium within each population using GENEPOP (v4; Raymond and Rousset, 1995) with 10,000 iterations of the Markov chain method. *P*-values were adjusted using the sequential Bonferroni procedure (Rice 1989). The presence of null alleles was also tested using GENEPOP.

Figure 5.1. Map of warru trapping sites on the A<u>n</u>angu Pitjantjatjara Yankunytjatjara Lands of South Australia with number of samples collected at each site.



5.5.4 *Mitochondrial sequencing*

A total of 140 samples were used for mitochondrial DNA (mtDNA) analysis. This included a subset of the APY Land samples across years (NW n = 53; AL n = 24; KA n = 26; KM n = 6; NWFW=9 and NWS n = 1) and the 22 MRR samples. The mtDNA control region (CR) was amplified in polymerase chain reactions (PCRs) using primers L15199M and H16498M (Fumagalli *et al.* 1997). Each 25µl PCR was carried out with 200-400ng of gDNA, 1 x reaction buffer, 2.5mM $MgCl_2$, 0.2mM dNTPs, 100ng/µl bovine serum albumin, 2pmol of each primer, 5µl of Q solution and Taq polymerase (0.5U). Thermocycling conditions were initial denaturation at 94°C for 2 minutes; 35 cycles of 94°C for 2min (denaturation), 60°C for 40s (annealing) and 72°C for 50s (extension); followed by a final extension for 5min at 72°C. PCR products were purified using USB® ExoSAP-IT® (Affymetrix) and then sequenced on an AB 3730*xl* DNA Analyzer (Applied Biosystems) by AGRF.

Mitochondrial sequences were edited using SEQUENCHER (v 4.10.1; Gene Codes Corporation) and aligned using MEGA (v5.05; Tamura *et al.*, 2011). DNASP (v5.10; Librado and Rozas, 2009) was implemented to estimate the number of polymorphic sites and indel sites. ARLEQUIN (v. 3.5; Excoffier and Lischer, 2010) was used to calculate the number of haplotypes (H), nucleotide diversity (π) and haplotype diversity (h) (Rozas *et al.* 2003).

5.5.5 *Population genetic structure and differentiation*

Genotypes of all individuals trapped and sampled across the six APY Lands sites (NW, AL, KA, NWFW, KM and NWS) between 2005 and 2012 were analysed using STRUCTURE 2.3.3 (Pritchard *et al.* 2000) to assess whether each site represented a discrete population. STRUCTURE utilises a Bayesian clustering method to group individuals into 'genetic clusters'. The number of genetic clusters (K) was estimated from 1 to 8, using admixture ancestry, independent allele frequencies, a burn-in length of 100,000 iterations and a run length of 200,000 Markov chain Monte Carlo repetitions, with 10 iterations for each value of K. The true value of K was estimated from the highest log-likelihood value of K and highest ΔK values (rate of change in the likelihood of K) (Evanno *et al.* 2005).

Microsatellite differentiation between populations was examined using pairwise F_{ST} (Wright 1951) in FSTAT (version 2.9.3; Goudet, 1995) based on 200 permutations. The extent of mtDNA differentiation (pairwise Φ_{ST}) between each APY Land site was examined using the distance method of Tamura and Nei (based on 110 permutations and Wright's *F* statistic;Wright, 1951) in ARLEQUIN.

5.5.6 Genetic diversity

Microsatellite and mitochondrial diversity was estimated for each population identified through STRUCTURE, F_{ST} and Φ_{ST} analyses. Individuals from each site were split into two capture periods: those sampled between 2005 to 2009 (in concordance with a previous genetic analysis using 6 microsatellite loci (Ruykys 2011) and those sampled between 2010 to 2012 (this group also included the genotypes of individuals sampled between 2005 to 2009 that were known to be alive in the populations between 2010 to 2012).

Allelic diversity (*AD*; average number of alleles per locus), allelic richness (*AR*; allelic diversity corrected for sample size) and F_{IS} (proportion of total inbreeding) were calculated using FSTAT. The F_{IS} value for each population was tested using Weir and Cockerham's estimator (Weir and Cockerham 1984) with 1,000 permutations. Observed and expected heterozygosity (H_O and H_E respectively), the number of rare alleles (*rA*: allele frequency less than 5%) and the number of unique alleles (*uA*) were calculated in GenAlEx6 (Peakall and Smouse 2006).

Significant differences in *AD, AR,* H_E and *rA* at each locus between a) populations within each time series and b) within each individual population between the two time series, were tested using Wilcoxon signed rank tests (SYSTAT v.9), with *P*-values corrected using the sequential Bonferroni method.

5.5.7 *Phylogenetic analyses*

The program MODELTEST 3.7 (Posada and Crandall 1998) was used to select the best-fit nucleotide substitution model for the aligned sequences using Akaike Information Criterion (AIC) values. The best fit model was then used to produce a consensus tree of mtDNA haplotypes using Bayesian inference algorithms in MRBAYES (v3.1.2; Huelsenbeck and Ronquist, 2005). The analysis was run using default settings as priors, a random starting tree and four Markov chains (three hot, one cold). Two independent analyses were run simultaneously for 2 million generations, sampling every 1000 generations. TRACER (v.1.5; Rambaut and Drummond, 2009) was used to check that convergence of parameter estimates and log likelihood values had occurred. Posterior probabilities were calculated after discarding the first 10% of the sampled trees as burn-in. In addition a maximum likelihood search, with an estimate of the proportion of invariable sites was conducted in RAxML (v7.0.3; Stamatakis *et al.*, 2008) to produce a consensus tree with bootstrap confidence at each node. Low genetic variation can be problematic in resolving nodes for phylogenetic analyses; therefore a haplotype network was also generated using TCS (v1.21; Clement *et al.*, 2000) to assess the relationships of mtDNA haplotypes.

5.5.8 *Population of origin assignment of individuals from new sampled localities*

The potential population of origin of warru sampled at the new sites (NWFW, KM and NWS) was investigated in the program GENECLASS (v2.0.h; Piry *et al.*, 2004). Using the Bayesian method of Rannala and Mountain (1997), individuals were assigned to one of the three reference populations (NW, AL, KA) if their probability of belonging was greater than 0.99. Putative first generation migrants were detected within each population using the method of Rannala and Mountain (1997) and probabilities computed using the method of Paetkau *et al.* (2004). The comparison took into account that each individual could belong to any one of the identified populations but that not all possible populations may have been sampled (L = L_home). Immigrants that did not satisfy the *P*-value for belonging to any of the populations were considered immigrants from unsampled colonies.

5.5.9 *Captive population comparisons*

Mitochondrial haplotypes of captive warru were inferred from known maternal haplotypes. Genetic diversity indices ($AD, Ar, H_O, H_E, rA, F_{IS,}$ h) were estimated as described above for the following captive warru groups:

a) NW captive founders

b) AL captive founders

c) Kalka captive founders

d) NW/AL captive founders ((a) and (b) combined – represents captive breeding structure)

e) Pintji founders (captive warru released to warru pintji (97-ha exclosure) 2011 – 2012)

f) NW/AL captive group at Monarto Zoo (present in 2012)

g) Kalka captive group at Monarto (present in 2012)

To determine the relative proportion of genetic diversity captured and retained in each captive group, genetic diversity indices were also estimated for the appropriate wild ‘comparative’ groups:

A1) NW wild population (all individuals trapped 2005 – 2012)

B1) AL wild population (all individuals trapped 2005 – 2011)

C1) KA wild population (all individuals trapped 2005 – 2012)

D1) NW/AL metapopulation ((a) and (b) combined as one population)

The total number of alleles (A_{TOT}) was also calculated for each group, in order to examine the percentage of wild alleles that were captured in each of the founding captive groups.

Wilcoxon signed rank tests were used to compare the genetic diversity within each current captive population (e, f and g) to their founding captive population (a, b, c and/or d) and to their relative wild population (A1, B1, C1or D1). *P*-values for multiple comparisons were corrected using the sequential Bonferroni method.

5.6 Results

5.6.1 *Microsatellite analyses*

A total of 184 wild individuals (89 males and 95 females) from across the four sampling locations (NW (3 sites), AL, KA and KM) were genotyped at 14 loci (5 individuals were removed as they failed to amplify at >50% of loci). Pa55 failed to amplify for the majority of samples and so was removed from the analysis. In addition, the 24 captive founders and 4 captive offspring were also genotyped at the 14 loci.

Microsatellite loci in each population were in HWE except for Me-17, G26-4 and Pa597 at NW; Me17 and Pa597 at AL; and Me-17 G26-4 and Me-2 at KA. Since Me-17 had significant homozygote excess at three of the four populations (NW, AL and KA), analyses were conducted with and without this locus. As the same pattern of population differentiation was detected, Me-17 was retained within the dataset. There was no evidence of significant linkage disequilibrium between any pairs of loci across the populations. Null alleles were not detected.

5.6.2 *Mitochondrial analyses*

A 672 base pair (bp) sequence of the CR was analysed for 140 MRR individuals. This consisted of 118 individuals from the APY Lands sites, plus an additional 22 individuals from across the taxon’s distribution. The sequence contained 69 polymorphic sites, 58 of which were parsimony informative and 6 indel sites. A total of fourteen mtDNA haplotypes were identified across the range (Table 5.1). Within the APY Lands, only two of the sites displayed haplotypic and nucleotide diversity: Alalka (h=8.3%, π=0.4%) and New Well (h=6.4%, π=0.3%). Kalka had a different haplotype to those found at New Well and Alalka but there was no haplotype diversity within the site (Table 5.1). Across the MRR, three additional sites (Walter James Range (WA), Arltunga Historical Reserve (NT) and the Davenport Range (SA)) displayed haplotype and nucleotide diversity with the highest diversity found across the Walter James Range (h=83.3%, π=0.9%) (Table 5.1). The two haplotypes present in the Davenport Range (SA) population differed by a single indel.

Table 5.1. Distribution and frequency of haplotypes identified in *Petrogale lateralis* MacDonnell Ranges race sampled across the taxon distribution.

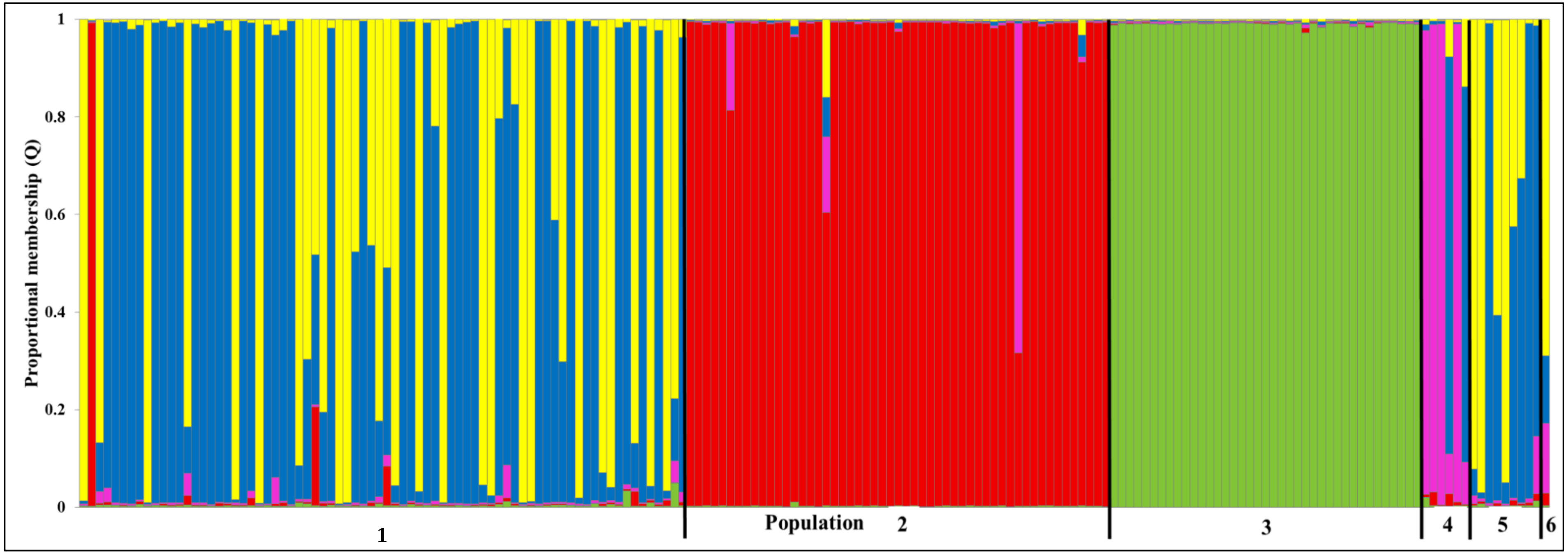
Site	**Location**								**Haplotypes**								
		n	A	B	C	D	E	F	G	H	I	J	K	L	M	N	*h* (± *S.D*)	π
New Well	APY, SA	62	60	2													0.0635 ± 0.042	0.00268
Alalka	APY, SA	24	1	23													0.0833 ± 0.075	0.00352
Kalka	APY, SA	26			26												0	0
Kaan̲ka Mangka	APY, SA	6		6													0	0
Sentinel Hill	APY, SA	1				1											0	0
Davenport Range	SA	7					3	4									0.5714 ± 0.12	0.00086
Walter James Range	WA	4							1	1	2						0.8333 ± 0.222	0.00856
Bell Rock	WA	1										1					0	0
Arltunga Historical Reserve	NT	5											2	2	1		0.800 ± 0.164	0.02801
Telegraph Station Historical Reserve	NT	2														2	0	0
Heavitree Gap	NT	2											2				0	0

5.6.3 *Population genetic structure*

STRUCTURE analyses indicated three (highest Δ*K*) or five (highest log likelihood) genetic clusters amongst the APY Lands warru populations. For both *K*=3 and *K*=5 a distinct KA and AL cluster was identified. KA individuals were assigned to the one cluster with high probability (>0.9). This was also the case for all AL individuals apart from one male who was assigned with high probability to the NW cluster. With *K*=3 a single NW cluster was identified which included all NW sampled warru (except one male assigned to the AL cluster), warru trapped at NWFW, 2 of the 6 warru trapped at KM and the lone female trapped at NWS (all were assigned with probability >0.9). The other four warru sampled at KM were not reliably assigned to any one of the clusters at $K = 3$ ($p < 0.8$).

At *K*=5 the NW cluster was sub-divided into three separate groups (Figure 5.2). Warru sampled at NW were placed into two clusters with 70% of individuals assigned with high probability to one of the two clusters. The remaining 30% of warru were assigned to both clusters with mixed ancestry (probability <0.8 for either cluster). Of the warru sampled at NWFW, five were reliably assigned to one of the two clusters whilst the other four had mixed ancestry. The lone female at NWS was assigned with mixed ancestry, as were two males trapped at KM. The remaining KM warru were assigned with high probability to a third separate cluster (>0.9).

Figure 5.2. STRUCTURE box plot showing membership (*Q*) for each APY Lands warru to one of five identified genetic clusters (blue, yellow, red, green and pink). Each individual is represented by a vertical bar. Solid black lines and corresponding numbers below plot indicate different sampling sites (1=New Well, 2=Alalka, 3=Kalka, 4=Kaa<u>n</u>ka Mangka, 5= New Well Far West, 6 = New Well South).



5.6.4 *Population differentiation*

All populations showed significant microsatellite differentiation (F_{ST}) (Table 5.2) except for NW and NWFW.

Table 5.2. Microsatellite differentiation amongst sampled warru sites on the APY lands identified through pairwise F_{ST}. * = significant pairwise comparison (*P*<0.05) after Bonferroni correction

F_{ST}	NW	AL	KA	KM	NWFW
NW		0.1620 *	0.1472 *	0.0672 *	0.0059
AL			0.2592 *	0.1600 *	0.1705 *
KA				0.1795 *	0.1610 *
KM					0.0796 *

Pairwise comparisons showed significant mitochondrial differentiation (ϕ_{ST}) between all populations apart from; NW and NWFW; and AL and KM (Table 5.3) as they share the same haplotype (Table 5.1).

Table 5.3. Mitochondrial (mtDNA) differentiation amongst sampled warru sites on the APY Landss identified through pairwise ϕ_{ST} . * = significant pairwise comparison (*P*<0.05) after Bonferroni correction

Φ_{ST}	NW	AL	KA	KM	NWFW
NW		0.9166 *	0.9153 *	0.9316 *	-0.0447
AL			0.9572 *	-0.0856	0.9366 *
KA				1.0000 *	1.0000 *
KM					1.0000 *

5.6.5 *Genetic diversity*

As a result of the genetic structure and genetic differentiation results, subsequent analyses of genetic diversity amongst the APY Lands populations consider four populations: NW (including NWFW individuals and the female from the south of the hill), AL, KA and KM.

Genetic diversity estimates at NW and KA did not differ significantly between the two time periods (Table 5.4) whilst AL had significantly lower AD and rA in 2010 – 2011 than 2005 – 2009 (Table 5.4). There was no evidence for significant inbreeding (FIS) for any of the populations (*P*>0.05). All populations had uA and rA present (Table 5.4).

Three haplotypes were detected across the four APY Lands populations (Table 5.1). Two of these haplotypes were present in both NW and AL between 2005 and 2009, although at each site, one haplotype dominated with the second haplotype occurring at a low frequency (h = 10% and 13% respectively) (Table 5.4). This reduced to only one haplotype at AL between 2010 and 2012. In addition, only one haplotype was detected for KA and KM individuals (Table 5.4).

Table 5.4. Mean (± s.e.) measures of diversity at 14 microsatellite loci and the mtDNA control region for four populations of *P. lateralis* (MacDonnell Ranges race) across two time periods (2005-2009 and 2010–2012).* Significant difference (P<0.05) between the time series within a population. Diversity measures provided are: allelic diversity (*AD*); allelic richness (*AR*); F_{IS} (proportion of total inbreeding); observed and expected heterozygosity (H_O and H_E); number of rare alleles (*rA*); number of unique alleles (*uA*); number haplotypes (*H*); nucleotide diversity (π); and haplotype diversity (*h*).

Population	*n*	*AD*	*AR*	H_O	H_E	*uA*	*rA*	F_{IS}	*n*	*H*	*h*	π
New Well 2005 - 2009	*40*	7.7 ± 0.6	5.1 ± 0.2	0.76 ± 0.03	0.79 ± 0.02	1.8 ± 0.6	2.4 ± 0.5	0.043	*20*	2	0.100 ± 0.088	0.00393
New Well 2010 - 2012	*61*	7.8 ± 0.6	4.9 ± 0.2	0.74 ± 0.03	0.77 ± 0.02	1.8 ± 0.4	3.0 ± 0.5	0.044	*41*	2	0.095 ± 0.061	0.00374
Alalka 2005 - 2009	*37*	6.2 ± 0.4	4.1 ± 0.2	0.63 ± 0.05	0.67 ± 0.03	1.1 ± 0.3	2.2 ± 0.3	0.078	*15*	2	0.133 ± 0.112	0.00524
Alalka 2010 - 2011	*26*	4.7 ± 0.3 *	3.8 ± 0.2	0.64 ± 0.05	0.67 ± 0.02	1.1 ± 0.3	1.0 ± 0.2 *	0.07	*14*	1	0.00	0
Kalka 2005 - 2009	*17*	4.8 ± 0.3	4.1 ± 0.2	0.63 ± 0.06	0.67 ± 0.03	1.6 ± 0.3	0.4 ± 0.2	0.134	*6*	1	0.00	0
Kalka 2010 - 2012	*29*	4.9 ± 0.4	3.9 ± 0.2	0.63 ± 0.06	0.67 ± 0.03	1.5 ± 0.3	1.0 ± 0.2	0.076	*21*	1	0.00	0
Kaanka Manka 2010 - 2012	6	4.6 ± 0.3	4.6 ± 0.3	0.79 ± 0.05	0.68 ± 0.03	0.5 ± 0.2	0	-0.063	6	1	0.00	0

NW had significantly higher *AD*, H_E and *rA* than all other populations in both time periods (Table 5.3). *AR* was significantly higher at NW than both AL and KA, which were not significantly different from each other (Table 5.4, Figure 5.3). However, *AR* at KM was not significantly different from NW and H_E did not differ significantly between AL, KA and KM (Figure 5.3). AL displayed significantly higher *rA* than KA in 2005-2009 but this significance was not present in 2010-2012, which saw an increase in *rA* at KA and a decrease at AL (Table 5.4, Figure 5.3).

Figure 5.3. Pairwise comparisons of genetic diversity between three populations of warru on the APY Lands (2005 – 2009) and a fourth population Kaanka Mangka (2010 – 2012). Significant differences are displayed by symbols as follows *AD*=allelic diversity; *AR*= allelic richness; H_E = heterozygosity; *rA*=rare alleles. Underscore indicates a non-significant difference.

2005 to 2009	**New Well**	**Alalka**
New Well		
Alalka	AD AR H_E __	
Kalka	AD AR H_E rA	A __ __ rA

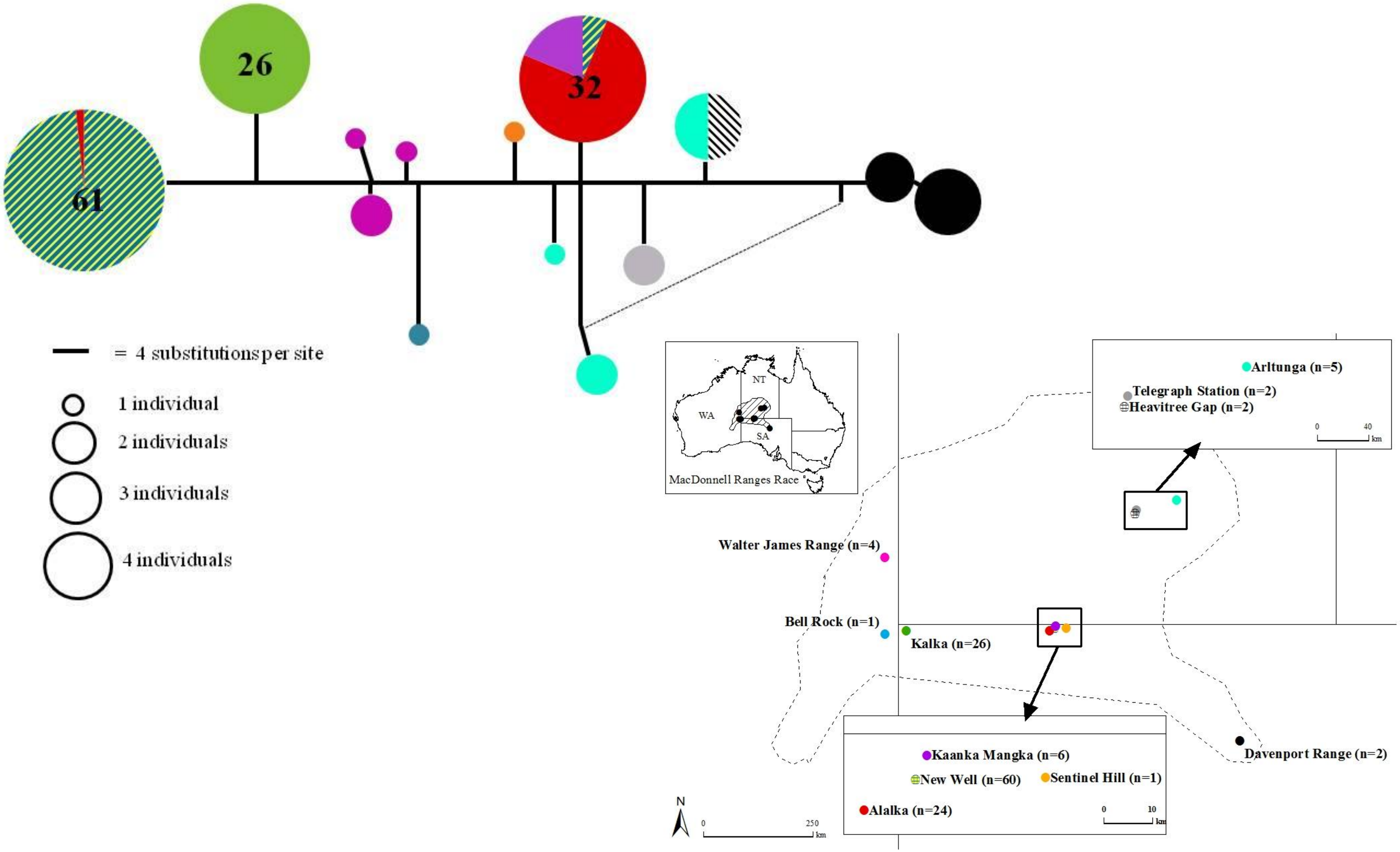
2010 to 2012	**New Well**	**Alalka**	**Kalka**
New Well			
Alalka	AD AR H_E rA		
Kalka	AD AR H_E rA	__ __ __ __	
Kaanka Maanka	AD __ H_E rA	__ __ __ rA	__ __ __ rA

5.6.6 *Phylogenetic analyses*

Fourteen CR haplotypes were identified across the MRR distribution (Table 5.1). Average pairwise sequence divergence between haplotypes was 3.9% (range 0.7 – 5.9%). For the three haplotypes identified within the current APY Lands populations, average sequence divergence was 3.8% (range 2.5 – 4.5%).

Maximum likelihood and Bayesian analyses both produced trees of similar topology which placed the 14 haplotypes in a monophyletic group with low probabilities and bootstrap confidence for the resolution of further nodes. Given the poor resolution of nodes, a haplotype network is presented in Figure 5.4. Each circle represents a haplotype and colours are site specific, thus one circle with multiple colours indicates haplotypes that were shared across sites (Figure 5.4). This indicates that while some individuals from WA and NT tend to group together within the network, there appeared to be no clear relationship between geographic and genetic distance (Figure 5.4).

Figure 5.4. Haplotype network of the 14 mitochondrial *CR* haplotypes detected across 10 sample sites of *Petrogale lateralis* MacDonnell Ranges race. Colours display sampling site (see map inset) and printed numbers or circle size represent the number of individuals with each haplotype. The dashed line denotes two substitutions per site.



5.6.7 *Population of origin assignment of individuals from newly sampled sites*

GENECLASS2 assigned two warru sampled at KM to NW as their population of origin with probabilities >0.99, but they were not detected as first generation migrants. The other four warru from KM could not be assigned to any of the known populations with a probability <0.01 suggesting they represent an unsampled population. All warru sampled at NWFW were assigned to NW as their source population with probabilities >0.99 and none were detected as first generation migrants.

Three first generation migrants were detected within the NW and AL populations. One male sampled at NW had a probability <0.01 of being an AL migrant and conversely one male sampled at AL had a probability <0.01 of being a NW migrant. One female sampled at AL was also not assigned to any of the other populations with a probability >0.8, suggesting she is a migrant from an unsampled site. No immigrants were detected for the KA population.

5.6.8 *Captive genetic diversity*

Of the A_{TOT} present in each wild population, 72% were harvested in captive founders from New Well (NW), 61% from Alalka (AL) and 71% from Kalka (KA) (Table 5.5). As NW and AL captive founders were subsequently combined, these individuals contained 75% of the alleles present in a combined NW/AL wild population. *AD* was significantly lower in all captive founder groups compared to their wild source population (Tables 5.5, 5.6 and 5.7). The captive KA group at Monarto Zoo consisted of five individuals. *AD* and H_E were significantly lower in this group in comparison to both the wild KA population and the original captive founders (Table 5.6). The group now represents 63% of the alleles present at KA (Table 5.5).

When corrected for sample size, *AR* was not significantly different between wild source and captive founders (Tables 5.5, 5.6 and 5.7). Rare alleles were not present in the KA founders but a subset was present in the combined NW/AL captive founders (Table 5.7). H_E of each captive founder group was significantly lower than the wild source except for those founders removed from AL (Tables 5.6 and 5.7). No significant evidence of inbreeding (F_{IS}) was detected within any of the captive founder groups (Table 5.5).

Table 5.5. Genetic diversity estimates at 14 microsatellite loci and the mitochondrial control region for founding captive populations and current captive populations of warru in comparison to each wild source population. *Significant differences between captive founders and source population for New Well and Alalka are displayed with a *. For other population comparisons, see Tables 6 and 7.*

Population	***n***	**AD**	**A_{TOT}**	**AR**	**Ho**	**He**	**rA**	**F_{IS}**	**n**	**NH**	**h**
New Well wild population	*85*	8.1 ± 0.6	113	4.3 ± 0.1	0.75 ± 0.03	0.78 ± 0.02	3.0 ± 0.6	0.045	*55*	2	0.07
New Well captive founders	*10*	5.8 ± 0.3 *	81	4.3 ± 0.2	0.68 ± 0.06	0.75 ± 0.02 *	0 *	0.149	*3*	1	0.00
Alalka wild population	*54*	6.6 ± 0.4	92	3.5 ± 0.2	0.63 ± 0.05	0.67 ± 0.02	3 ± 0.4	0.077	*23*	2	0.09
Alalka captive founders	*8*	4.0 ± 0.3 *	56	3.4 ± 0.2	0.68 ± 0.06	0.64 ± 0.03	0 *	0.011	*6*	1	0.00
New Well/Alalka metapopulation	*139*	9.6 ± 0.9	134	4.6 ± 0.2	0.7 ± 0.03	0.81 ± 0.01	4 ± 0.8	0.135	*78*	2	0.48
New Well/Alalka captive founders	*18*	7.2 ± 0.5	100	4.5 ± 0.2	0. 68 ± 0.04	0.78 ± 0.02	1.0 ± 0.3	0.167	*9*	2	0.50
Pintji founders (reintroduced)	*14*	6.2 ± 0.4	87	4.3 ± 0.2	0.68 ± 0.04	0.76 ± 0.02	1 ± 0.3	0.138	*7*	2	0.47
New Well/Alalka captive group at Monarto	*4*	3.9 ± 0.5	54	3.9 ± 0.5	0.70 ± 0.07	0.65 ± 0.04	0	0.143	*2*	2	0.50
Kalka wild population	*40*	5 ± 0.4	70	3.5 ± 0.2	0.62 ± 0.06	0.68 ± 0.03	1 ± 0.2	0.096	*24*	1	0.00
Kalka captive founders	*6*	3.6 ± 0.3	50	3.6 ± 0.3	0.59 ± 0.08	0.60 ± 0.04	0	0.143	*2*	1	0.00
Kalka captive group at Monarto	*5*	3.1 ± 0.3	44	3.0 ± 0.2	0.56 ± 0.09	0.57 ± 0.04	0	0.126	*2*	1	0.00

Table 5.6. Significant pairwise differences in four genetic diversity estimates denoted by the presence of a symbol (allelic diversity – *AD*; allelic richness – *AR*; expected heterozygosity – H_e; rare alleles - *rA*) between a wild source population (Kalka wild), harvested captive founders (Kalka captive founders) and the currently maintained captive population (Kalka captive group Monarto) of *P. lateralis* MacDonnell Ranges race. Non-significant differences are denoted by __.

	Kalka wild	**Kalka captive founders**
Kalka wild		
Kalka captive founders	AD __ H_E rA	
Kalka captive group Monarto	AD __ H_E __	AD __ H_e __

There was no difference in the *AR* of the NW/AL combined breeding group, and those individuals released for reintroduction in comparison to the NW/AL metapopulation (Table 5.5, Table 5.7). However, the two current captive groups (reintroduced group in the warru pintji and group maintained at Monarto Zoo) both have significantly lower *AD*, H_e and *rA* in comparison to the wild metapopulation (Table 5.7).

Table 5.7. Significant pairwise differences in four genetic diversity estimates denoted by the presence of a symbol (allelic diversity – *AD*; allelic richness – *AR*; expected heterozygosity – H_e; rare alleles - *rA*) between a wild source population (New Well/Alalka metapopulation), harvested captive founders (New Well, Alalka captive founders), the individuals from this breeding group that were reintroduced (Pintji reintroduction founders) and the remaining captive population (New Well/Alalka Monarto) of *P. lateralis* MacDonnell Ranges race. Non-significant differences are denoted by __.

	New Well/Alalka Metapopulation	**New Well/Alalka captive founders**	**Pintji reintroduction founders**
New Well/Alalka metapopulation			
New Well/Alalka captive founders	AD __ H_E rA		
Pintji reintroduction founders	AD __ H_E rA	AD __ H_E __	
New Well/Alalka Monarto	AD __ H_E rA	AD __ H_E rA	n/a

5.7 Discussion

An understanding of natural population genetic structure and differentiation is important when considering captive breeding and reintroduction programs for the management of threatened species (Hazlitt *et al.* 2006b; Jamieson and Lacy 2012). Threatened species by their nature are likely to have experienced localised extinction and recolonisation processes which can significantly impact the contemporary genetic structure of remnant populations (McCauley 1991). Genetic markers that can provide insight into historical genetic structure and population connectivity are therefore very useful (Johnson, 2000; Hedrick and Miller, 1992). Previous microsatellite information on the structure of populations of *P. lateralis* MacDonnell Ranges race (MRR) on the APY Lands led to the mixing in captivity of individuals from the Musgrave Ranges (New Well and Alalka populations) whilst keeping Kalka animals as a separate genetic group (Ruykys 2011). Using microsatellite and mitochondrial markers, this study confirms significant contemporary genetic differentiation between the main populations on the Lands, but also provides evidence for additional structure and gene flow across the Musgrave Ranges, and broader historical connectivity across the MRR. As a result, recommendations are made to manage future captive and reintroduced warru populations as one genetic group to minimise inbreeding and genetic drift and to ensure that natural diversity and structure is maintained (Jamieson 2011).

5.7.1 *Contemporary genetic structure of warru on the APY Landss*

Microsatellite analyses indicated that warru on the Lands are structured into three main clusters: New Well (NW), Alalka (AL) and Kalka (KA), with the newly sampled sites at Kaa<u>n</u>ka Mangka (KM), New Well Far West (NWFW) and New Well South (NWS) included within the main NW cluster. These three main clusters are consistent with previous analyses, using six microsatellite loci, of samples collected at NW, AL and KA between 2005 and 2009 (Ruykys 2011). The three clusters display high population differentiation for both microsatellite alleles and mitochondrial haplotypes. Strong population structuring, even across short distances (e.g. 12km separates NW and AL) is a common feature of *Petrogale* populations (Hazlitt *et al.* 2006b; Telfer and Eldridge 2010) and is thought to be the result of innate behaviours of *Petrogale* which lead to low male dispersal and high female philopatry (Eldridge *et al.* 2010; Hazlitt *et al.* 2006a; Sharp 1997).

This study only found evidence for the successful dispersal of two males between NW and AL and the dispersal of one female to AL from an unsampled population. No evidence of dispersal was documented for KA. This low level of gene flow supports the conclusion that dispersal events are rare within *Petrogale* (Eldridge *et al.* 2010; Pope *et al.* 1996; Sharp 1997). The two male dispersers were also detected in the study of Ruykys (2011), and led to the conclusion that these two colonies formed part of a Musgrave Ranges metapopulation. As a result, the captive breeding founders from NW and AL were maintained as one genetic group and the KA founders were kept separately, despite significant microsatellite differentiation between all three populations (Ruykys (2011) and this study).

The new population at KM (just 5km north of NW) included two male dispersers from NW and four individuals that were not assigned to any of the sampled colonies as population of origin. As a result, KM shows significant microsatellite differentiation from the other populations although it was not identified as a separate cluster within some STRUCTURE analyses. Interestingly KM warru share the most common mitochondrial haplotype detected at AL, the potential reasons for which are discussed later. Warru sampled at NWFW were not significantly differentiated from NW. This genetic connectivity is not unsurprising given that the sites are separated by 1.7km of continuous rocky habitat which is shown to reduce differentiation in other *Petrogale* populations (Potter *et al.* 2012a). The presence of new genetic diversity at KM and evidence of warru movement between NW and KM shows promise for the future adaptive potential of warru. Movements such as these are likely to decrease the chances of inbreeding and reduce genetic drift between populations, both of which are linked to extinction risk in highly fragmented populations of threatened species (Gaggiotti 2003; Nei *et al.* 1975). The detection of unsampled genetic diversity also indicates the existence of other warru populations within the Musgrave Ranges which warrants further investigation.

Despite apparent low levels of connectivity and small minimum population size estimates (range n = 10 at KM to n = 40 at NW), genetic diversity found in the APY Lands populations is similar to other *Petrogale* studies (Eldridge *et al.* 2010). In comparison to the study years of 2005 to 2009, diversity estimates at all three populations were similar between 2010 and 2012 and there was no evidence of significant inbreeding or increases in inbreeding across the time series. A lack of inbreeding despite small population size has been found in other rock-wallaby populations (Hazlitt *et al.* 2006b; Pope *et al.* 1996; Telfer and Eldridge 2010) with strong breeding group structure proposed as an inbreeding avoidance mechanism (Hazlitt *et al.* 2006a; Piggott *et al.* 2006). The evidence of sub structuring at NW (STRUCTURE analyses at *K*=5 assigned individuals to two genetic clusters with mixed ancestry) warrants further investigation to conclude if inbreeding avoidance is driven by the presence of spatially separated breeding groups. Whilst the maintenance of genetic diversity and lack of significant inbreeding is encouraging for the adaptive potential and likely persistence of the APY Lands populations, periodic genetic monitoring should be conducted to determine whether gene flow at the present low rate will have long term impacts on the genetic diversity of each population (Stockwell *et al.* 1996).

5.7.2 *Historical genetic structure of the APY Lands warru*

This study represents the first analysis of mtDNA diversity across the MRR, enabling the structure observed within APY Lands warru to be placed within a broader context.

This study identified four mtDNA *CR* haplotypes across the APY Lands (this includes one haplotype at the now extinct Sentinel Hill population) with an average sequence divergence (SD) of 3.4%. Although each site was dominated by a single haplotype (Table 5.1), some haplotypes were shared between populations (Figure 5.4;Table 5.1). The low haplotype diversity at each site indicates that contemporary female and male movements between these populations are limited and may also be an artefact of declining population size (Browning *et al.* 2001) which has been documented through spotlighting and scat quadrats since monitoring commenced in 2000 (Read and Ward 2011).

Unlike other *Petrogale* studies (Browning *et al.* 2001; Pope *et al.* 1996; Potter *et al.* 2012b), within the APY Lands there was no apparent correlation between geographic distance and mtDNA sequence divergence (SD). For example, the haplotypes at KA and NW (300km apart) differed by 2.5% SD, whereas 4.5% SD separated the dominant haplotypes at Alalka and New Well (12km apart). This level of SD at a small spatial scale (12km) is unusual in comparison to studies of *P. xanthopus* colonies that found

0.42% SD at distances of 10-70km apart (Pope *et al.* 1996) and 0.8% SD between colonies of *P. brachyotis* 4km apart (Telfer and Eldridge 2010). Strong phylogeographic structure in other *Petrogale* species is indicative of biogeographical barriers, leading to reduced gene flow which influences haplotype distribution (Potter *et al.* 2012b). However, the presence of two divergent haplotypes (A and B - 4.5%SD) within both the NW and AL populations and no correlation between genetic and geographic distance suggests that long term isolation is unlikely to be the evolutionary explanation for the observed divergence pattern within the APY Lands.

When placed into the broader context of the *P. lateralis* MRR, a lack of strong phylogeographic structure is also apparent. Whilst some samples from the NT and WA tend to group together within the haplotype network, there was no evident association between geographic and genetic distance. Byrne *et al.*(2008) outlined two common phylogeographical patterns within Australian arid-zone biota. The majority of arid-zone species appear to show multiple clades with high diversity, indicating that species have contracted and expanded from multiple refugia during evolutionary history whilst low diversity in more vagile species has been used to suggest more recent expansion (Byrne *et al.* 2008). The fact that the 14 MRR haplotypes could not be resolved beyond a monophyletic group and do not display a geographical and genetic distance pattern suggests a potential combination of these two patterns. The low diversity between the MRR haplotypes suggests relatively recent separation (Byrne *et al.* 2008) whilst the spatial patterning of these different haplotypes indicates that historical migration rates during cycles of local contraction and expansion must have been higher than observed within contemporary populations. High historic levels of female mediated gene flow would have resulted in a lack of spatial separation of haplotypes within ancestral populations. Random dispersal to recolonise habitat following periods of isolation during the glacial maxima of the Pleistocene, could therefore have resulted in the phylogeographic pattern observed in the MRR. A similar phylogeographic pattern has been documented in arid zone populations of *Petrogale xanthopus* (Potter, S. and Eldridge, M. pers. comm.). It is therefore possible that this pattern is common to arid zone *Petrogale* populations and warrants further investigation.

5.7.3 *Reflection of contemporary and historical genetic structure in captive warru populations*

The proportion of wild genetic diversity captured during the establishment phase of a captive colony has a lasting impact on the adaptive potential, and hence likely persistence, of reintroduced populations established from these colonies (Jamieson 2011); (Biebach and Keller 2010). Assessment of the representation of contemporary and historic genetic diversity and structure within a captive group is therefore essential to determine breeding strategies and release individuals that will maintain genetic variability captured in founding individuals (Stockwell *et al.* 1996), and accurately reflect natural population structure (Hazlitt *et al.* 2006b; Jamieson and Lacy 2012).

Warru captive founders had significantly lower allelic diversity, heterozygosity and number of rare alleles than their wild source populations. No rare alleles were captured in the founder groups, which is consistent with theoretical predictions of population bottlenecks suggesting that rare alleles will be most impacted (Nei *et al.* 1975) and is commonly documented in vertebrate translocations (Stockwell *et al.* 1996). This study further supports bottleneck theory, which also predicts that after rare alleles, allelic diversity will be second most impacted, followed by heterozygosity (Nei *et al.* 1975). Of the allelic diversity in the source population, 28% was lost in the removal of New Well captive founders, 39% at Alalka and 29% at Kalka, whilst heterozygosity was reduced by 4% in New Well and Alalka founders and 12% in Kalka founders. These results are unsurprising, given that only 6-10 PY were removed from each of the three genetically differentiated sites and some of these PY were removed from the same mother in consecutive years (Warru Recovery Team, unpublished data). These numbers are well below the 20 – 50 captive founders suggested in order to capture >95% of genetic diversity of source populations (Frankham *et al.* 2002; Weeks *et al.* 2011). Due to the limited diversity in mitochondrial haplotypes in the remnant populations, 100% of mtDNA diversity was captured within the founders. Furthermore, the combining of NW/AL individuals into one genetic group has facilitated the mixing of the two maternal haplotypes present in these colonies, which has actually produced a haplotype diversity index within this group akin to presumed historical levels. Whilst this shows promise for the retention of historical structure, the percentage of allelic diversity lost in this study is akin to a natural bottleneck that occurred as a result of a population crash of *P. penicillata* at Jenolan Caves (26% of alleles, 10% heterozygosity and 72% of rare alleles were lost) (Eldridge *et al.* 2004). Similar patterns of allelic diversity loss have also been observed in translocations of bighorn sheep (*Ovis canadensis*) (Fitzsimmons *et al.* 1997) and bridled nailtail wallabies (*Onychogalea fraenata*) (Sigg 2006). The results of this study clearly highlight the bottleneck effect on genetic diversity, which is commonly associated with establishing and maintaining captive breeding and translocated groups from a small number of wild founders (Eldridge *et al.* 2001; Lennon *et al.* 2011).

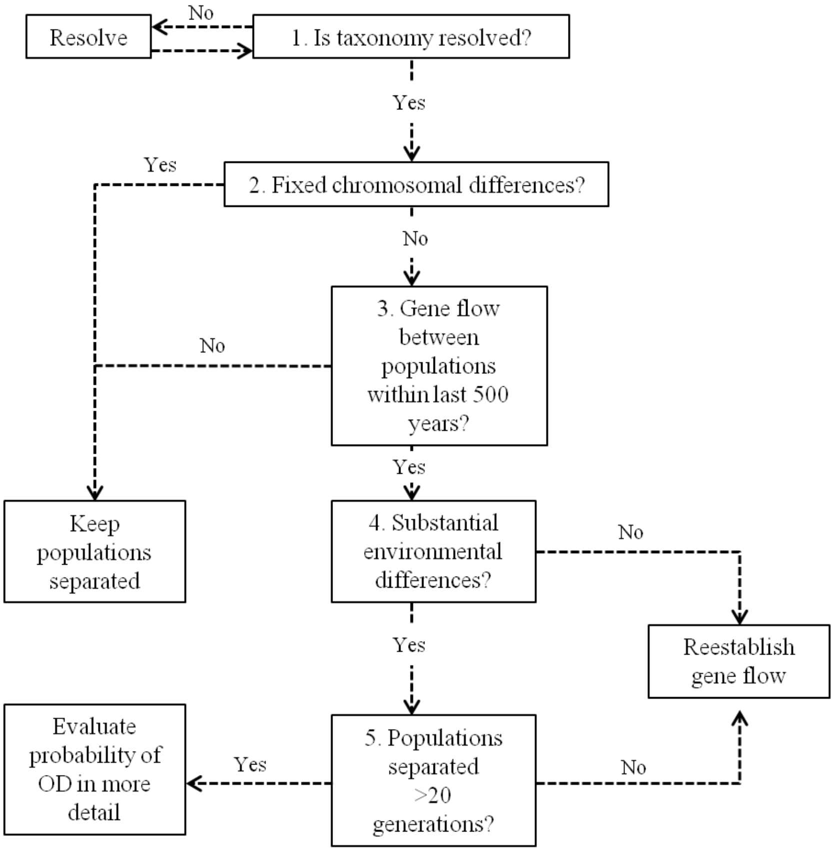
A drastic reduction in allelic diversity following a bottleneck can limit the adaptive potential of populations, even when heterozygosity remains relatively high (Nei *et al.* 1975). Management of the captive founders as two separate genetic groups, deaths of genetically diverse individuals and the reintroduction of 76% of the NW/AL group have increased genetic drift between the captive and wild source populations. The currently maintained groups at Monarto Zoo represent just 40% of the allelic diversity of the NW/AL metapopulation and 63% of the allelic diversity at KA. As a result of space limitations at Monarto Zoo, no attempts were made to breed KA individuals until October 2012 (five years after foundation) and minimal breeding was conducted between NW/AL individuals. Furthermore, three of the five individuals (one male, two females) within the KA group are half-siblings, which limit the options for pairings that will avoid inbreeding. Indeed, early, rapid population growth rates have been shown to be important in reducing inbreeding levels and genetic drift in reintroduced populations of the alpine ibex *(Capra ibex ibex*) (Biebach and Keller 2010). The reintroduced population in the warru pintji currently represents 65% of the NW/AL metapopulation diversity and also contains 3 half siblings, which has already led to inbreeding (R West, unpublished data). Whilst the level of inbreeding is presently not significant, maintaining the current captive groups will likely lead to further inbreeding and genetic drift within a few generations (Sigg 2006). As both genetic diversity and population size are correlated with population fitness (Reed and Frankham 2003), the reduction in genetic diversity and small population size within the captive warru groups is cause for concern and requires active management.

5.7.4 *Recommendations for future genetic management of captive and reintroduced warru populations*

The lack of phylogeographic structure and low sequence divergence in *CR* haplotypes across the *P. lateralis* MRR suggests that historical gene flow occurred across warru populations on the APY Lands. This is consistent with information from early explorers and Anangu informants who declared the warru as a ‘common’ animal, that was ‘swarming the hills’ (Finlayson 1961; Nesbitt and Wikilyiri 1994). The contemporary high microsatellite differentiation between the APY Lands populations and low mitochondrial diversity at each site is therefore a likely consequence of reduced gene flow following post-decline isolation and may not represent natural population structure (Browning *et al.* 2001; Potter *et al.* 2012a).

Knowledge of the historical gene flow patterns across the APY Lands would suggest that future management of captive and reintroduced warru populations should mix warru from KA with the NW/AL individuals. Reticence to mix differentiated populations in captive management is driven by concerns of inducing outbreeding depression which can also lead to a reduction in fitness (Frankham *et al.* 2011). However, the risk of outbreeding depression is often substantially less than the impacts of inbreeding depression and genetic drift and thus should be considered thoroughly to ensure that management options are not going to be restricted (Weeks *et al.* 2011). The decision framework of Frankham *et al.* (2011) (Figure 5.5) was used to weigh the risks of outbreeding depression in the captive warru populations.

Figure 5.5. The decision framework presented in (Frankham *et al.* 2011) to predict outbreeding depression



1. and **2.** The warru of the APY Lands are part of the distinct MacDonnell Ranges chromosomal race of *Petrogale lateralis* (Sharman *et al.* 1989).

3. At the time of European settlement this genetic race occupied the largest distribution of any of the *P. lateralis* groups (Pearson 2012) and is reported to have occurred across the APY Lands (Finlayson 1961; Nesbitt and Wikilyiri 1994). The mtDNA results of this study supports the long-term connectivity of populations across the taxon's range and suggest that limited contemporary gene flow across the APY Lands is most likely a result of the dramatic contraction in distribution and abundance that has occurred over the last 80 years (Read and Ward 2011).

4. Whilst geology differs between the Musgrave and Tomkinson Ranges (Robinson *et al.* 2003), the rocky escarpments offer similar vegetation assemblages, including *Ficus brachypoda* (Fig) and *Pandorea doratoxylon* (Spearbush), both preferred food plants of warru (Geelen 1999). Locations experience similar climate (Bureau of Meteorology) and warru captured at both sites display similar morphology, timing of breeding and developmental characteristics (Ruykys 2011).

Due to a lack of substantial environmental differences, the decision framework would therefore propose re-establishing gene flow between the populations (Frankham *et al.* 2011). The mixing of the NW/AL and KA founders and offspring could be a practical and cost-effective method of increasing the present genetic diversity within the captive populations, whilst also representing historical genetic structure within future reintroduced groups. However, it must be noted that captive populations will still continue to require the introduction of new genetic material to negate the effects of small effective population size across multiple generations (Weeks *et al.* 2011). Failure to do this has been documented in the bridled nailtail wallaby (*Onychogalea fraenata*), where a lack of new genetics to the captive population saw the continued decrease in heterozygosity and allelic diversity of translocated populations despite repeated supplementations (Sigg 2006). The microsatellite results of this study suggest that unsampled genetic diversity could be captured by adding new individuals from KM to the captive populations. In addition, periodic reviews of the genetic diversity within and between captive and reintroduced populations will enable the most appropriate management to be implemented (Stockwell *et al.* 1996).

The current study indicates the importance of securing large founder groups for captive warru colonies to avoid population bottlenecks and ensure that a high proportion of source variation is captured to minimise genetic diversity loss. It also highlights that genetic diversity within captive groups should be fully assessed to enable refinement of founder choice, breeding protocols and release group selection (Arrendal *et al.* 2004; Biebach and Keller 2010; Earnhardt 1999; Goossens *et al.* 2002; Jamieson 2011). For example, post release genetic assessments of reintroduced griffon vulture *(Gyps fulvus)* populations enabled genetic drift from source populations to be monitored and led to the refinement of captive breeding protocols and release group selection to maintain genetic diversity in reintroduced groups (Le Gouar *et al.* 2008a). The use of markers to highlight ancestral connectivity are useful in determining whether contemporary structure is actually indicative of the scale on which these threatened populations used to be independent (Pope *et al.* 1996; Potter *et al.* 2012a). This knowledge can have implications for the breeding management of captive and reintroduced groups and thus research to clarify this should be given high priority. This information can not only ensure the selection of genetically variable reintroduction groups but allows for consideration of natural population connectivity and gene flow when selecting sites and strategies for reintroduction (Le Gouar *et al.* 2008a). Limited dispersal in *Petrogale* means that reintroduced groups are unlikely to move and recolonise new areas very quickly. Isolating reintroduced groups from other known populations will remove the potential for gene flow and hence lead to effective population sizes below that required to maintain their adaptive potential (Weeks *et al.* 2011). Genetic variation has been successfully retained in reintroduced populations of the gray wolf (*Canis lupus*) where migration from native populations could occur (Forbes and Boyd 1997). Future warru and other *Petrogale* reintroductions should therefore aim to establish genetically diverse populations that are near to extant colonies and/or other reintroduced groups. This will allow reintroduced populations to naturally disperse, thus increasing the chance of establishing viable, resilient populations with high adaptive potential that are able to persist into the future with minimal management.

5.8 References

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CHAPTER 6: PREDICTING THE VIABILITY OF REINTRODUCED WARRU (BLACK-FOOTED ROCK-WALLABY) POPULATIONS.

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Rangers collect five captive warru from Pukatja airstrip to release into the warru pintji, July 2012. Photo: L Olds

6.1 Preamble

This chapter presents a population viability analysis (PVA) for warru reintroduction. Using the observed demographic rates presented in chapter 2 and the PVA software, *VORTEX*, a baseline model was constructed to predict the future growth of the warru pintji population. Simulations were then implemented to test the impact that harvesting groups of varying size from the pintji population would have on a) the growth of the pintji population and b) the establishment probability of a hard release population. This chapter provides guidance on release group sizes, timing of releases and future monitoring that will be required to evaluate model predictions, revise parameter estimates and improve model accuracy to assist future adaptive management.

6.2 Statement of Authorship

Title of paper	**Predicting the viability of reintroduced warru (black-footed rock-wallaby) populations.**							
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Author contributions

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Name of Principal Author	**Rebecca West**		
Contribution to the paper	Designed and developed model. Ran all scenarios and conducted analyses. Wrote manuscript.		
Signature		Date	28/10/2013

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6.3 Abstract

A clear priority for any reintroduction project is to select release strategies that will establish a self-sustaining population. However, there is often little prior information or experience on which to base these critical decisions. Population viability analysis (PVA) offers a useful tool for managers to examine the potential outcomes of alternate management options to facilitate structured decision making. The reintroduction of captive warru (black-footed rock-wallaby, *Petrogale lateralis* MacDonnell Ranges race) to the Anangu Pitjantjatjara Yankunytjatjara (APY) Lands of South Australia involved a transitionary period of a soft-release into a 97-ha predator-free enclosure (the warru 'pintji') on the APY Lands. It was therefore necessary to predict the growth rate of the pintji population in order to determine sustainable harvest schedules for hard release groups (releases to unfenced natural habitat). Models were constructed using the PVA software *VORTEX* with input parameters derived from demographic rates observed in the first 24 months post-captive release and previously calculated estimates of wild warru populations. The baseline demographic parameters resulted in positive deterministic growth rates for populations within the pintji (r = 0.19) and for hard-release reintroductions outside the enclosure (r = 0.063). Within the pintji, *VORTEX* simulations of different removal strategies highlighted that supplementation of additional captive warru and delaying harvest by at least 1 year would increase the realised growth rate and reduce the probability of the population going extinct. In addition, simulations of hard release group sizes estimated that groups of at least 10 warru would be preferable to reduce the probability of extinction below 10%. Sensitivity testing of the model input parameters highlighted the need for further monitoring to improve understanding of juvenile survival rates and the impact of predation on age-specific survival as population growth rates and extinction probabilities were most sensitive to variations in these parameters. The results of this study provide a baseline around which managers can evaluate future growth rates of the pintji and hard release populations. Refinement of model input parameters through continued monitoring of releases may improve the predictive accuracy of the models so that they can be used to facilitate adaptive management approaches for future warru reintroductions.

Keywords: reintroduction, *Petrogale*, population viability analysis

6.4 Introduction

Reintroduction is a popular, yet often ‘risky’ option for the recovery of threatened species. In concept, reintroduction offers a viable strategy to extend the range and size of populations threatened with extinction, yet the outcomes have been highly variable and frequently resulted in failure (Fischer and Lindenmayer 2000; Griffith *et al.* 1989; Short 2009). This is because conservation managers often have to make important decisions regarding the ‘when?’, ‘how many?’ and ‘where?’ of reintroduction with very little, or in some cases no, knowledge of the basic biology, response to new habitat and population demographics of a species post-release (Converse *et al.* 2013; Nichols and Armstrong 2012; Runge 2013). This can result in the use of release protocols that cause over-dispersal from the release site (Le Gouar *et al.* 2008), release of group sizes that do not enable establishment (Bester and Rusten 2009), or over-harvesting of source populations (Todd *et al.* 2002), all of which can result in functional extinction of either reintroduced or source populations. As reintroduction programs are typically expensive, both in terms of monetary cost and the risk to the founders, success is paramount. Although outcomes can never be guaranteed, the likelihood of success can be increased if uncertainty in responses to management actions are assessed and used to drive formal decision making (Converse *et al.* 2013).

Population models offer a useful framework in which managers can address uncertainty in reintroduction decisions (Converse *et al.* 2013). Using estimates of demographic rates and life history traits, population viability analysis (PVA) predicts the current viability of a population and the likely extinction of that population over particular time scales and under varying management scenarios (Brook *et al.* 2000). In the context of reintroduction, PVA can offer insight at multiple stages of the decision process (Armstrong and Reynolds 2012). During planning, PVA can be used to assess whether reintroduction is a suitable conservation strategy. For example, feasibility assessments for reintroduction have been conducted using PVA for the European beaver (*Castor fiber*), in Scotland (South *et al.* 2000) and the African wild dog (*Lycaon pictus*) in South Africa (Bach *et al.* 2010). The factors likely to affect the growth rate of a released population can be examined with PVA and the predictions applied to estimate optimal release group sizes, intervals of release and spatial configuration of release sites (Converse *et al.* 2013). Estimates of future population size and probability of long-term persistence can also be derived from PVA models (Armstrong and Reynolds 2012), incorporating additional factors such as genetic diversity and inbreeding depression into these estimates (Possingham *et al.* 1993). Integrated within adaptive management processes, PVA is therefore capable of providing quantitative measures for reintroduction success against which management actions can be evaluated (Lindenmayer *et al.* 1993) and guidance for when management intervention may be required.

The use of PVA in reintroduction is a new and emerging field, with the majority of papers on the subject published in the last 15 years (Armstrong and Reynolds 2012). Whilst many advocate that PVA should be integral to guiding research and monitoring programmes for recovery of threatened species (Lindenmayer *et al.* 1993; Morris *et al.* 2002), there has been much scepticism surrounding the accuracy of PVA predictions (Ball *et al.* 2003; Ellner *et al.* 2002). This is because the predictive capability of PVA is governed by the robustness of the input data (Brook *et al.* 2000). As many reintroductions involve threatened species for which empirical data are lacking, the inherent limitations of PVA can be exacerbated (Lindenmayer *et al.* 1993). However, if PVAs are viewed firstly as relative estimates rather than absolute (Beissinger and Westphal 1998); secondly incorporate a level of uncertainty in the absence of reliable data (Coulson *et al.* 2001; Ellner *et al.* 2002) and thirdly are part of an adaptive management strategy that uses continued monitoring to refine model input parameters (Dimond and Armstrong 2007; Possingham *et al.* 1993), then PVA can be a useful tool in decision analysis surrounding reintroduction (Coulson *et al.* 2001).

A captive breeding for reintroduction programme was initiated for the warru (black-footed rock-wallaby, *Petrogale lateralis* MacDonnell Ranges race) in 2007 through the cross fostering (Taggart *et al.* 2010) of pouch young from wild dams trapped on the A<u>n</u>angu Pitjantjatjara Yankunytjatjara (APY) Lands to surrogate *P. xanthopus* mothers at Monarto Zoo, South Australia. The goal of the Warru Recovery Team is to use reintroduction to increase the distribution and abundance of warru on the APY Lands (Read and Ward 2011). This process occurred in two stages. Firstly, captive warru were transferred to a 97-ha predator exclosure (‘the warru pintji’) on the APY Lands in 2011 and 2012 to establish a source population for future releases. Secondly, it is planned that the warru pintji population will be harvested to provide groups for release at new unfenced sites (hard release) on the APY Lands. In order to ensure that removal of groups from the warru pintji population would be both a) sustainable in terms of the continued viability of the warru pintji population and b) be of a suitable size to increase the likelihood of establishment of the release group, it was critical to predict the growth of the pintji population and how group size would impact establishment and success of hard release populations. Although a number of successful wild to wild translocations have occurred for *P. lateralis lateralis* in Western Australia and *P. lateralis pearsoni* in South Australia (Pearson 2012), this was both the first use of captive warru and the first use of an intermediate soft-release protocol for reintroduction which therefore created a level of uncertainty in the expected outcomes.

The current study implemented a PVA approach to assist future management decisions through prediction of:

1. The annual mean population size of warru in the pintji given the observed survival and reproductive rates in the first 24 months post-release;
2. The factors most likely to impact the growth rate of the warru pintji population;
3. How harvesting of different group sizes and timing of removal will impact the pintji population size and extinction probability; and
4. How founding group size and variation in demographic rates will affect establishment (extinction) probability for hard release groups.

6.5 Methods

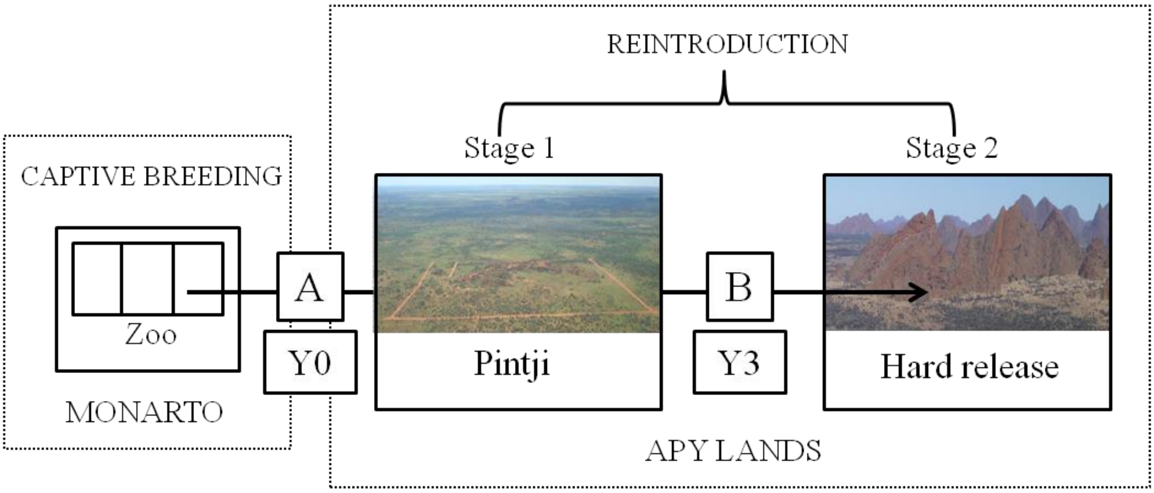
6.5.1 *Simulating population dynamics*

A simulation approach was used to predict warru population dynamics, using the individual-based simulation model of *VORTEX* (v9.999; Lacy and Pollak 2012). *VORTEX* computes population dynamics by following the fate of each individual in a population (Lacy 2000). Each simulation steps through a series of events that relate to the life cycle of a species (such as mate selection, reproduction and mortality) and applies each event to an individual by randomly sampling from a binomial distribution of the mean probability for each event. This incorporates demographic stochasticity (fluctuation in birth rates, death rates and sex ratios) within the model. The fate of each individual is monitored, the outcomes tallied at the end of each year, and then used to calculate the change to the overall population. The impact of environmental stochasticity (annual variation in the probabilities of birth and death) on overall growth rate can also be examined by sampling the annual population means from a binomial distribution. In addition, *VORTEX* includes genetic stochasticity by simulating random inheritance of alleles at a specified number of loci. Inbreeding depression can then be incorporated into simulations by modelling one allele as a recessive lethal equivalent with first year survival reduced for homozygous individuals. These features therefore enable the impact of demographic, environmental and genetic stochasticity on population dynamics to be examined and tracked through time. For this reason *VORTEX* is commonly used to predict the growth rate and persistence of reintroduced populations (Armstrong and Reynolds 2012).

6.5.2 *Model design*

The model was designed to represent the two stages of the warru reintroduction program (Figure 6.1). Two populations were created in *VORTEX*. The first population was at the site of the ‘pintji’, the 97-ha exclosure on the Lands. This population was initiated in 2011 (model year ‘0’) through transfer of captive bred warru from Monarto Zoo (Figure 6.1). The dynamics of the zoo population were not included in the current model. The pintji population could be supplemented with additional individuals from the zoo (A, Figure 6.1) or harvested (B, Figure 6.1) to provide groups for the second population, ‘hard release’. The Warru Recovery Team plans to commence hard reintroductions in 2014 (model year 3) and hence this was the first year that the model removed a group from the pintji population. To avoid error propagation, populations were modelled over a short timeframe of 10 years (2011 – 2021) (Beissinger and Westphal 1998), with 1000 iterations of the model in each year. Extinction of either population was defined as only 1 sex remaining.

Figure 6.1. Graphical representation of the *VORTEX* model used to simulate warru reintroduction to the APY Lands. Individuals were removed from the zoo to initiate the pintji population (fenced enclosure on the Lands) at Year 0 (2011). In the model, the pintji population was harvested at Year 3 (2014) to initiate a hard release population (not fenced). The model allowed for further individuals to be supplemented to the pintji population from the 'zoo' but the viability of the zoo population was not included in the model.



6.5.3 *Parameter estimation*

Each population had its own parameterisation within *VORTEX* to reflect the fact that demographic rates were expected to vary between the populations. Stage 1 consisted of captive-bred individuals residing within a fenced enclosure (absence of introduced predators and competitors) whilst individuals released at stage 2 would be 'hardened-off' individuals transferred to a managed (predators, competitor and weed control) but unfenced site. Demographic parameters used for the simulation of the pintji population (stage 1) were mostly derived from values observed during the post-release monitoring period of 24 months (ref. Ch. 2). Mortality and breeding estimates for the simulation of the hard release groups (stage 2) were adjusted to the demographic rates observed in three wild remnant populations (Ward *et al.* 2011) which were also managed but unfenced populations. Variation between parameter estimates and the deterministic growth rates (projected exponential growth rate in the absence of stochastic fluctuation) for the two populations are displayed in Table 6.1 and justified below.

Table 6.1. Population parameter estimates used in the basic scenario model (*VORTEX*) for warru released into the warru pintji. The same parameter estimates were used for hard-release populations, except where adjustments are indicated. Deterministic growth rates (projected from life table calculations using the input values) are provided for each population for later comparison with stochastic predictions.

Parameter	Estimate	
	Pintji population	***Hard release population***
Mating strategy	Polygynous	
First age reproduction	1 year	
Breeding pool		
Females 1 year	88%	
Females 2 years +	100%	94%
Males	60%	55%
# young per female per year	1.5	
Sex ratio at birth	50%	
Maximum age of reproduction	10 years	
Density dependent reproduction	No	
Mortality 0-1year	55%	
Mortality 1-2years	9%	25%
Mortality 2 years+	9%	25%
Carrying capacity	50	250
Dispersal	No	
Inbreeding depression	No	
Deterministic growth rate	0.198	0.063

6.5.3.1 *Life history*

Warru development is categorised into four stages: ‘pouch young’, ‘juvenile’, ‘sub-adult’ and ‘adult’ (Ward *et al.* 2011). Following a gestation period of ~ 30 days, females give birth to one young (Jones 2001). Permanent pouch emergence (PPE) occurs at around 6 months after which individuals are termed ‘juvenile’, passing through a sub-adult phase before adulthood at ~ 2 years of age (Ward *et al.* 2011). As VORTEX simulations run in discrete annual cycles, age-specific mortality and reproduction rates were defined for three age classes: juveniles (<1 year olds), sub adults (1-2 years of age), and adults (>2 years). Both sub-adults and adults are capable of breeding (Ward *et al.* 2011) with captive records confirming both sexes can breed at 15 months of age (M Post, pers. comm.) and an ~11 month old female was carrying a pouch young in the warru pintji (ref. Ch. 2). Within the VORTEX annual cycle, first age of reproduction was therefore determined to be 1 year for both sexes and thus reproductive rates only applied to sub-adult and adult age classes. Lifespan was estimated at 10 years based on average captive longevity of other *Petrogale* species (D. Taggart, pers. comm.) and long-term trapping records of warru (WRT, unpublished data) with no apparent reduction in fecundity with increasing age.

6.5.3.2 *Reproductive rates*

Warru exhibit a polygynous mating structure with genetic analysis of wild paternity determining that 55% of sampled males contributed to the breeding pool (Ruykys 2011). Genotyping of young born in the warru pintji revealed that 60% of the released males sired a young in the first 24 months (R West, unpublished data). As in many other macropodids, warru exhibit embryonic diapause, meaning that removal/weaning of one young will lead to the birth of a diapause young within 28-34 days (Jones 2001). This results in a potentially continuous reproductive cycle with births occurring throughout the year, depending on environmental conditions (Jones 2001; Ruykys 2011; ref. Ch. 2). As PPE occurs at an average of 176 days (Ruykys 2011), and gestation period is 30 days, female warru can potentially raise 1.5 young within a 12 month period. As *VORTEX* simulates life history events in discrete years, a breeding rate of 1.5/year was reflected by implementing a maximum number of ‘broods’ per female of 2/year, with 50% of females giving birth to 2 young and 50% giving birth to 1 pouch young. Pouch young sex ratio was not found to differ from parity in wild (Ward *et al.* 2011) or reintroduced warru (ref Ch. 2). Adult females (≥2 years) demonstrate high reproductive rates, with 100% of pintji females breeding in the first year post release and 94% documented as the average breeding rate at the wild colonies (Ward *et al.* 2011). Annual trapping records also reveal that 88% of sub-adult wild females were trapped with pouch young (WRT, unpublished data). Due to only 1 sub-adult female record in the warru pintji this rate was applied to both populations. Density dependent reproduction was not examined in the model as it is unlikely to occur within the small starting sizes of either population (Converse *et al.* 2013).

6.5.3.3 *Mortality rates*

Annual juvenile mortality (0-1 years) within the warru pintji was calculated from the number of documented births (n = 12) and the number of independent juveniles (approximately 1 year old) that were observed subsequently (n = 5: 3 trapped, 2 on remote camera – ref Ch. 2). As a result, a mortality rate of 55% was applied to all juveniles in the warru pintji. A slightly lower wild juvenile mortality rate of 49% was estimated from annual mark-recapture data (Ward *et al.* 2011). However, this estimate was also derived from a limited sample size and hence the higher rate of 55% observed in the warru pintji was also applied to the hard release population to avoid overestimation of survival within this age category (see below for how uncertainty in the estimated juvenile mortality rate was considered through sensitivity analysis). In calculating annual adult mortality in the warru pintji, mortalities that occurred immediately post-release (n = 6) were ignored as they were attributed to translocation-induced stress (a post-release effect). Survival estimates that include post-release effects can lead to pessimistic predictions of vital rates and so their use is discouraged (Armstrong and Reynolds 2012; Converse *et al.* 2013). Only 1 adult death was documented within the following year in the warru pintji population out of a total of 11 adults. This low mortality rate is not unexpected given the absence of introduced predators or competitors and so was applied to both age classes (adult/sub-adult) within the pintji. To account for the fact that hard release groups would be subject to additional pressures, the highest adult mortality estimate of the three wild colonies (25%) (Ward *et al.* 2011) was applied to the hard release population.

6.5.3.4 *Genetic processes*

VORTEX models genetic drift by simulating the inheritance of two alleles at 1 locus (Lacy 2000). The default simulation assigns each animal two unique alleles at year 0 and then monitors the random assignment of these alleles to each offspring to calculate an estimate of genetic diversity at the end of each simulation (Lacy 2000). However, as allele frequency data were available for the captive warru group at 14 microsatellite loci (R West, unpublished data), these frequencies were used to assign alleles to each adult in both populations within the *VORTEX* model.

6.5.3.5 *Initial population size and carrying capacity*

An initial population size of 10 warru was used to start the pintji simulation. This equated to those warru that survived the translocation at stage A, Figure 6.1. The initial population had the following age structure, 5 females (1x1year, 1x3year, 1x4year, 2x5 year) and 5 males (3x1 year, 2x5 year). The initial population size of the hard release group equalled the group size harvested at stage B (Figure 6.1), the age composition of which was selected by the simulation at random from across the age range of the adult population. Carrying capacity of the warru pintji was set at 50, the estimate provided in the site selection report, which was based on the availability of shelter sites (Ward *et al.* 2010). Carrying capacity for the hard release populations was set at an arbitrary value of 250 as the actual site had not yet been selected. This value was high enough to ensure that density-dependent effects would not affect simulations of hard release groups in the time frame modelled (7 years post- release).

6.5.4 _Model simulations_

Following parameter determination, three simulation stages were conducted.

6.5.4.1 *Simulation 1: Growth of the warru pintji population*

Using the parameters specified in Table 6.1, the warru pintji population was modelled alone (i.e. without supplementation or harvesting events at A or B, Figure 6.1). The mean extant population size (*N*) at the end of each year of the simulation (2012 – 2021) was inspected to determine whether the population would reach the estimated carrying capacity and whether the predicted population size matched the observed trapping rates in the warru pintji. The stochastic growth rate (*r*) (calculated as the log of *N* in one year over *N* in the previous year of the simulation) and probability of extinction were also examined.

To investigate the impact that uncertainty in the input parameters would have on model projections and the factors most strongly determining the future population dynamics of the warru pintji, 12 sensitivity scenarios were implemented as listed below.

Carrying capacity

1. Reduction (K=30)
2. Absence (K= 500)

Inbreeding depression

In the inbreeding depression feature, *VORTEX* replaces one of each adult allele with a recessive lethal allele and models inbreeding (having both recessive alleles) as a reduction in fitness for first year animals that are inbred.

3. Inbreeding depression: Yes.

Breeding pool

4. Reduction in female breeding pool (both age classes reduced to 75%)

First age of reproduction

Whilst it is documented that first year females can carry pouch young, the variability in the month of onset of breeding within the first year is unknown.

5. Postponed until 2 years of age.

Mortality

6 – 8. 10% increase across each age class (6: adult, 7: sub-adult and 8: juvenile)

As it is possible that the observed juvenile survival rates are an underestimate due to detection error, scenario 9 was implemented to investigate this.

9. 10% increase in juvenile survival

Environmental stochasticity

VORTEX models changes to reproduction, mortality and carrying capacity that might result from fluctuating environmental conditions by adjusting the standard deviation of the specified distribution (Lacy 2000). As rainfall has been linked to juvenile survival rates of warru (Ward *et al.* 2011) and female reproductive output is influenced by environmental conditions in other rock-wallaby species (Wynd *et al.* 2006) this was simulated for warru populations.

10. 5% environmental variation
11. 10% environmental variation

Supplementation

Supplementation is proposed as a management strategy to increase the pintji population growth rate. As a result of breeding at Monarto Zoo in 2013, there will likely be six 'excess' warru (5 females, 1 male) available for transfer to the warru pintji in 2014. Given the previously observed 40% loss due to captive to pintji translocation (ref Ch. 2), it was assumed that only 4 of these individuals are likely to establish within the warru pintji.

12. 3 females and 1 male supplemented in Year 3 (2014)

6.5.4.2 *Simulation 2: Harvesting a hard release population from the warru pintji*

Using the 'obtain supplements from harvests' special option within *VORTEX*, group sizes ranging from 6 to 20 warru were harvested from the pintji population in Year 3 (2014) and translocated to a hypothetical hard release site. It has been recommended that reintroduction groups of polygynous species are female biased (Sigg *et al.* 2005) so each group size was specified in a ratio of 60:40 (F:M). During harvesting, *VORTEX* selects, at random, adults of each sex (age >1year) to make up the specified release group size. However, if there are an insufficient number of adults of either sex within the modelled population that year, the simulation will only harvest the available adults, which may produce a release group size that is smaller than the specified release group size. To examine this, the proportion of either sex that could not be harvested in each simulation was extracted from the output and compared to the target number. The probability of extinction and mean population size by 2021 for both the pintji and hard release population were examined for each target group size. Two additional scenarios were implemented within this simulation to determine the effect of a) postponing the first harvest/reintroduction until 2015, and b) supplementing the pintji population with the 4 individuals available in 2014, prior to a harvest in 2015.

6.5.4.3 *Simulation 3: Establishment probability for hard release groups*

As hard releases have not been conducted before on the APY Lands, there is a high degree of uncertainty around the appropriate input parameter values for the hard release groups. In order to examine how variability in these estimates may impact the establishment probability of hard release groups, four sensitivity analyses were conducted for a small (n = 6), medium (n = 10) and large (n = 14) group size. For each sensitivity analysis, probability of extinction and mean group size in 2021 (i.e. 7 years post release) were examined.

A. Translocation loss

Examined at 0, 10, 20 and 30% of harvested individuals ‘lost’ (i.e. did not survive) during the translocation process.

B. Environmental variation

Inter-annual variation in reproduction and mortality rates due to fluctuating environmental conditions were tested by imposing standard deviations of the specified distribution at 0, 5, 10 and 15%.

C. Population growth rate

The hard release parameter estimates produce a deterministic population growth rate of 0.063. As juvenile survival is still not well understood, this sensitivity analysis adjusted the juvenile survival estimate to produce population growth rates of 0, 0.05, 0.1, 0.15, 0.2, 0.25 and 0.3.

D. Increased predation

It is suggested that cat predation could be limiting population growth in the wild warru colonies (Ward *et al.* 2011) and cats have been shown to predate other rock-wallaby populations (Spencer 1991). However, it is not known how often cats take juvenile warru and/or whether this rate is already reflected in the low juvenile survival rates. As predation is commonly reported as a cause of failure in mammal translocations (Short 2009), this scenario imposed additional mortality on juvenile and sub-adult age classes by adding a probability of removal from the population by a predator at 0, 0.2, 0.4 and 0.6 probability. The additional predation probability was not applied to the adult age classes (>2 years) as spotlight shooting sessions on the APY Lands are detecting cats of mass 2 - 4kg, and it is presumed that individuals of this size would be unlikely to take down an adult rock-wallaby (J Read, pers. comm.).

6.6 **Results**

6.6.1 *Simulation 1: Growth of the warru pintji population (no removals for hard-release)*

The baseline pintji population scenario produced a stochastic growth rate of *r*=0.19±0.1 (mean ± SD) hence a 19% annual population increase resulted in a mean population size of 46 individuals after 10 years (Year 2021) (Table 6.2; Figure 6.2). The mean population size estimate in Y1 of the simulation (2012) was 13 which equates to the ‘known to be alive’ estimate for the warru pintji population that year (ref. Ch. 2; Figure 6.2). The baseline scenario population had an extinction chance of 0.1% (Table 6.2). This chance of extinction increased above 0.1% when the percentage of females breeding was reduced to 75%, juvenile mortality increased by 10% or environmental variation in vital rates increased to 10% (Table 6.2), but still remained below 2% in all of these scenarios. A decrease in juvenile mortality to 45% increased the growth rate to *r*=0.25 and reduced the probability of extinction to zero (Table 6.2). The supplementation scenario also reduced the probability of extinction to zero but had little effect on the stochastic growth rate (Table 6.2).

Figure 6.2. Mean (+SD) annual population size of the warru pintji population on the APY Lands as determined in *VORTEX* using the basic model parameter estimates (Table 6.1). The dashed line represents the proposed carrying capacity of the warru pintji site.

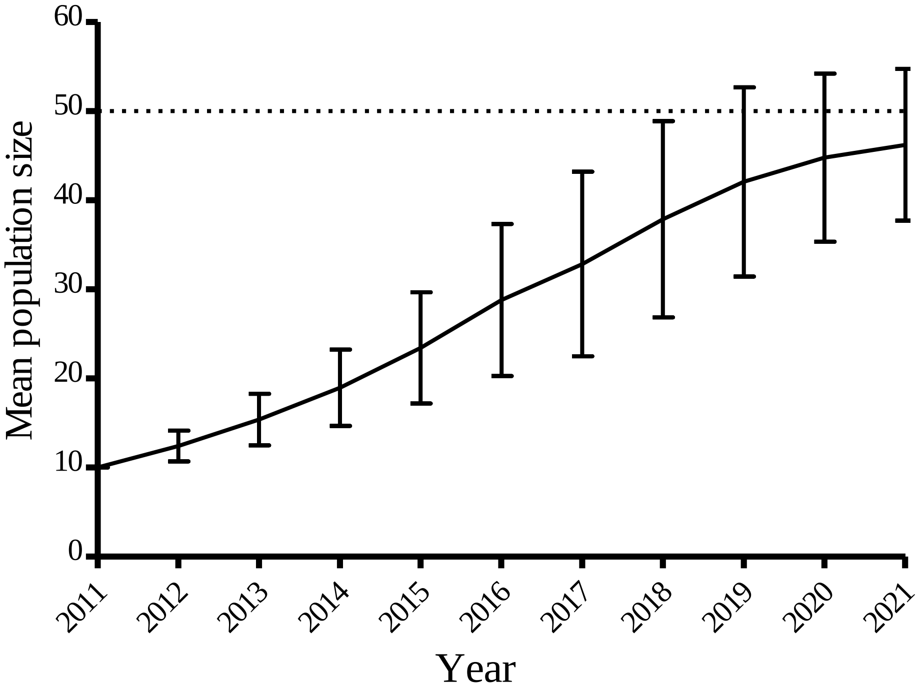


Table 6.2. The results of 1000 iterations over 10 years for the warru pintji population (inside the 97ha enclosure). Basic scenario (estimates in Table 6.1) and 12 sensitivity scenarios (one parameter is varied at a time) are shown. Results provided are: stochastic population growth rate (*r* ± sd), probability of extinction (%) (*PE*), final population size (*N* ± sd), genetic diversity (*GD* ± sd) and the mean number of years to extinction for iterations that went extinct (*TE*). Growth rates are specified without truncation by carrying capacity. Final population size is a mean across all extant and extinct populations.

Scenario	***r***	**SD (*r*)**	**PE**	**N**	**SD(N)**	**GD**	**SD(GD)**	**TE**
Basic	0.188	*0.115*	0.1	46.2	*8.79*	0.872	*0.037*	6
1 Carrying capacity = 30	0.188	*0.118*	0.3	29.37	*3.52*	0.862	*0.036*	7
2 No carrying capacity	0.191	*0.115*	0.1	75.5	*33.57*	0.876	*0.035*	6
3 Inbreeding depression	0.166	*0.118*	0.3	43.43	*10.2*	0.878	*0.03*	7.7
4 75% of all females breed	0.118	*0.131*	1	34.05	*13.19*	0.86	*0.039*	8.3
5 0 first year females breed	0.133	*0.12*	0.3	37.82	*11.37*	0.876	*0.034*	7.7
6 Adult mortality ↑10%	0.127	*0.145*	1.4	35.89	*13.89*	0.834	*0.06*	8.1
7 Sub-adult mortality ↑10%	0.168	*0.125*	0.1	43.87	*10.38*	0.866	*0.04*	7
8 Juvenile mortality ↑10%	0.121	*0.129*	0.7	35.15	*13.36*	0.863	*0.046*	8.6
9 Juvenile mortality ↓10%	0.249	*0.103*	0	49.46	*4.29*	0.877	*0.029*	n/a
10 5% environmental variation	0.189	*0.132*	0.2	44.75	*8.8*	0.871	*0.035*	4.5
11 10% environmental variation	0.181	*0.187*	0.5	38.67	*11.16*	0.862	*0.044*	7
12 Supplement with 4 (3F, 1M) in 2014	0.191	*0.111*	0	46.96	*7.58*	0.874	*0.033*	n/a

6.6.2 *Simulation 2: Harvesting a hard release population from the warru pintji*

The probability of extinction for the hard release group declined with increasing founder group size although this relationship then reached an asymptote at approximately 2% for group sizes greater than 15 (Figure 6.3a). Extinction risk of the pintji population increased with increasing hard release group size up to a probability of 90% if a group size of 20 was harvested in 2014 (Figure 6.3a). This risk could be reduced if the harvest did not take place until 2015 or further reduced if 4 individuals were supplemented to the pintji population in 2014 and then the harvest was conducted in 2015 (Figure 6.3a). Postponement of the harvest and supplementation also increased the mean pintji population size (Figure 6.3b). Target group size was most likely to be met if hard release group sizes were 6 or 7 warru (Figure 6.3c). The likelihood that the required number of females and males for the target release group size were available in the pintji population decreased with increasing target release group size, with females less likely to be available than males (Figure 6.3c). This therefore produced slower post-release growth rates of the ‘larger’ release group sizes because the simulation had not actually been able to harvest and release the larger specified group size (Figure 6.3b).

6.6.3 *Simulation 3: Sensitivity analysis for hard release groups*

Simulation results were relatively robust to uncertainty in translocation success, environmental stochasticity and population growth rate (Figure 6.4). There was little difference in the probability of extinction for medium (n = 10) and large (n = 14) group sizes across all three analyses (Figure 6.4a-c). Translocation losses similar to those observed in the pintji (30%) predicted an extinction risk of 46% for small groups (n = 6) but could be reduced to less than 20% through the release of groups of at least 10 individuals (Figure 6.4a). Environmentally induced variation in demographic rates produced the least discrepancy in estimated mean population size, although extreme changes (15%) would also place a high risk (33%) of extinction on small group sizes (Figure 6.4b). Increased growth rates produced the greatest reduction in extinction risk and increase in mean population size (Figure 6.4c). A stochastic growth rate akin to the warru pintji (r=0.2) could lead to a mean population size of 50 animals in 2021 if the founding group size was large (Figure 6.4c). Significantly, uncertainty of mortality rates for juvenile and sub-adult individuals generated the greatest variation in simulation results (Figure 6.4d). Simulations predicted that even large group sizes would be unlikely to persist if mortality rates of these age classes, assumed to be a result of predation, are greater than expected (Figure 6.4d).

Figure 6.3. The probability of (a) extinction (*PE*), (b) mean population size (*N*) and (c) harvest failure for the warru pintji population in relation to group size harvested, timing of the harvest (2014 or 2015) and if supplementation occurred. *PE* and *N* for the hard release population if harvested in 2014 are also displayed in relation to founding group size.

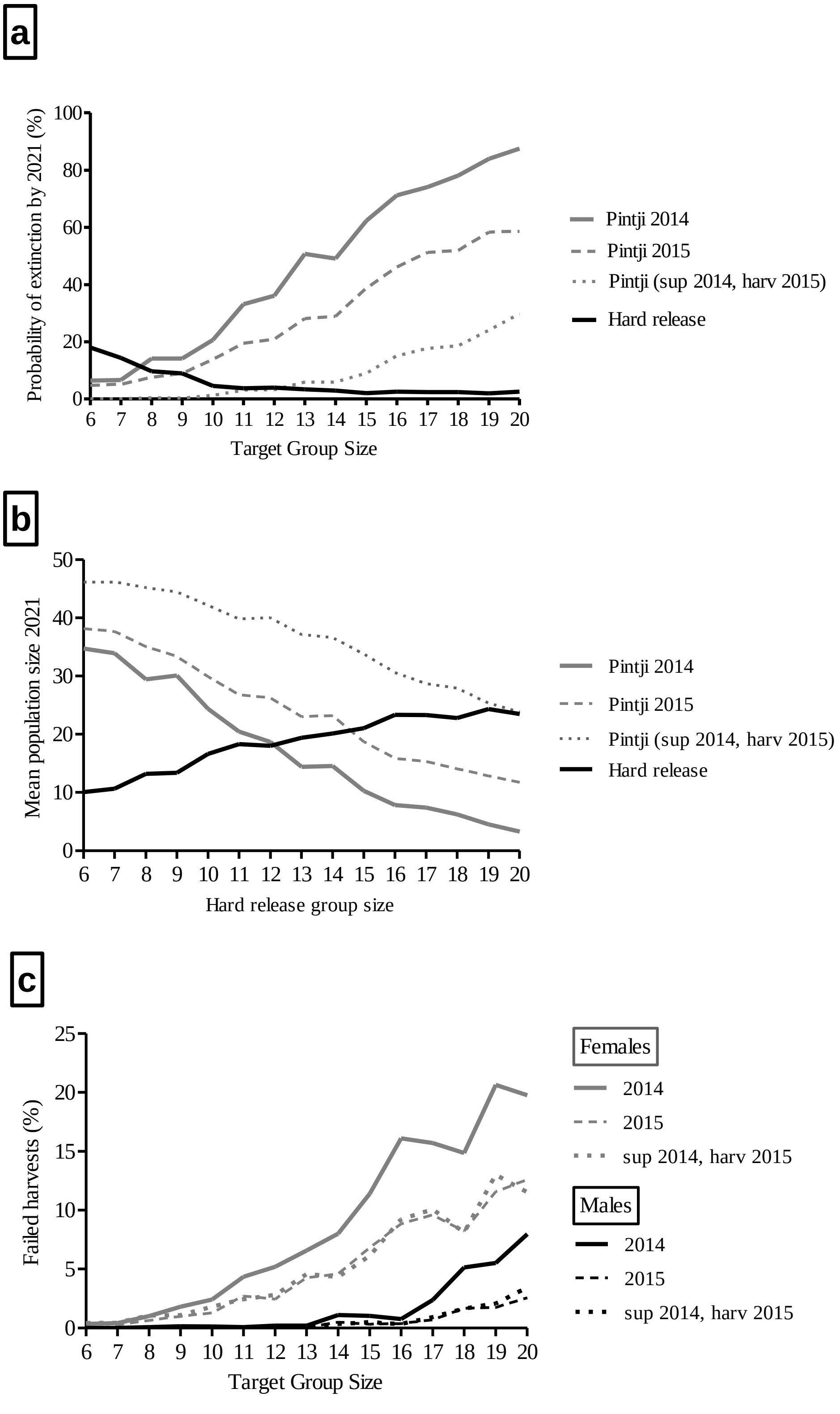
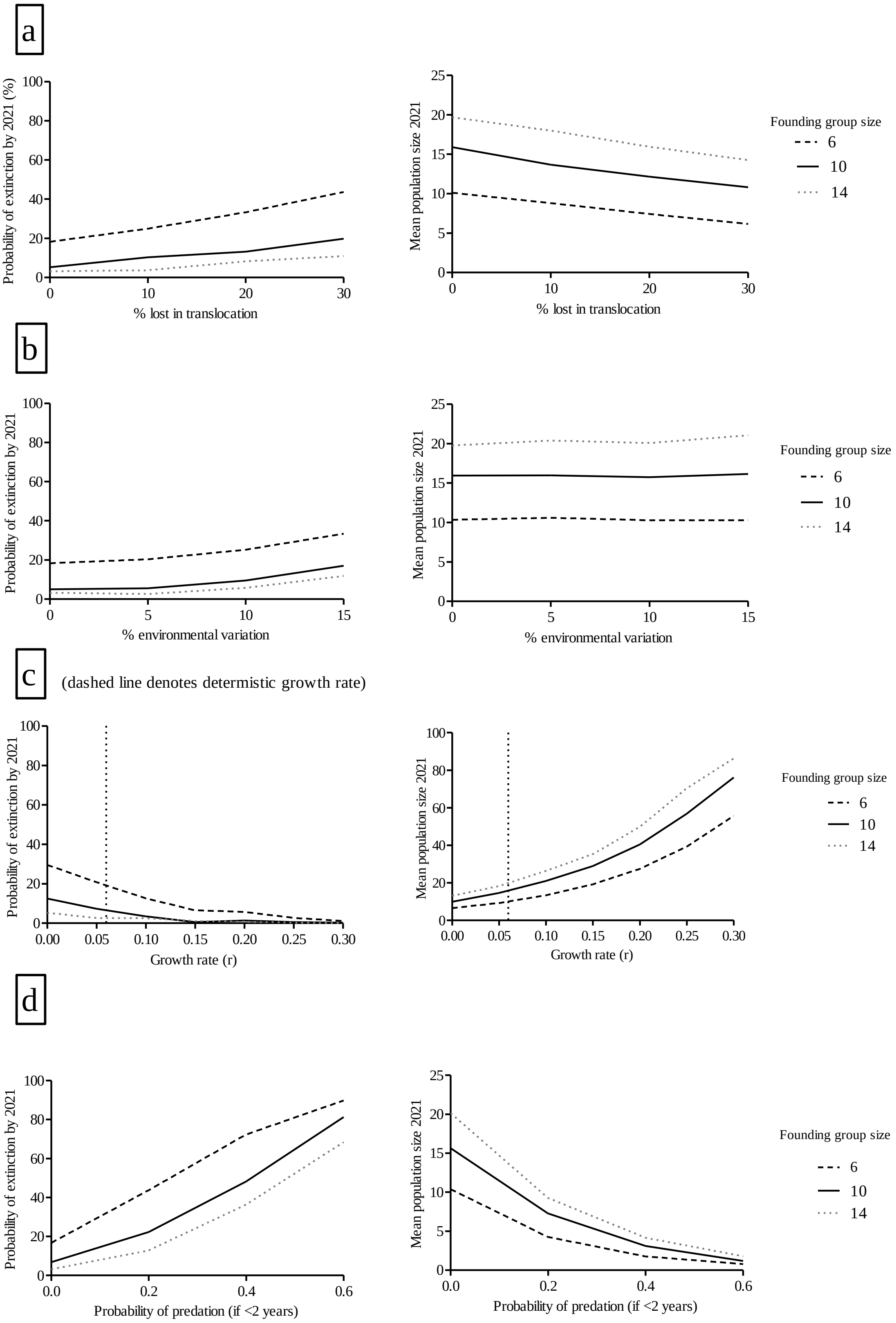


Figure 6.4. The probability of extinction and mean population size of hard release groups of warru by 2021 for three different group sizes under varying conditions a) the percentage of individuals lost as a result of translocation b) the percentage variation on vital rates caused by fluctuating environmental conditions c) variation in population growth rate as a function of adjustment to juvenile survival and d) the probability that a juvenile or sub-adult warru is removed from the population through predation.



6.7 Discussion

6.7.1 *Growth of the reintroduced population*

A fundamental question addressed using PVA is whether a population is expected to persist (Armstrong and Reynolds 2012). The survival rates of warru following soft-release showed that it was possible to transfer warru successfully from captive to natural habitat within a predator – exclosure fence (ref Ch. 2). Whilst immediate survival is clearly critical for persistence, more importantly a population must demonstrate 'establishment', reflected through successful breeding by founders and their offspring, at rates that lead to positive population growth (Armstrong and Reynolds 2012). Using the post-release demographic data for captive warru, simulation predicted a stochastic annual growth rate (*r*) of 0.188 and a negligible chance (0.1%) of extinction in the next 10 years. The similarity between the stochastic *r* (0.188) and the deterministic *r* (0.198) growth rate for the warru pintji population suggest that stochastic factors are likely to be of minimal threat to the persistence of the pintji population based on the current input parameters. Demographically viable populations are said to be those which have a 10% or lower probability of extinction (IUCN 2012). It is therefore reasonable to conclude that if demographic rates remain similar to those observed; the current pintji population has become established and has a high chance of persistence over the next 10 years.

The stochastic growth rate of 0.19 predicted a mean population size of 13 individuals in 2012 (Figure 6.2) which equalled the known-to-be-alive population estimate for the warru pintji that year (ref Ch. 2). If current survival and reproductive rates are maintained, this growth rate could result in a mean pintji population size of 75 individuals by 2021, assuming the population is not restricted by a carrying capacity. However, given the availability of den sites it is unlikely that the pintji will support this number of individuals (Ward *et al.* 2010). As resource depletion in exclosure reintroductions has been a cause of failure in other macropodid translocations (see review of Clayton, 2011), it will remain critical to monitor population growth rate to determine whether density dependent regulation will occur (Armstrong and Ewen 2002). Although one year of data was enough to estimate a growth rate for reintroduced populations of the North Island saddleback (*Philesturnus rufusater*), an increasing number of years of monitoring data improved the accuracy of the viability predictions

(Gedir *et al.* 2013). Continued data collection in the warru pintji should therefore be used to reassess and refine the predictions of the current PVA.

Sensitivity analyses are useful to target monitoring towards those factors to which population dynamics are found to be most sensitive, so that parameter estimates can be improved (Morris *et al.* 2002; Possingham *et al.* 1993). Sensitivity analyses highlighted that variation in the percentage of females breeding and juvenile survival rates of warru will have the greatest impact on population growth. The slowest growth rate (r=0.118) was obtained when only 75% of females bred and was fastest (r=0.25) if juvenile survival increased by just 10%. The current trapping regime appears to be an effective method to assess reproductive rates within the warru pintji but juvenile survival rates are more challenging to obtain. Radio-tracking is shown to be the most accurate method for survival estimation in adult macropodids (Fisher *et al.* 2000) but due to ethical guidelines for body mass percentage, radio collars cannot be fitted until individuals are 2kg or ~ 2 years old and hence cannot be tracked whilst juvenile or sub-adult. In free living *Petrogale* populations mark-recapture estimates are usually compounded because juveniles, particularly males are more likely to disperse (Eldridge *et al.* 2001; Sharp 1997) which can underestimate or sex bias juvenile survival rates. However, given that dispersal is restricted within the pintji, monitoring using a combination of trapping, remote camera and line transect methods may provide opportunity to improve the accuracy of these juvenile survival estimates over the next few years.

The sensitivity analysis also suggested that future genetic monitoring of the warru pintji population is warranted. The lack of variation in genetic diversity between scenarios is not surprising given that in growing populations, the rate of inbreeding is expected to be larger than the rate of genetic drift (Keller *et al.* 2012). The inclusion of inbreeding depression in the simulation had a minor impact on population growth rate and final population size. *VORTEX* models inbreeding depression as a reduction in first year survival for inbred individuals. It is therefore likely to have had a minimal impact on the growth rate of the pintji population within the timeframe modelled because the founding individuals of the pintji population were selected for their genetic diversity (ref. Ch 5). However, the limited number of individuals released means that matings between related individuals is likely to occur within a short time frame. Indeed, uncle *x* niece matings were detected in the genotyping of the first year independent juveniles at

14 microsatellite loci (R West, unpublished data). Given the enclosed nature of the warru pintji and inability for natural dispersal and migration to take place, supplementation and routine genotyping of offspring will therefore be required to facilitate adaptive management of genetic diversity to avoid the negative consequences of inbreeding depression (Ralls *et al.* 1979).

6.7.2 *Harvesting source populations for reintroduction*

Sustainable harvesting options need to be determined in species relocation programmes through monitoring of the source population (Sheean *et al.* 2012), yet few papers appear to examine this (Armstrong and Seddon 2008). The need to monitor the source population and predict the impacts of harvesting was highlighted in translocations of eastern barred bandicoots (*Perameles gunnii*) (Todd *et al.* 2002). Repeated translocations of 120 individuals from an estimated population of 500 contributed to the source population becoming functionally extinct (Todd *et al.* 2002). Although these removals were not held solely responsible for population extinction (Todd *et al.* 2002), decreasing population size has shown to increase population vulnerability to extreme events and factors limiting population growth (Caughley 1994).

The PVA simulations revealed a clear trade-off between the competing goals of promoting the viability of both the pintji and hard-release populations. Given the current growth rate of the pintji population, harvesting more than 7 individuals in 2014 will lead to a probability of extinction greater than 10% (Figure 6.3). As a hard release group size of 7 or less is predicted to have an extinction risk of 20% under baseline demographic estimates, this management option is not conducive to meeting the objective of establishing a self-sustaining population. Supplementation of individuals from Monarto Zoo in 2014 and a harvest in 2015 predicted the best management option for the pintji population in terms of reduced extinction risk, increased population size and the ability for the target group size to be harvested from the pintji population (Figure 6.3). For example, if the warru pintji is supplemented in 2014 then harvested in 2015, a target hard release group size of 15 is likely to be feasible since only 6% of female harvests failed and 0.5% of males (Figure 6.3c). This hard release group would have a probability of extinction of 2.1% and a mean group size of 21 by 2021 (7 years post release), whilst the warru pintji population would still retain an acceptable probability of persistence (91%) and a mean group size of 33 (Figure 6.3). However, this supplementation option assumes that at least 4 of the 6 captive warru available for translocation will successfully establish within the pintji. Whilst this is a reasonable assumption given previously observed rates of translocation loss (ref Ch. 2), higher translocation loss will affect the capability of this strategy to increase the growth rate of the warru pintji population and hence will need to be determined. If the growth rate of the pintji population can be increased through supplementation then this would allow for larger group sizes and/or higher rates of harvest for hard release populations. As harvesting will also manipulate the density of individuals within the warru pintji, continued monitoring post-harvest may elicit density-dependent regulation of warru populations, which is currently poorly understood. For example, pre- and post-harvest monitoring determined that juvenile survival in translocated North Island Robin (*Petroica longipes*) populations was regulated by density dependence (Dimond and Armstrong 2007). If density dependence is limiting population growth in the warru pintji then it should mean that further groups could be harvested for hard release without damaging the viability of the source (pintji) population (Armstrong and Ewen 2002).

6.7.3 *Establishment of a hard-release group*

Theory predicts that increasing founder size will decrease the extinction risk of reintroduced populations but that this benefit will asymptote after a certain group size (Griffith *et al.* 1989). This was demonstrated in simulations of greater bilby (*Macrotis lagotis*) reintroductions which found a plateau in declining extinction risk above group sizes of 30 founding individuals (Southgate and Possingham 1995). For simulated reintroduced warru populations, extinction risk also decreased as founding group size increased, with extinction risk falling below 10% for all group sizes greater than 10 (Figure 6.3). This reached a plateau to 2% extinction risk for any group size greater than 15 (Figure 6.3). Group sizes of 6 had an extinction risk of 20% which would define the population as ‘Endangered’ within IUCN categories (IUCN 2012) and hence should be avoided unless additional supplementations with only slight delay can be conducted. Simulations suggest that there are only minor improvements in establishment probability from a hard - release of more than 10 warru (Figure 6.3a) and that the persistence of the pintji population will most likely be compromised if a group of greater than 10 were harvested, especially in 2014 (Figure 6.3a). A group size of 10 would appear to be a suitable starting point for a release group size. However, in addition to founding group size, an array of post-release effects will also influence the establishment probability of the release group (Armstrong and Reynolds 2012) and need to be considered to determine an optimal number of individuals to release (Converse *et al.* 2013).

The initial probability of establishment will be influenced by the percentage of warru that survive translocation. The process of translocation produces multiple stressors for animals (Dickens *et al.* 2010) and so it is usual to observe elevated mortality levels immediately post release. Indeed, all mortalities of captive warru occurred within 9 weeks of release (ref. Ch 2). It is expected that hard releases of warru will not experience such high rates of translocation loss as individuals are now at least adapted to wild resources, but these individuals will be exposed to introduced predators for the first time, and this naivety may increase the number of mortalities observed (Balmford *et al.* 1996; Mathews *et al.* 2005). In addition, translocation loss may occur due to over dispersal from the release site which can decrease establishment success as observed in reintroduced populations of griffon vultures (*Gyps fulvus*) (Le Gouar *et al.* 2008). However, the establishment probability for a hard release group of 10 warru was robust to uncertainty in the percentage of warru lost due to translocation and furthermore to ambiguity in environmental variation of breeding and survival rates (Figure 6.3). Uncertainty in population growth rate also had little impact on the probability of extinction for group sizes of 10 – 14 but if increased to r=0.15 (a result of a decrease in juvenile mortality from 55% to 42%) would lead to an extinction probability of less than 1% and mean population sizes of between 28 (if founding group = 10) up to 35 (if n = 14) by 2021 (Figure 6.4c). However, the ability for management actions to effectively increase population growth rate can be the greatest challenge to reintroduction (Armstrong and Reynolds 2012).

The survival and successful recruitment of juveniles will be one of the major drivers in reintroduced warru population growth rate. An increased probability of mortality of 0.2 for juvenile and sub-adult warru resulted in a greater than 10% extinction risk even for release group sizes of 14 (Figure 6.4d). Therefore, if survival rates of juveniles and sub-adults are lower than those expected for hard release populations, successful establishment will be unlikely (Figure 6.4d). Modelling of reintroductions of bridled nailtail wallabies concluded that even low predation rates could cause group sizes of up to 50 individuals to have a high risk of extinction (McCallum *et al.* 1995). It is presumed that predation is the key limiting factor for juvenile warru survival (Ward *et al.* 2011) and is why an understanding of the predator dynamics at future release sites is a key focus for the Warru Recovery Team (Read and Ward 2011).

An alternate option to increase population growth rates may be by the supplementation of additional adults from the warru pintji to the hard release sites. Whilst this is not a sustainable management strategy, the pintji is unlikely to be able to support the removal of group sizes >15 individuals in the first instance and so subsequent supplementations of small groups as they become available in the warru pintji could be a potential strategy to avoid extinction of newly released populations. Indeed, continued supplementation was determined to be necessary to increase the chances of long-term survival in reintroduced groups of Przewalski's horses (*Equus ferus przewalskii*) (Slotta-Bachmayr *et al.* 2004).

6.7.4 Management implications and future research

The current study suggests that warru reintroductions have a reasonable chance of leading to an established population if the initial founder group size is >10 and if less than 10% of individuals are lost due to the stresses of translocation. However, the simulations also indicate that the likely establishment of any release group will be highly susceptible to post-release effects, particularly juvenile and sub-adult survival. Monitoring of reintroduced populations will therefore need to be frequent, sensitive and sustained so that warning signs of failure, such as low recruitment, can be detected and management adjusted. In addition, the pintji population size should be calculated biannually to ensure that harvests for hard release have minimal impacts on the viability of the source population. These data can then be used to refine the models presented in the current study. This is a particularly important process in the use of PVA to assist reintroduction decisions. For example, the parameters of a PVA model used to guide reintroduction protocols for *P. penicillata* in Victoria have recently been revised as a result of poor post-release establishment (survival and breeding) which suggested that the input parameters taken from studies of wild *Petrogale* were significant over-estimates for reintroduced individuals (Weeks 2012).

Further refinement of the parameter estimates in the hard release model for warru could be achieved by obtaining the current population estimates of previously translocated groups of wild *P. lateralis* in Western Australia (Pearson 2012). Although detailed data on age-specific survival and reproductive rates are not available for these populations, PVA could be used to retrospectively estimate the demographic rates post-release as a function of the initial group size and measured population growth. These estimates would contribute to the empirical data on *P. lateralis* responses to reintroduction, which would also assist future decisions regarding release group size and composition. In addition, movement and genetic data post-release could be incorporated into the current models to help to address the metapopulation level question within *Petrogale* reintroduction biology of ‘what is the optimal allocation of translocated individuals among sites?’ (Armstrong and Seddon 2008).

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CHAPTER 7: GENERAL DISCUSSION

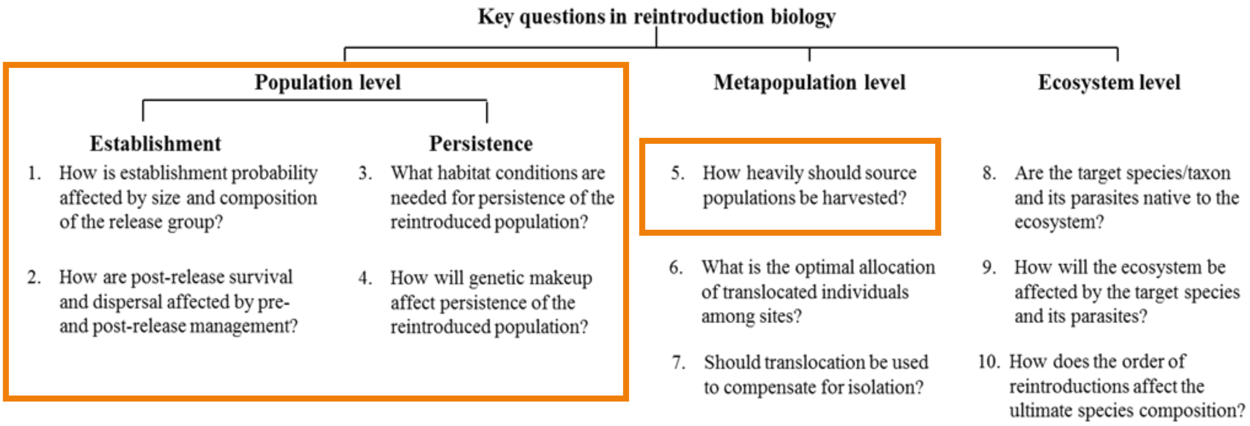
Warru rangers Bronson Bennett and Anthony Minutjukar conduct the weekly radiotracking check of the reintroduced warru population. Photo: R West

> *'We were climbing hills and not finding warru. Warru disappeared from Wamitjara. Now we bring more warru from the zoo and let them breed in the pintji. When there are lots of young ones we will let them go, one big family, back on the hills.'*
>
> *Tjariya Stanley, warru minyma, Traditional Owner, Pukatja*

7.1 The reintroduction biology of warru – key findings

The Warru Recovery Team considered captive breeding for reintroduction to be a key method to enable the conservation status of South Australian (SA) populations of warru (black-footed rock-wallaby, *Petrogale lateralis* MacDonnell Ranges race) to be down listed from ‘Endangered’ to ‘Vulnerable’ under the SA NPWS Act (1972) (Read and Ward 2011). The IUCN Reintroduction Guidelines (IUCN/SSC 2013) encourage feasibility assessments prior to any reintroduction attempt, although these are often confounded by an absence of basic data on the ecology and reintroduction biology of the target species (Converse *et al.* 2013a). In 2010, a 97-ha predator exclosure (known as the ‘warru pintji’) was constructed around an historic warru site on the Anangu Pitjantjatjara Yankunytjatjara (APY) Lands of SA, in which to conduct a soft-reintroduction of captive warru (Muhic *et al.* 2012). A lack of reintroduction biology knowledge for warru meant that the implementation of this initial soft-release protocol, using captive founders, was an opportune time to investigate the likely outcomes of reintroduction. Releases of captive warru commenced in 2011 and the five data chapters within this thesis outline the range of monitoring and analysis methods that were used to evaluate reintroduction as a tool in the context of warru recovery. The results are synthesised in the following pages in terms of their contribution towards addressing five of the ten key reintroduction biology questions identified by Armstrong and Seddon (2008) (Figure 7.1).

Figure 7.1. The ten key questions in reintroduction biology proposed by Armstrong and Seddon (2008), highlighting the five questions addressed in the current study.



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

The release protocol for the reintroduction into a predator – exclosure was that of three separate releases of 5 (3M, 2F), 6 (3M; 3F), and 5 (2M; 3F) warru, with the first two releases occurring four months apart and the third release 12 months later. The size and group composition was determined by the availability of suitable captive individuals and their genetic relatedness. In addition, the first release used solely captive-born individuals to test the suitability of the soft-release area without the risk of losing captive founder genetics. Successful reproduction has been documented in both the founders and their offspring (Chapter 2), suggesting that in the absence of introduced predators, population establishment (Seddon *et al.* 2012) has been achieved through a relatively small number of founders (n = 10 survived translocation). There was no effect of age, sex or weight on post-release survival and an even sex ratio was maintained (5F, 5M) despite the mortalities. All females were breeding within six months of their release. This suggests that female-biased release groups could further increase population growth rates, as has been suggested for other species that exhibit polygynous mating strategies (Sigg *et al.* 2005). However, assessment of the impact that a skewed sex ratio will have on subsequent genetic diversity, as well as persistence of the release group and the warru pintji population (see Question 4) is required.

Using the data on survival and reproduction following soft-release and previous data on wild warru population dynamics (Ward *et al.* 2011), Chapter 6 implemented a population viability analysis to predict the influence of group size on the establishment probability of hard reintroductions of warru. The results suggest that establishment after hard-release will be most likely if at least ten individuals, with a slight female bias (60:40) are released. In the current study, 37% mortality following captive to wild translocation was observed, thus the number of individuals that survive the translocation event from the pintji to the wild will also influence the establishment probability of any hard release groups. Therefore group sizes larger than ten may be required to achieve this effective initial population size.

2. How are post-release survival and dispersal affected by pre- and post-release management?

The founders for this warru reintroduction were all captive-reared warru, being either cross-fostered (Taggart *et al.* 2010) from the wild (up to 4 years in captivity) or captive-born during initial breeding trials (~2 years in captivity). Individuals were housed in scrub enclosures, provided with small shelter structures, kangaroo pellets, lucerne hay and water *ad libitum*, and browse where available (mainly lucerne tree, *Cytisus proliferus* and willow, *Salix sp.*). Frequency of resource provision in captivity was not adjusted prior to release. All released males were housed separately (due to excessive fighting) and although females were routinely housed in groups of two to three, these were not necessarily familiar groups prior to release.

Loss of fitness following lengthy periods in captivity is a common concern for translocations using captive founders, and can lead to a failure to exhibit wild-type behaviours and may result in elevated mortality (Mathews *et al.* 2005). Mean survival rate of captive warru following release was 63% with all mortalities occurring within nine weeks of release. This suggests that translocation-related stress factors may have affected survivorship (Chapter 2). In those surviving warru, swift adaptation to semi-wild conditions was demonstrated through maintenance of health and condition post-release (Chapter 2), appropriate selection of hill habitat, and the establishment of overlapping home ranges typical of wild individuals (Chapter 3). This facilitated high rates of female reproduction (Chapter 2). These results indicate that captive warru were capable of quickly developing wild-type behaviours. The use of a soft-release management strategy may have facilitated rapid acclimatisation and establishment of surviving released individuals, although how well that translates to improving subsequent hard-release survival rates is still unknown for warru and debated for other species (Short 2009; Wolf *et al.* 1996). In this study warru remained in soft-release conditions for a longer time than in most other reintroduction projects, and will continue to do so, with the first hard releases unlikely to occur before 2015 (four years after soft-release). The relative influence that adaptation to captivity and the time spent in a predator - free enclosure will have on survival rates may be determined through survival monitoring following hard release, although additional factors such as increased predation risk are likely to confound interpretation.

A direct conclusion about the role of supplementary water on the survival of released individuals is not possible from the data collected and presented here. However, the higher drinking rates at supplementary water points, observed in both wild and reintroduced warru during the drier winter, period suggest that this management action could help to alleviate resource pressure for reintroduced individuals when natural free water is unavailable and forage moisture content is reduced (Chapter 4).

No evidence of attempted dispersal from the release site was documented, with GPS locations of released warru centred on the rocky outcrop within the warru pintji (Chapter 3). This has been further evidenced by consistent trapping of all surviving founders up to 30 months post release (R West, pers. obs.). Wild *Petrogale* have been shown to demonstrate strong habitat preferences (Telfer and Griffiths 2006; Waldegrave-Knight 2002) and innate behaviours of high site fidelity (Eldridge *et al.* 2010; Hazlitt *et al.* 2006; Sharp 1997). The lack of dispersal and high survival rates in reintroduced warru supports both the site attribute scoring system used to select a suitable release site (Ward *et al.* 2010) (see Appendix 1A) and the management practices put in place both prior to and following warru release, e.g. removal of all introduced predators and euro (*Macropus robustus*) and reductions in European rabbit (*Oryctolagus cuniculus*) densities.

3. What habitat conditions are needed for the persistence of the reintroduced population?

Population modelling using data gathered on the survival and reproductive output of captive warru post-release predicted a positive stochastic growth rate of 0.188 (Chapter 6). The model also predicted that the warru pintji population had a high probability of persistence (>10% probability of extinction), suggesting that the current site is providing the conditions needed for growth (Armstrong and Seddon 2008) and possesses the characteristics of quality warru habitat. Physically, the site was selected because of the presence of a large area of complex rocky habitat (≥10ha) providing high numbers of shelter sites (≥10shelters/ha) and an abundance of dietary items such as fig and spearbush (Ward *et al.* 2010) (see Appendix 1B). The fence excluded all introduced predators and, as a result of continued management, by mid - 2012 was cleared of euros (presumed to be a major competitor). In comparison, the growth rate for hard-release groups (derived from demographic rates of the wild populations) was predicted to be much slower at 0.063 (Chapter 6). This difference in growth rates was driven by the fact that population modelling of the wild warru colonies provided estimates of adult and sub-adult mortality that were 16% higher (Ward *et al.* 2011) than those observed within the warru pintji. The most obvious habitat condition differences between the two sites is the complete absence of cat, fox, wild dog and euros within the warru pintji, suggesting that densities of these predators and potential competitors are important habitat conditions for population growth (Osborne and Seddon 2012). This is unsurprising given that predation has been shown to have a significant impact on other *Petrogale* population dynamics (Kinnear *et al.* 2010) and competition for shelter, water and dietary overlap has been previously documented between warru and euros (Creese 2007; Read and Ward 2011; Ruykys 2011).

The inclusion of elevated complex shelter sites as a hard-release site selection criterion may assist in reducing predation pressure for reintroduced warru populations. The home ranges of wild warru at New Well displayed low use of the flats and high fidelity to the hill with core areas centred about the rocky outcrops at the upper elevations of the hill (Chapter 3). Elevation and geological complexity have been shown to be significant predictors of current warru occurrence, with preference displayed for elevations above 900m, suggested to be most likely a result of reduced accessibility to introduced predators and areas where structural complexity is greatest (Ruykys 2011). The most recent warru extinction at Wamitjara, where the most complex boulder piles are located at the base of the hill (maximum 50 – 100m elevation) (R West, pers. obs.) also suggests that elevation of shelter sites may assist persistence of reintroduced populations in the presence of introduced predators.

The role of water in warru population persistence still requires investigation. Historical distribution records suggest that warru occurrence was influenced by the presence of drainage features, natural soaks and springs (Ruykys 2011) with rockholes common features of previously occupied sites but not all extant sites (Read and Ward 2011). Rockholes are known to be focal areas for predator activity (Hume *et al.* 2004; James *et al.* 1999) and thus, where present in warru habitat, may have increased this threat to warru populations. Water turnover experiments have shown that *Petrogale* populations can clearly survive in the absence of reliable free water (Hume *et al.* 2004; Lapidge and Munn 2012), but juvenile warru survival (Ward *et al.* 2011) and population growth rates of *Petrogale xanthopus* have been positively related to the availability of free water through rainfall events (Lethbridge and Alexander 2008). The significant negative association between warru drinking rates at supplementary water points and natural water availability (Chapter 4) suggests that the presence of free water, particularly during dry periods, is likely to aid persistence of reintroduced warru populations. However, it is possible that this will need to be provided via small supplementary points rather than seeking habitat that provides permanent free water; to avoid increasing predator pressure on reintroduced populations.

4. How will genetic makeup affect persistence of the reintroduced population?

The captive warru population was established from 24 pouch young cross-fostered from three colonies on the APY Lands and maintained as two separate genetic groups representing the Musgrave Ranges (n = 18 founders) and Tomkinson Ranges (n = 6 founders) metapopulations (Read and Ward 2011). Microsatellite and mitochondrial analyses (Chapter 5) were conducted to examine the impact of this management action on the adaptive potential of these captive groups in the context of wild population diversity and genetic structure. The results reinforce the importance of securing large founder groups for captive colonies (Frankham *et al.* 2002). The removal of only six to ten founders from each site resulted in an average capture of only 67% of the wild source genetic diversity, including the complete loss of rare alleles (Chapter 5), akin to a natural population bottleneck (Nei *et al.* 1975). In addition, some pouch young were harvested from the same mother in consecutive years, which resulted in half and full siblings within the captive groups, and therefore limited the effective captive population size.

Although the level of inbreeding within the captive group was not significant, continued breeding of this group was likely to increase genetic drift from the wild source populations and the likelihood of inbreeding (Sigg 2006). As severe levels of inbreeding have been shown to impact the fitness of *Petrogale lateralis* island populations (Eldridge *et al.* 1999), it is likely that increases in inbreeding levels would ultimately affect the persistence of reintroduced warru populations. A lack of phylogeographic structure within mitochondrial haplotypes suggested greater historical connectivity across warru populations in central Australia. Therefore, a low risk of outbreeding depression was predicted (Frankham *et al.* 2011) and the maintenance of the east and west populations as separate genetic groups was considered unnecessary. As a result, Chapter 5 recommended that future releases use a combination of captive founders from both Range systems to increase the genetic diversity and thus the adaptive potential of reintroduced populations. This can be achieved through the cross-breeding of animals from these groups in captivity and the release of founders from all of these sites into the warru pintji. Future supplementation of the captive population should select founders from sites with unique genetic diversity, such as the newly identified Kaa<u>n</u>ka Mangka site in the Musgrave Ranges (Chapter 5). This could be achieved through the periodic use of cross-fostering during annual trapping surveys or the supplementation of wild caught individuals directly to the pintji population.

5. How heavily should source populations be harvested?

The captive warru population was established through the cross-fostering of pouch young from wild mothers (Taggart *et al.* 2010), which avoided the removal of adult animals from the *in situ* population, a common concern in the establishment of captive populations (Frankham *et al.* 2002b). However, the genetic results of the current study stress the importance of harvesting a suitable number of pouch young from unrelated individuals in order to capture sufficient levels of source population genetic diversity. The current study also began to address the question of appropriate harvest schedules of the warru pintji population to provide hard-release groups. Population modelling in Chapter 6 suggested that the harvest of ten individuals (question 1) for hard release would be best to take place in 2015 to minimise the chance of extinction for the warru pintji population. Supplementation of the population with additional captive founders will further decrease the probability of extinction, in addition to increasing the genetic diversity within the warru pintji population (question 4).

7.2 Reintroduction as a tool for the recovery of warru

The results of the current study suggest that, in the absence of introduced predators, reintroductions using captive founders are capable of re-establishing populations of warru on the APY Lands and that these populations will exhibit positive population growth rates, indicating a good chance of persistence. The outcomes of this soft reintroduction combined with genetic analyses and the population modelling of proposed hard releases can provide the following guidance on reintroduction protocols and monitoring for the next stages of this project:

- Dissolve the separate genetic groups within captivity and establish Musgrave/Tomkinson Range breeding pairs.
- Supplement the warru pintji in 2014 with at least six individuals, including, where possible, Kalka individuals. However, use intensive radiotracking and remote camera analysis to monitor aggression between established and released individuals and intervene if social integration appears unlikely (remove individuals and place into the small holding pen within the warru pintji).
- Include elevation of ideal shelter sites (based on structural complexity) within the site selection ranking system.
- Where possible, select release sites that allow dispersal between reintroduced and *in situ* colonies (longest dispersal distance documented for warru is currently 12km).
- Hard releases should not commence until 2015 at the earliest and should harvest a minimum group size of ten individuals from the warru pintji with a slight female bias if possible.
- Releases should be conducted in spring or autumn to avoid a) the dry winter when water content of pasture and free water availability are reduced and b) extreme temperatures during summer.
- Provide supplementary water in small reservoirs (8 – litre volumes sufficed in the current study) on a monthly basis during the first three months post-release and during winter and drought periods to assist adaptation and alleviate limiting resource periods which may decrease survival of reintroduced individuals or lower recruitment rates.
- Place remote cameras at supplementary water points to assist in the monitoring of warru presence, condition, reproductive status and recruitment.
- Fit radio collars (VHF or GPS) to released adult warru and use heat shrink tubing to uniquely mark antennas to facilitate individual identification via

remote camera. This can be complemented by placement of ear-tags on right ears for males and left ears for females.

- In addition to a mortality latching feature, radio collars where possible should include a ‘time since death’ feature (a series of beeps that indicates hours and minutes since mortality latching) to provide a fine scale timeline of mortalities post-release.
- Radiotracking should be conducted daily for the first two months post release to determine site fidelity and estimate initial translocation mortalities. If these rates reduce the effective population size below ten, further supplementation should be considered to reduce extinction probability. In addition, intensive radio tracking will allow the rapid detection of mortalities and timely retrieval of carcasses, thus increasing the possibility of a conclusive post-mortem.
- Biannual trapping (including blood analyses) should be conducted for at least the first two years post-release to provide detailed data on health, reproduction and recruitment. Modelling indicates that deterministic growth rates below 0.1 will have a high probability of extinction (>10%) and this will need to be routinely assessed.

Positive population growth in hard-release warru populations will be essential to the success of the next phase of this project. Failure of extant warru populations to increase in size is currently attributed to predation of juveniles by cats (Ruykys 2011). Comparative reintroduction biology between hard and soft releases will enable an assessment of the role that introduced predator pressure will play in population establishment, the levels at which predator abundance must be maintained to achieve population growth, and the likely value of reintroduction as a recovery method for warru. This is further discussed below as a future research direction.

7.3 Implications for reintroduction monitoring and management

This thesis provides a case study of the significant contribution to species reintroduction biology that can be made by appropriate, targeted and intensive monitoring of both reintroduced and remnant populations. While intensive monitoring involving multiple strategies requires significant time and monetary investment, this case study demonstrates the critical role of these data in evaluating the potential of reintroduction to recover a threatened species. A multi-faceted monitoring approach also increases the ability for adaptive management to improve the outcomes of reintroduction, particularly as no single monitoring method in this study provided a complete assessment of the status of the reintroduced population. The refinement of management techniques during this process can also be applied to the recovery of *in situ* remnant populations in addition to the management of future reintroductions.

Genetic assessments of remnant populations need to be further encouraged within captive breeding for reintroduction programmes. The use of markers, to assess both the historic and contemporary genetic structure and diversity of a species, is critical to evaluating the percentage of source diversity represented within a captive population and ensuring that breeding management suitably reflects natural population structure. The detailed genetic assessment conducted in this study has had significant implications for captive breeding management and reintroduction release protocols for warru. Programs currently attempting to manage small groups of a threatened species as separate genetic populations based on contemporary population structure should, where possible, assess the appropriateness of this management strategy, by examining historical gene flow between populations and evaluating the risk of outbreeding depression (Frankham *et al.* 2011).

For species at high risk of predation by feral carnivores, there may be great value to conducting the first stage of reintroduction as a long - term soft introduction into an area of wild habitat protected by a predator exclusion fence, despite the high costs of construction and maintenance. This management strategy is likely to be particularly appropriate for critical weight range mammals, released from captivity, whose naivety combined with body size, can make them highly susceptible to predation (Balmford *et al.* 1996; Johnson 2006). In Australia, present and future control agents are unlikely to be able to eliminate introduced predators completely, the presence of which within a reintroduction site dramatically decreases establishment probability (Moseby *et al.* 2011; Short 2009). A loss of large numbers of released animals to predation confounds assessment of the capability of the species to adapt to wild habitat and other habitat conditions necessary for population growth (Moseby *et al.* 2011). In the absence of threats, such as introduced predators, this can be fully examined during long-term soft-reintroduction through the implementation of pre- and post-release medical screening and post-release trapping. Post-release survival and reproduction data obtained through trapping and radiotracking can be used to directly assess the quality of the selected habitat (Armstrong and Seddon 2008) and thus evaluate release site selection strategies. This can also be examined through pre- and post-release health assessments for reintroduced individuals, although there are only a few examples of studies that have conducted this (Mathews *et al.* 2006). Health screening using blood biochemistry analysis can be useful as a proxy measure of adaptation to wild diet and the provision of appropriate resources at the release site, particularly as dietary composition and habitat assessment studies are time-consuming. Such assessments during a long-term soft reintroduction will enable managers to determine whether hard reintroduction, in combination with control of threatening processes, will be a suitable conservation tool for the species.

There are many examples of reintroduction projects in which supplementary resources are reported to have been provided, but their use has not been fully evaluated (Hardman and Moro 2006; Richards and Short 2003). While supplementary resources may play an important role in population establishment, they can discourage individuals from seeking natural resources, adjust space use (Moseby and Bice 2004) or increase other threats (Treby *et al.* 2007). Comparing the use of supplementary resources by reintroduced and *in situ* populations in relation to changes in natural resource availability can address some of these concerns. For arid zone taxa in particular, there is likely to be a trade-off between water presence and predator pressure (Hume *et al.* 2004). As demonstrated in this study, remote cameras can be used to determine visits to supplementary resources by both the target and sympatric species, and can therefore be used to evaluate the effectiveness of supplementary provision.

Limited numbers of captive individuals often make it impossible for managers of threatened species to conduct replicated, controlled experiments, thus hindering any reliable conclusions regarding the effect of group size and composition on establishment probability (Seddon *et al.* 2007). The current thesis provides evidence for the merit, in these situations, of collecting detailed survival and reproduction data from small trial releases of individuals and supplementing this with data from remnant populations. These data can be used to develop predictive population viability models (PVA) to evaluate the possible outcomes of alternate management strategies using the results of previous releases. These predictions can then be integrated within an adaptive management decision framework, and are likely to significantly improve future reintroduction decision-making processes (Converse *et al.* 2013b; Runge 2013).

Based on the monitoring schedule that was implemented in this study, and the conclusions that could be drawn from the data collected, a monitoring framework to assess the establishment success of a reintroduced population in the first 24 months post-release is presented (Figure 7.2). This schedule incorporates the recommendations provided above, to conduct genetic and blood sampling prior to release and biannually post-release. Daily intensive tracking in the first two months post-release should ensure that failure as a result of early mortalities or long distance dispersal from the site can be detected and adaptively managed. Rapid retrieval of carcasses during this period should also assist determination of the causes of mortality. In addition, the use of transmitters with a mortality sensor and time-since-death features will provide a fine-scale timeline of mortality that can be examined for patterns in post-release mortality events. Remote camera monitoring can also supplement these methods by helping to determine population estimates and also evaluate the role of supplementary resource points. If a population is deemed to have established during this period (breeding by founders and offspring is detected; Seddon *et al.* (2007)), it is likely that the monitoring intensity can be scaled back, although at the very least, an annual trapping census including genetic monitoring should be maintained.

The value of community involvement in reintroduction programs (Kleiman *et al.* 1994) was apparent in this study. A key objective of the Warru Recovery Plan is to provide training and employment opportunities for A<u>n</u>angu within the context of warru recovery (Read and Ward 2011). Currently nine permanent rangers and seven minyma (senior ladies) are employed to oversee the key decisions surrounding warru recovery and to conduct the daily on-ground activities (such as radio tracking, predator monitoring and fire management). All of these permanent rangers and minyma, along with a number of casual rangers (of which 150 casual hours were supported by grants obtained by R West), were involved in the data collection and routine monitoring for this study. A standard operating procedure (SOP) and accompanying training program for new rangers/trappers were developed (Appendices 2 and 3). The training program uses life-sized plush toy warru models, which enabled new rangers to practice and build confidence in the marking, pouch young securing (using suturing) and collar fitting techniques prior to conducting these procedures on live animals. In addition, extensive radio-tracking training was provided to assist mortality detection post release. The SOP may be of particular use to managers of other *Petrogale* trapping programs and the training concept could be adapted to be used in other community based reintroduction projects (Appendices 2 and 3).

Figure 7.2. A suggested monitoring schedule for the first 24 months of a reintroduction attempt, including assessments to be conducted pre-release. Zero (0) indicates the time of release. TSD = time since death (a series of beeps indicates time since collar switched to a mortality signal).

Month	**Trapping**	**Radiotracking**	**Remote camera**
PRE	Mass Bloods Genetics	Attach VHF collars with TSD	Establish at resource points
0		daily	weekly
1			
2			
3		weekly	
4			
5			
6	▨		▨
7			
8			
9			▨
10			
11			
12	▨		▨
13			
14			
15			▨
16			
17			
18	▨		▨
19			
20			
21			▨
22			
23			
24	▨		▨

7.4 Future directions

This thesis has made significant progress towards understanding the reintroduction biology of warru, an arid zone, critical weight range, threatened species. The five data chapters provide a baseline of knowledge for five key reintroduction biology questions, which can be built upon in the future through continued monitoring of the warru pintji population and future hard reintroductions. The synthesis of these outcomes may prove useful to managers of other *Petrogale* reintroductions, enabling generalised trends to be identified and by highlighting knowledge gaps. Further areas of research that will benefit the warru recovery project (and address the five remaining questions in the framework), and future reintroduction of other *Petrogale* and arid zone species are outlined below.

The most important question surrounding the potential use of reintroduction to recover warru populations is: how do hard-released individuals respond to introduced predators and native/introduced competitors? Pre-release monitoring of predator and potential competitor densities at the selected release site, combined with implementation of control methods (baiting and spotlight shooting) and subsequent evaluation of their effectiveness in reducing predator/competitor densities, will be essential to address this question. Combined with survival and reproductive monitoring of released warru, continued post-release predator/competitor control and monitoring may be able to determine the thresholds of abundance of these species that are required to achieve successful warru reintroduction. This, in turn, could provide guidance for other arid zone species reintroductions. Furthermore, predator dynamics around extant warru colonies are not well understood and would also benefit from targeted research to inform adaptive management.

In addition to population growth rates, long term persistence can be facilitated through genetic diversity (Biebach and Keller 2010; Jamieson and Lacy 2012). While genetic diversity of a population can often be enhanced through the supplementation of individuals, this is not a sustainable management option for warru and ideally should be promoted via natural dispersal between reintroduced and proximal *in situ* colonies. The development of natural dispersal capabilities within captive warru, and their success in the presence of introduced predators, is yet to be determined, therefore initial movement data and regular genetic monitoring of neighbouring populations should be implemented. Data on dispersal rates and their effect on population genetic diversity could be used to extend the current PVA model to examine key questions 6 and 7, ‘What is the optimal allocation of translocated individuals among sites?’ and ‘Should translocation be used to compensate for isolation?’ (Figure 7.1).

The warru pintji also provides an opportunity to study the accuracy of the current PVA model and the impact of density and carrying capacity on population growth rates for *Petrogale*. A continued annual population census could be used to examine the impact that the removal of warru groups for hard releases and supplementations from the zoo/wild and population density will have on population growth rate, health and condition of individuals. As resource depletion has been implicated in the failure of other enclosure reintroductions (see review of Clayton, 2011), it will be important to determine the carrying capacity within the facility. Establishment of remote cameras around the fence perimeter and continued track monitoring may assist in the detection of changes in space use by warru. This will not only provide further information for metapopulation level question 5 within *Petrogale* reintroduction biology but will also supplement limited knowledge on the role that density factors have in the regulation of wild *Petrogale* population dynamics.

Furthermore, the warru pintji now offers the potential to investigate the reintroduction biology of other arid zone species, particularly critical weight range mammals in the absence of introduced predators. Reintroductions into the Arid Recovery Reserve (Roxby Downs, South Australia) have made significant progress towards understanding these behaviours (Moseby and Bice 2004; Moseby and O’Donnell 2003; Moseby *et al.* 2011) which could be further investigated through replicated releases of similar species within the warru pintji. Traditional owners have highlighted particular interest in bringing back *ninu* (greater bilby, *Macrotis lagotis*) and *wayuta* (brush-tailed possum, *Trichosurus vulpecula*). Not only would this extension of the project provide opportunities to explore questions within ecosystem-level restoration frameworks (Ewen and Armstrong 2007) and questions 8-10 within the reintroduction biology framework (Armstrong and Seddon 2008), but it would also contribute to the historical and contemporary cultural beliefs and practice (the Tjukurpa or “dreaming” of A<u>n</u>angu). The successful outcomes observed in the current project have strengthened contemporary Tjukurpa, with an inma (song) developed by the warru minyma when pouch young left the lands to be cross-fostered. A new verse was added when warru were first returned to the pintji in 2011 and has been sung multiple times during each pintji trapping trip since. The continued management and research of reintroduced populations within and outside the warru pintji will not only contribute to the reintroduction biology of arid zone species and evaluation of specific management strategies, but will also continue to fulfil the aspirations of Aṉangu for positive landscape-scale environmental outcomes in this incredible and remote part of the world.

The warru trapping team and three warru from the 'training troop', July 2013. These plush toys were made to assist ranger and project staff training in the techniques for monitoring warru. Photo: R West

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APPENDICES

Appendix 1A: Warru pintji site selection attributes and scoring system

Appendix 1B: Warru pintji site selection results

Appendix 2: Warru Trapping Standard Operating Procedure summary

Appendix 3: Warru handling training program

Appendix 1A: Warru pintji site selection attributes and scoring system.

ID	Criteria	Criteria Level	Essential?	Score
A	Accessible to Anangu workers	Reasonable distance to a potential Warru ranger team	Yes	4
		Likely to detract some Warru rangers from working there		2
		Ranger team(s) / Anangu unwilling or unable to work at site		Exclude
B	Anangu clearance	Site cleared by Traditional Owners and APY Executive and anthropological clearance	Yes	
		Site **NOT** cleared by Traditional Owners and APY Executive and anthropological clearance		Exclude
C	Warru presence / absence	Warru currently resident	Yes	Exclude
		Warru present in last ten years.		2
		Warru present in last 50 years.		2
		Warru never present		1
D	How many individuals could be sustained at the site?	0-15	Yes	1
		15-30		2
		30-60		3
		60+		4
E	Will fencing the site affect dispersal movements between other locations?	Site would definitely exclude movements between to active locations	Yes	0
		Site between active and historical location		1
		Site between two historical locations		2
		Site adjacent to active location		2
		Site isolated		3
F	Dispersal / connectivity between colony sites (ability to start metapopulation)	Continuous rock faces to medium / large dispersal sites	Not applicable – Only for reintroduction	Exclude
		Continuous rock faces to small dispersal sites		Exclude
		Some connectivity to rock faces to medium / large dispersal sites		Exclude
		Some connectivity of rock face to small dispersal sites		Exclude
		No connecting rock faces / outcrops but medium / large habitat within 500m		NA
		No connecting rock faces / outcrops but small within 500m		NA
		Isolated site		NA
G	Food plants available	Abundant / Very common	Yes	10
		Common		6
		Uncommon		3
		Rare / absent		Exclude
H	Size of area with suitable habitat (in particular shelter sites)	Approx 10 ha or more	Yes	4
		0 – 10 ha		2
		None		Exclude

Appendix 1A: contd.

ID	Criteria	Criteria Level	Essential?	Score
I	Number of ideal shelter sites per ha. Ideal = labyrinth/crevice, fits 1-2 people, low –medium exposure, in / adjacent to other areas of high complexity.	10 or more	Yes	10
		0-10		5
		None		Exclude
J	Management of predation	The site greater than 4km from a community (Yes / No)	No	
		A continuous track network within 10km is or can be established (Yes / No)		
		An on site baiting ring can be established (Yes / No)		
		A multiple layer of feral predator defence be established (Yes / No)		
		Is baiting currently occurring within 1 km of the site (Yes / No)		
K	Bait take	No / low	No	
		Medium		
		Consistently High		
L	Fire Risk	High Buffel grass / spinifex fuel loads in the flats		0
		Potentially high Buffel grass / spinifex fuel loads following rain in the flats		1
		Low Buffel / spinifex fuel loads in the flats		2
		No Buffel or spinifex in the flats		3
M	Fire control	Tracks encircling site	Yes	2
		Tracks passing site		1
		No tracks accessing site		0
N	Competition from Rabbits	No / low rabbit densities at site and controllable	Yes	5
		Rabbits in medium – high density and difficult to manage		0
O	Competition from other macropods	Low or no threat due to low densities or absence	Not Applicable, Kanyula to be removed.	NA
		Medium threat due to medium densities		NA
		High threat due to high densities		NA
P	Can a predator proof fence be 'easily' established around site? DT)	Yes, easily	Y	10
		Yes, with minor difficulty		5
		No		Exclude

Appendix 1A: contd.

ID	Criteria	Criteria Level	Essential?	Score
Q	Ability to manage the site safely (Available communications, vehicle access, terrain at sites, travel time etc.)	Good communications (Yes / No)	Yes	4
		Vehicle Access (Yes / No)		4
		Travel Time < 1 hour		4
		Travel time 1-2 hours		2
		Travel Time > 2 hours		0
R	Human Usage	Low human use, managed easily	Yes	5
		Existing moderate level of usage		3
		High usage, difficult to manage		Exclude
		Major human use area		Exclude
S	Accessibility (is the site easy to access by the community for visits etc) and for fence checking, colony maintenance activities	Not far off a well maintained road so that impacts associated with increased activity are minimised.		4
		Access Road likely to get boggy or degraded in the wet or prone to erosion with increased use.		0
T	Potential to form large, complex metapopulation	> 10 potential &/or historical) refuge areas within 10 km and well connected by rugged terrain	No – For reintroduction.	
		5-9 potential &/or historical) refuge areas within 5km and well connected by rugged terrain		
		1-4 other potential &/or historical) refuge areas within 5km		
		No other potential refuge areas within 5 km		

For full document see: Ward M. J., Read J., Partridge T., Taggart D. A. and Dent A. (2010) Warru pintji (fence) site selection. Unpublished report to the Warru Recovery Team. Department for Environment and Heritage: Adelaide, Australia.

Appendix 1B: Warru pintji site selection results.

Site 21 was the highest scoring and was given anthropological clearance. This is the site of the ‘warru pintji’. See Ward M. J., Read J., Partridge T., Taggart D. A. and Dent A. (2010) Warru pintji (fence) site selection. A report to the Warru Recovery Team. Department for Environment and Heritage: Adelaide, Australia.

		Site										
		05	**08**	**10**	**11**	**12**	**14**	**18**	**21**	**22**	**24**	**25**
A	Accessible to Anangu workers	4	4	2	4	2	2	2	4	4	4	4
B	Anangu clearance	?	?	Y	?	y	?	?	y?	y?		
C	Warru presence / absence	Yes Exclude	Yes - Exclude	2	2	2	2	2	2	2		
D	No. of individuals			4	4	4	3	3	4	4		
E	Fencing and dispersal			3	3	3	3	2	3	3		
F	Dispersal											
G	Food plants			6	10	6	6	6	10	10		
H	Size of suitable habitat			4	4	2	2	4	4	4		
I	No. of shelter sites			5	5	5	5	5	10	5		
J	Predation											
K	Baiting											
L	Fire risk			2	2	2	2	2	2	2		
M	Fire control			1	1	2	0	1	2	2		
N	Rabbit competition			5	5	5	5	5	5	5		
O	Macropod competition											
P	Fencing viability			5	No - exclude	5	10	5	10	10	No - Exclude	No - Exclude
Q	Management			4		4	2		4	4		
R	Human Usage			5		5	5	High - Exclude	5	5		
S	Accessibility			4		4	0		4	4		
T	Metapopulation potential											
	Comments						attributes assess from imagery	Major thoroughfare				
	Exclude?	Yes	Yes		Yes			Yes			Yes	Yes
	Score	**Exclude**	**Exclude**	**52**	**Exclude**	**51**	**47**	**Exclude**	**69**	**64**	**Exclude**	**Exclude**

Appendix 2: Warru Trapping Standard Operating Procedure summary.

WARRU PROCESSING AND DATASHEET CHECKLIST

STRESS CHECK 1

START STOPWATCH

1. Remove warru from trap and bag

2. Weigh warru + bag – record total weight (kg)

3. Vitamin E injection

less than 5kg	0.05ml
5kg	0.1ml
5kg+	0.15ml

4. Retrap warru

4.1 Microchip – record last 6 digits

4.2 Record ear tag colour inner and outer

4. New warru

4.3 Microchip

Mchip sticker on sheet

4.4 Ear biopsy

Low down ear

Clean with ethanol

Use Cardboard

Label with mchip sticker

4.5 Ear tag

Check available colours

Trim ear tags

Tease apart after insertion

STRESS CHECK 2

5. Place/replace collar (if required)

Check frequency

Record original and tuned

2 fingers space

CHECK WARRU WEIGHT

VHF WARRU > 2.5KG

GPS WARRU > 3.0KG

STRESS CHECK 3

Tip of nose to back of head

6. Headlength mm

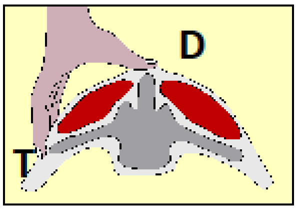
Heel to tip of longest toe (no nail)

7. Pes mm

Base to bone tip (no excess hair)

8. Tail cm

Appendix 2: contd.



9. Record condition score

Condition score	D and T assessment
1	T prominent
2	T can be felt as bump
3	T covered – i.e. can still feel when pressed but can't be seen
4	D can be felt when pressed
5	D and T well covered

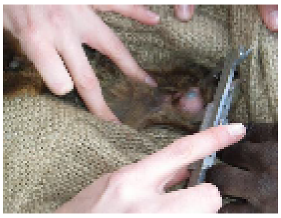
STRESS CHECK 4

10. 1 Males/Wati

Record maximum diameter of testes in mm

10.2 Females/Kungka

If no PY: record pouch condition and teat



PY – DON'T TOUCH OR REMOVE

Record headlength (HL) in mm

If HL > 12mm, sex PY

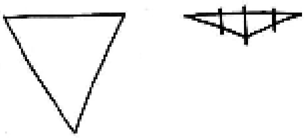
If furred – place microchip and take biopsy

Male – 2 bumps

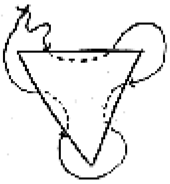
Female – 1 bump + V

If HL 30 - 60mm, secure PY even if not thrown.

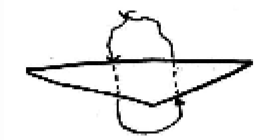
HL 30 – 40 mm 3 staples



HL 41-60mm Purse string suture



HL 41-60mm + mother stressed mattress suture



If HL 60mm+, leave mother and PY in bag.

11. Release into cave/crevice.

Record release speed and total time.

12. Weigh bag and calculate warru weight (kg)

Appendix 3: Warru handling training program

WARRU HANDLING TRAINING PROGRAM

New handler name: .... Start date: ....

Trainers must demonstrate each technique to new handlers using the plush toy/wild warru. New handlers should have the opportunity to try the technique under the trainer's supervision with the plush toy warru. Each technique should then be practiced on a wild warru during the trapping trip. Trainers should give a skill rank at each attempt and sign and date in the box.

	TRAINER: rank skill 1 to 5 AND INITIAL				
PROCESSING TECHNIQUE	TOY WARRU		WILD 1	WILD 2	WILD 3
Remove warru from trap					
Place warru in to catch bag					
Hold warru for processing					
Administer vitamin E injection					
Head measurement					
Pes measurement					
Tail measurement					
Take a condition score					
Administer microchip					
Take ear biopsy					
Place ear tag					
Fit a collar					
Tune collar frequency					
Take a scrotal measurement					
Hold in pouch check position					
Take small pouch young headlength					
Staple a pouch					
Purse string suture a pouch					
Mattress suture a pouch					
Release a warru from the bag					

SKILL RANKING

1	CAN'T PERFORM
2	CAN PERFORM WITH ASSISTANCE
3	CAN PERFORM WITH SUPERVISION
4	CAN PERFORM ALONE
5	CAN TRAIN OTHERS

Where possible, new handlers should reach a skill rank of 3 using the plush toy warru, before attempting on a wild warru.