Conservation biology of greater bilby (Macrotis lagotis) populations translocated to the temperate zone of South Australia



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Thesis Abstract

Many threatened Australian marsupial species had distributions that encompassed large parts of the Australian continent prior to European occupation. However, as their remnant populations are now confined to relatively small parts of Australia, our knowledge on their ecology is limited to their extant range or captive populations. This thesis aimed to explore whether aspects of the biology of the greater bilby (*Macrotis lagotis*) differed in a temperate environment from what has been recorded in arid zone populations, and sought to gather information that will aid in the future establishment and management of translocated temperate zone populations. This study used a comparative approach to investigate the population dynamics, breeding biology, and behaviour of greater bilby populations translocated to two sites in temperate South Australia, Venus Bay Conservation Park and Thistle Island.

Regular field surveys were conducted between August 2007 and April 2010. Mark-recapture and spatially explicit capture-recapture analyses indicated a high density, stable bilby population occupied Thistle Island, which had a previous history of clearance and grazing, but was free of exotic predators. In contrast, the Venus Bay Conservation Park population declined over the study period. Adult survival rates were similar in both populations; however, the Venus Bay population had low recruitment rates and low numbers of subadults despite high adult female fecundity, likely due to high levels of predation by feral cats after pouch emergence. Births of pouch young in both populations were seasonal (June to January) with a cessation of breeding in autumn, which differed from what has been recorded in arid zone populations. Females produced up to three litters per annum, and the mean head length of females that successfully raised twins was significantly larger than that of females who raised a single pouch young.

Spool-and-line tracking of bilbies at Venus Bay Conservation Park revealed individuals used multiple burrows and regularly moved between diurnal burrows. Spatial analysis using burrow locations revealed males had significantly larger burrow ranges than females, and that burrow range sizes in this temperate zone population were similar to those recorded in an arid zone population. Active burrow density at the site was at least 2.75 to 5 times the density of bilbies at the site. Parentage analysis using 10 microsatellite loci concluded that a small number of males in the two populations dominated the breeding in the areas they were captured. In both populations, nearly all females bred within a breeding season,

whereas only a maximum of 20% of the males that we captured had sired pouch young we sampled in the area. Body mass and relative testes size of males were poor predictors of reproductive success. However, genetic relatedness values of breeding pairs were lower than expected by chance in the Venus Bay population.

The thesis suggests a previously widespread species such as the greater bilby can adapt its reproductive strategy to suit local conditions. However, burrow use behaviour and the mating system operating at the sites were similar to what has been recorded in arid zone populations. Given female body size influenced fecundity in the study, releasing large females at the start of the breeding season in temperate areas may maximise the initial rate of increase of a translocated greater bilby population. Female-biased founder groups may also be appropriate given that only a small proportion of males were found to breed in a breeding season. This thesis suggests that greater bilbies and their burrows were once a common part of the southern Australian landscape prior to their local extinction. Therefore, greater bilbies should be considered as part of any ecosystem restoration projects within southern temperate areas of their former range.

Thesis declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, 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 in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide.

I acknowledge that copyright of published works (Chapters 2, 3 and 4) contained within this thesis resides with the copyright holders of those works. I give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

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Karleah Berris

Signed …… …… Date: 2/09/2024

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Chapter 1: General Introduction



Venus Bay Conservation Park
Photo by Karleah Berris

"It's surely our responsibility to do everything within our power to create a planet that provides a home not just for us, but for all life on Earth."

Sir David Attenborough

1.1 Mammal extinction and decline

Mammalian species globally are declining at a rapid rate due to human-induced changes to our planet (Brook *et al.* 2003; Karanth *et al.* 2010; Hoffmann e*t al.* 2011; Palmeirim *et al.* 2020). As a result, the mammal diversity patterns we now observe across the world have been significantly influenced by human-driven extinctions (Faurby and Svenning 2015). Humans may have been manipulating global diversity as far back as the late Quaternary, and recent studies suggest global megafauna extinctions during this period were more likely to be driven by human impacts than climate change (Sandom e*t al.* 2014; Bartlett *et al.* 2016; Andermann *et al.* 2020). However, extinctions do not tell the whole story. Across the globe, many species that still persist are suffering population declines, range contractions and local extinctions from at least parts of their former range (Ceballos *et al.* 2017; Tomlinson *et al.* 2023). It is unlikely that this trend will abate in coming years. The global human population is projected to increase further in the coming decades (Cohen 2003) and this will likely lead to an intensification of pressures on high biodiversity areas, further land use change and further extinctions (Venter *et al.* 2016; Lui *et al.* 2024).

The causes of mammal extinctions and ongoing declines are complex and vary between countries, habitats and species. Deforestation and land-use change are key drivers of mammal decline in forest habitats (Brook *et al.* 2003; Harper *et al.* 2007; Tracewski *et al.* 2016). Deforestation can lead to fragmentation of remaining habitat, which can increase extinction risk in mammals and leave patches of habitat that are too small to support populations (Gibson *et al.* 2013; Crooks e*t al.* 2017). Human activity within remnant habitat, such as grazing, fire management and recreation activities can also lead to habitat degradation and decline of mammal populations (Legge *et al.* 2011; Griffiths *et al.* 2015; Shamoon *et al.* 2018). Targeted capture of mammal species for bushmeat hunting and illegal poaching is further reducing population sizes and causing local extinctions (Ripple *et al.* 2016; Palmeirim *et al.* 2020; Rija *et al.* 2020). Invasive species, which have arrived on foreign shores with humans, also play a major role in extinction and population decline of native species, particularly in Australia (Doherty *et al.* 2017; Radford *et al.* 2018; Murphy *et al.* 2019; Woinarski *et al*. 2019). In addition to these factors, climate change is now recognised as a significant threat to the persistence of many mammal populations globally (Urban 2015; Baisero *et al.* 2020; Evaldt *et al.* 2024).

1.1.1 Australian mammal decline and the loss of ecosystem engineers

Mammalian extinctions during recent centuries have been particularly severe in Australia, with ~35 % of all global mammal extinctions since the year 1500 representing Australian endemic species (Woinarski *et al*. 2015). Numerous species that were once widespread across the continent now occupy a mere fraction of their historic range prior to European colonisation (Ford 2014; Woinarski *et al*. 2015; Tomlinson *et al.* 2023). Ninety-one Australian native mammal species are now absent from at least half of the bioregions that they occupied prior to the arrival of Europeans (Burbidge e*t al.* 2008). There is mounting evidence that introduced predators, which arrived with Europeans, have played a key role in native mammal declines, and are continuing to drive species closer to extinction (Doherty *et al.* 2017; Radford *et al.* 2018; Murphy *et al.* 2019; Woinarski *et al*. 2019). A large number of Australian endemic mammal species are within the ideal prey weight range for feral cats (*Felis catus*) and red foxes (*Vulpes vulpes*) (Woolley *et al.* 2019). In general, ground-dwelling species within the 'critical weight range' (CWR) of between 35 g and 5.5 kg are most at risk of extinction in Australia (Burbidge and McKenzie, 1989; Johnson and Isaac, 2009).

The impacts of the severe decline in mammalian diversity across Australian landscapes are largely unknown, but evidence is mounting that many of these mammal species played important roles in ecosystem function (Fleming *et al.* 2014; Michael *et al.* 2022). Much of Australia was once occupied by mammals that dig during foraging activities. Digging species are a type of ecosystem engineer, and their activities have been shown to be important for soil function, fungi dispersal and native seedling germination (Fleming *et al.* 2014; Dundas *et al.* 2018; Davies *et al.* 2019; Decker *et al.* 2019). Soil carbon sequestration has been shown to be higher in the presence of native digging mammals, which could result in higher water holding capacity of soils and greater plant establishment and growth (Decker 2020). Ecosystem engineers have also been shown to increase the rate of leaf litter breakdown, which is linked to reduced flame heights and reduced spread of fires (Hayward e*t al.* 2016). The widespread contraction of digging mammals in Australia has likely led to a deterioration of ecosystems and soil function across the continent (Fleming *et al.* 2014; Decker 2020). Therefore, conservation and restoration of Australia's mammalian fauna is also important for the restoration of our unique Australian ecosystems.

Ten Australian marsupial species and one monotreme were recorded to dig burrows for shelter (Fleming *et al.* 2014). While digging during foraging activities mostly influences soil function, leaf litter and plant germination, the construction of burrows has direct impacts on other fauna species that do not dig. Numerous studies have now shown that non-digging species will use the burrows of digging mammals as shelter if they are available (Wood Jones 1924; Copley 1999; Haby *et al*. 2013; Ostendorf *et al*. 2016; Hofstede and Dziminski 2017; Thornett *et al*. 2017; Dawson *et al*. 2019). Burrows often have stable climatic conditions and can provide a critical refuge during and after bushfires (Newsome *et al*. 1975; Long 2009; Chapman 2013; Linley *et al.* 2024). The loss of burrowing species, and their burrows from across Australia may have reduced non-burrowing species access to shelter from predation, extreme temperature and fire. Therefore, the re-establishment of populations of digging species could improve conservation outcomes for non-digging burrow commensal species.

The loss of native digging mammals may have less pronounced impacts on ecosystems if non-native invasive mammals can fulfil the ecological roles that native species once filled. However, this has not been the case in Australia. As an example, the European rabbit (*Oryctolagus cuniculus*) was introduced by Europeans during the mid to late 1800's and spread rapidly across the southern half of the Australian continent (Myers 1970). European rabbits play important ecological roles in Europe, where they have positive effects on plant species diversity and soil fertility, and their digging and burrowing habits create shelter for various other fauna species (Delibes-Mateos *et al.* 2008). However, in Australia, it is a different story. European rabbits have significant negative impacts on the recruitment and survival of Australian native plant species, and their diggings fail to show the same benefits to native seedling recruitment as diggings made by Australian native mammals (Lange and Graham 1983; James *et al.* 2011; Bird *et al.* 2012; Mutze 2016). Therefore, to restore these ecosystem processes across Australian landscapes, the most effective approach will likely be to reduce or remove invasive species like rabbits and protect or rehabilitate populations of native ecosystem engineers (James *et al.* 2011; Cooke 2012).

1.1.2 The local situation – mammal decline in South Australia

Local extinctions recorded for South Australia provide an example of how range contractions of mammal species are shaping our current fauna assemblages. Thirty out of 114 mammal species are estimated to have become extinct in the state since the time of

European colonisation (Robinson *et al.* 2000; Table 1.1). Twelve species that are locally extinct in the state are also nationally extinct (Robinson e*t al.* 2000), leaving 18 species locally extinct in South Australia that still have remnant natural populations existing in other states. Bandicoot (Order Peramelemorphia; Family Peramelidae) and bilby species (Family Thylacomyidae) have fared the worst, with seven out of the eight species that once occurred in South Australia now locally or nationally extinct (Kemper 1990; Robinson e*t al.* 2000). Overall, it is estimated that South Australia's current mammalian assemblage (based on remnant populations of native species) represents only 74% of the species that occurred at the time of European colonisation (Table 1.1).

Table 1.1 The total number of mammal species recorded in South Australia at the time of European colonisation, and the number of species whose natural populations became extinct in the state post-colonisation. Data are from Robinson *et al.* (2000).

Order	Common name	Total species recorded	Number of species locally extinct
Monotremata	Platypus and echidna	2	0
Dasyuromorphia	Carnivorous marsupials	26	5 (19%)
Peremelemorphia	Bandicoots and Bilbies	8	7 (88%)
Notoryctemorphia	Marsupial mole	1	0
Diprotodontia	Koala, wombats, possums and macropods	30	10 (33%)
Chiroptera	Bats	23	1 (4%)
Carnivora	Carnivorous eutherian mammals	1	0
Rodentia	Rodents	23	7 (30%)
Total		114	30 (26%)

Species that have become extinct in the state are primarily ground-dwelling marsupials, many of which are digging species (ecosystem engineers). In South Australia there were 17 digging species present in the state at the time of European arrival, and it is likely these

species played a role in soil and ecosystem function (Fleming *et al.* 2014). However, natural populations of 12 (71%) of these digging species no longer occur in the state. Given the importance of digging mammals for several ecosystem processes, it is likely that effective ecosystem function of South Australian landscapes is severely compromised due to the loss of these mammals.

1.2 Conservation Biology and Management Practices

The aim of conservation management programs for declining mammal species is to prevent extinction of species and preserve the habitats that are critical for their survival. But with so much of the world's mammalian fauna in peril and limited financial resources allocated to conservation, the first step is often an assessment of which species are most at risk of extinction and require urgent intervention. Globally, The International Union for Conservation of Nature (IUCN) compiles information on species and assesses their conservation status through the 'Red List of Threatened Species' (IUCN 2024). Species are assessed against several criteria, including population trajectory, geographic distribution and total population size. Species that are identified as threatened with extinction are given a status of either 'Critically Endangered', 'Endangered' or 'Vulnerable' to rate their threat level (Croteau and Mott 2011). Similar processes exist in many countries under local legislation. For example, in Australia the conservation status of species is assessed under the 'Environment Protection and Biodiversity Conservation Act 1999' (EPBC Act).

Once species are determined to be at risk of extinction, there are several conservation management approaches that are commonly applied in an attempt to prevent the extinction of a species. The main conservation management practices are outlined below, with particular reference to those commonly employed in Australia.

1.2.1 Protected areas

Protected areas make up more than 16% of the earth's terrestrial and inland water areas, and as the global protected areas network grows it encompasses more ecosystem types and becomes more representative of the full range of earth's ecosystems (IUCN 2021). Within protected areas that are well defined, well-resourced and legally protected, lower levels of human induced impacts are often recorded (IUCN 2021). For some mammal species, almost all of their current range is now within protected areas, however adequate resourcing and effective reserve management can be crucial to species persistence within

them (Watson *et al.* 2014; Pacifici e*t al.* 2020). Some regions struggle to prevent human induced threats within protected areas, such as hunting of large game in Africa (Craigie *et al.* 2010). In some instances, protected areas themselves can contain focal points of human activity that affect threatened species due to tourism and human interest in exploring these natural areas (e.g. da Silva *et al.* 2018). However, protected areas still remain a vital tool for the protection of threatened mammals and their habitats (IUCN 2021). In Australia, ecologically important areas are protected as part of the National Reserve System, and more than 22.1 % of Australia's land area is protected under this scheme (DCCEEW 2023a), which is higher than the global average. Threatened species in Australia with a greater distributional overlap with protected areas have recorded better conservation outcomes (Taylor *et al.* 2011).

1.2.2. Laws and regulations

Threatened species can be afforded legal protection at both a national and international level. Legal protection can restrict or prohibit the capture, killing, disturbance or trade in threatened species. For example, species where illegal poaching and wildlife trafficking is a key threatening process can be listed under the Convention on International Trade in Endangered Species (CITES) of wild fauna and flora, which is an international treaty restricting trade of listed species (CITES 2022). Legal protection can also apply to habitat that is critical to the survival of a threatened species. In Australia, threatened species are identified and protected under the EPBC Act. Although the EPBC act aims to protect species and their habitats, some criticisms of this legislation have been that listings under the Act are taxonomically biased and that a lack of enforcement makes the Act ineffective at protecting threatened species habitat (Walsh *et al*. 2013; Ward e*t al.* 2019).

1.2.3 Raising public awareness

Public awareness can be important for threatened species conservation for several reasons. As many species have a geographic range that overlaps with private land, public awareness and landholder engagement is also important to promote management of threatened species across different land tenures. Landholder awareness and attitudes towards threatened species can vary between regions or even between neighbouring landholders, and management of threatened species on private land is dependent on landholders understanding of, and appreciation of, local threatened species and the legislation that protects them (Kamal *et al.* 2015; Olive and McCune 2017). Positive

engagement and relationships between landholders, researchers and conservation managers can result in long-term beneficial conservation outcomes (Bridle e*t al.* 2021). Raising awareness more broadly through Zoos or television publicity can also increase public donations for featured species (Fukano e*t al.* 2020).

1.2.4 Species specific management actions

In some instances, protected areas, legislative protection, and public awareness are not enough to halt the decline in a species population trajectory. In these cases, more intensive management of species may be required. Management can be *in situ*, where the conservation management actions take place where remnant populations occur, with the aim of maintaining viable wild populations. *In situ* management actions can include invasive species control (Dueñas *et al.* 2021), the provision of artificial refuges such as human-made structures to replace dens or nests (Cowan e*t al.* 2021; Watchorn e*t al.* 2023) and revegetation to increase or connect remnant habitat (Craig *et al.* 2017). Many threatened species are also the subject of *ex situ* conservation programs which involve conserving species outside their remnant natural habitat and can include captive breeding and translocation. The purpose of conservation translocations is generally to create or re-create populations of a target species that are self-sustaining with minimal intervention (Seddon *et al.* 2012). Translocations can be used to establish populations of species within their former range, outside their historic range to create insurance populations, or to supplement existing populations that are declining (Seddon e*t al.* 2012). Translocations have been used globally for mammalian species, and over the years the success of translocations has been growing as causes of failure have been increasingly identified (Morris *et al.* 2021; Gross *et al.* 2024). Translocations are widely used in Australia, despite evidence to suggest they are less successful in the Oceania region than in Europe and North America, likely because of the widespread occurrence of invasive species in this region (Morris *et al.* 2021). Due to the importance of translocations to Australian conservation programs, the following section will explore the current use of, and lessons learnt from translocations in Australia.

1.3 Translocations in Australia

At least 54 Australian native mammal species have been the subject of translocation programs (Short, 2009). For a state like South Australia, where numerous mammal species are locally extinct, translocations are a particularly valuable tool that can be used to re-

establish populations of a species within its historic range. It is widely recognised that if the key threats that led to the original decline of a species cannot be eliminated or managed, translocation to an area is not recommended and is unlikely to be successful (IUCN, 1987; Kleiman 1989; Perez *et al*. 2012). Many Australian species that have suffered massive range declines now exist in small, isolated or fragmented populations (Woinarski *et al.* 2015). Therefore, captive breeding is sometimes a necessary step to provide enough founder individuals for translocations (e.g. Moro 2003; Moseby and O'Donnell 2003).

1.3.1 Causes of translocation failure

Translocations have been widely implemented for medium-sized marsupials to repatriate species to parts of their former range. However, success has been variable (Short 2009; Morris e*t al.* 2015). Many Australian translocation failures have been attributed to introduced predators, and the inability of our native mammals to survive in the presence of these species (Moseby *et al.* 2011a; Moseby e*t al.* 2015a). Both foxes and feral cats can impact the population persistence of translocated populations of medium sized marsupials, and both of these introduced predators are likely to have been a major cause of the original decline of many species (Moseby *et al.* 2015b; Radford e*t al.* 2018; Moseby *et al.* 2021). Intensive lethal control of introduced predators at key sites can lead to improvements in native mammal abundance (Dexter and Murray 2009; Robley *et al.* 2014). Current lethal control techniques for foxes are relatively effective at reducing their abundance. However, lethal control of feral cats in open populations does not always lead to reduced feral cat activity at control sites (Moseby and Hill 2011; Lazenby *et al.* 2015). Because introduced predators are so widespread across the continent, and are able to rapidly recolonise areas, the efficacy of small-scale control programs is questionable in the long-term, given that even low predator densities can have significant impacts on native mammal populations (Saunders *et al.* 2010; Moseby *et al.* 2015b; Doherty *et al.* 2017). Further complicating matters is evidence that feral cats can increase in areas where fox numbers are reduced through lethal control (Marlow *et al.* 2015).

More recently, there have been improvements in control techniques for feral cats (Johnston *et al.* 2020; Moseby *et al.* 2020) and a significant effort in feral cat control in Australia (Garrard *et al.* 2020). Despite these advances, feral cats are still estimated to be present across > 99.8 % of Australia's land area (Legge e*t al.* 2017), leaving very few places where translocations can take place in the absence of introduced predators. There is

evidence from some areas of Australia that suggests dingos may suppress fox and feral cat activity (Letnic *et al.* 2012, Schroeder *et al.* 2015). However, much of the available research has found little or no relationship between dingo and feral cat abundance, indicating that increasing dingo populations is unlikely to be a solution for the impacts of feral cats (Allen *et al.* 2014). Medium-sized marsupials such as bilbies and bandicoots are also recorded prey items of dingos (Fleming e*t al.* 2022), therefore their populations likely still experience top-down effects of predation in the presence of dingoes. Therefore, to date, feral predator control has largely been carried out at small scales with varying levels of success, and landscape wide control or eradication of these invasive species is still unachievable.

1.3.2 Translocations to 'safe havens'

To improve conservation outcomes for threatened species, 'safe havens' have been proposed to provide areas where risks to species persistence can be removed. Safe havens for medium-sized marsupials in Australian have largely taken the form of either offshore islands or fenced exclosures on mainland Australia where introduced predators have been removed entirely, or at the very least, effectively managed (Legge e*t al.* 2018). The number of these safe havens around Australia has increased significantly in recent decades (Ringma e*t al.* 2018). More than 101 islands and 17 exclosures, representing an area of 2498 km^2, now help protect 32 different mammal species from extinction (Legge e*t al.* 2018). Populations of native mammals within safe havens that exclude introduced predators have fared significantly better in recent decades than unprotected populations (Tulloch *et al.* 2023). These safe havens have not only provided additional sites for translocations of medium-sized marsupials, but they are also beneficial to *in situ* populations of native mammals (Moseby *et al.* 2009; Roshier *et al.* 2020). The success of safe havens has provided further evidence of the devastating impact of invasive species on Australian native mammals. The Australian Government's 2015 Threatened Species Strategy and Threatened Species Action Plan 2022-2032 both have a large focus on managing feral cats and creating more safe havens for threatened species. Therefore it is likely that the use of safe havens will continue to increase.

1.3.3 Management of 'safe haven' populations

There is no doubt that safe havens have increased the long-term security of some of Australia's most imperilled medium-sized marsupials. However, confining once widespread species to relatively small and isolated areas can sometimes have unintended

consequences for populations, including increased susceptibility to environmental catastrophes, overpopulation, and reduced gene flow leading to reduced genetic variation within populations (Hayward and Kerley 2009; Helmstedt *et al.* 2014; Moseby e*t al.* 2018; White *et al.* 2020a). These potential issues generally mean that in order to maintain a viable population of a target species within a 'safe haven', without the loss of genetic diversity, ongoing management of the site and the population is required.

Populations of medium-sized marsupials within safe havens are essentially closed populations, which are unable to disperse outside the boundaries of their fenced reserve. Small founder groups and genetic isolation from other populations can result in significant reductions in genetic diversity over time or unintended genetic divergence between populations (White *et al.* 2020a; Nistelberger *et al.* 2023). A reduction in genetic diversity in small, isolated populations could lead to a heightened risk of extinction due to reduced fitness of the population (Weeks *et al.* 2015). Given the small geographic range of most safe havens, stochastic events such as droughts and bushfires could also lead to population crashes as individuals are unable to disperse to refuge areas or vacate the 'safe haven' area if conditions deteriorate. These reductions in population size, could produce bottlenecks, further reducing genetic diversity. Reduced gene flow and low diversity in a population can, however, be mitigated in translocated populations by sourcing founders from large, genetically diverse populations, encouraging rapid population growth and simulating gene flow between populations through supplementation (Margan *et al.* 1998; Jamieson and Lacy 2012; Weeks *et al.* 2015). However, this means rather than a once-off release, translocated populations may require ongoing supplementation with individuals from other populations.

A lack of dispersal opportunities can also lead to overpopulation. For example, a reintroduced population of herbivorous burrowing bettongs (*Bettongia lesueur*) became overabundant within a fenced conservation reserve, resulting in a reduction in palatable plant species and a reduction in activity of another reintroduced herbivorous species (Moseby e*t al.* 2018). A lack of native predators was a possible cause, and this case highlights the need to consider translocations of all trophic levels to promote ecosystem function (Moseby e*t al.* 2018). It could also mean individuals in high density populations require removal and translocation to other populations to reduce population size.

1.3.4 Risks and opportunities for 'safe haven' populations

Populations within safe havens face some risks long-term. Often exotic predators are completely excluded from safe havens, hence populations of threatened species do not need to develop anti-predator behaviours, and there is concern this could heighten prey naiveté of native mammals to exotic predators (Moseby *et al.* 2016; Bytheway and Banks 2019). Controlled predator exposure and avoidance training is an emerging field of research and has been shown to result in behaviour changes in individuals when exposed to exotic predators (West *et al.* 2018; Ross *et al.* 2019). However it is not yet known whether the behaviours elicited by training are learned by subsequent generations and whether there is any effect on long-term population persistence in the presence of exotic predators. Fences are also not impenetrable; incursions of introduced predators into safe havens or persistent low levels of exotic predators (that have never been completely eliminated) can cause the failure of translocations into safe havens (Bradley *et al.* 2015; Short 2016).

Translocated populations in safe havens also provide opportunities to research the aspects of the biology of species when exposed to different climatic conditions and ecosystems. Some Australian species have not been present in landscapes for more than 100 years, and safe havens provide the first opportunity to study once wide-ranging species in some parts of their former range. In many cases, our knowledge of the biology of a species comes only from studies where its remnant populations now occur, and it is not known whether aspects of their ecology, including behaviour, mating systems, habitat preferences and/or diet of species varies in response to different variables such as climatic zone, vegetation or landscape type. Therefore safe havens have real value in not only protecting populations of medium-sized marsupials, but also increasing our knowledge to improve management of small isolated populations.

1.4 A case study –the greater bilby

The greater bilby (*Macrotis lagotis*; Reid, 1837; henceforth referred to as the bilby) is the only extant member of the family Thylacomyidae, which diverged from other bandicoots in the mid-late Oligocene (Westerman e*t al.* 2012). The bilby is one of the medium-sized marsupials that has experienced a massive range contraction following European colonisation, and translocations to safe havens have played a major role in subsequent recovery efforts for the species (Bradley *et al.* 2015). In this section, the timeline and

possible causes of the original decline of bilbies in Australia, conservation efforts to date, the biology of the species, and current knowledge gaps are outlined.

1.4.1 Historic and continued decline

At the time of European colonisation, bilbies were recorded across a range of arid, semi-arid and temperate ecosystems in Australia (Southgate 1990a). In South Australia, bilbies were regarded as common and even abundant in the southern temperate parts of its range in the 1890s (Wood Jones 1924). However, by the early 1900's bilbies had disappeared from the southern parts of their range (Wood Jones 1924). The species continued to decline rapidly throughout the early 1900s, and the last specimens collected in South Australia were in 1933 in the far north-west of the state (Kemper 1990). Bilbies were also recorded in southern Western Australia, but also declined in these areas and they are now only present in the northern and central arid zone of Western Australia (Abbot 2001, 2008; Bradley *et al.* 2015). Natural populations of bilbies are now presumed extinct in South Australia and New South Wales, and are restricted to parts of Western Australia, Northern Territory and south-west Queensland (Bradley *et al.* 2015). Bilbies are now estimated to occupy approximately 20% of their former range (Southgate 1990a; Bradley *et al.* 2015) and are listed as Vulnerable to extinction under the *EPBC Act 1999.*

Key threats to the bilby are similar to those of other ground-dwelling critical-weight range marsupials, and include predation by introduced red foxes (Finlayson 1961; Southgate 1990a), predation by feral cats (Lollback *et al.* 2015), grazing by introduced livestock or rabbits (*Oryctolagus cuniculus*) (Southgate 1990a; McDonald *et al.* 2015), and possibly inappropriate fire regimes (Southgate and Carthew 2006). The relative importance of these threats is unknown, and it could be that threats played different roles in different parts of the country and at different times. For example, in southern Australia it has been suggested that rabbits and foxes were a primary cause of the decline of bilbies (Finlayson 1961; Southgate 1990a). However, more recent research seems to indicate feral cats could have played a much larger role in the decline of bilby populations than previously recognised (Moseby *et al.* 2011a; Bradley *et al.* 2015; Moseby *et al.* 2015b).

1.4.2 Conservation of the greater bilby

Two previous Recovery Plans (see Bellchambers and Johnson 1991; Pavey 2006) have guided the conservation and recovery actions implemented for bilbies in Australia, with an updated Recovery Plan released in 2023. In both of the previous adopted plans, captive

breeding and translocation were key recovery actions recommended. In the 2006 Recovery Plan, a thorough assessment of conservation actions to date was made and seven specific recovery objectives were defined:

1. Reduce impact of predation by introduced carnivores.

2. Maintain genetic diversity through a captive breeding program.

3. Establish self-sustaining populations within the former range.

4. Monitor trends in occurrence and abundance.

5. Assess the impact of predators, fire and other threatening processes on bilby populations.

6. Inform and involve the community and all stakeholders in the recovery process.

7. Continue to manage the recovery process through a national recovery team.

In March 2015, the Greater Bilby Recovery Summit was held, which collated information on recovery efforts to date and outlined a new plan for the recovery and conservation of bilbies (Bradley *et al.* 2015). At the time of the Summit, bilbies had been reintroduced to 12 sites; four in South Australia, four in Western Australia, two in the Northern Territory, one in Queensland and one in New South Wales (Bradley *et al.* 2015). Four of those translocations had failed, and the cause in each case was attributed to predation by exotic predators (Bradley *et al.* 2015). At this Summit, it was also recognised that the current knowledge on bilby ecology came from a very small number of sites, mostly in the arid zone, despite the species historic range encompassing vastly different areas of Australia (Bradley e*t al.* 2015). Since the Summit, translocations of bilbies have taken place at a further four sites; three in New South Wales and one in the Northern Territory (DCCEEW 2023b). In the most recent Recovery Plan (2023), undertaking more translocations, particularly to safe havens, and facilitating genetic exchange between translocated populations were key actions identified and will likely be central to bilby conservation in the future.

1.4.3 The biology of the greater bilby

Bilbies are strictly nocturnal, and shelter in deep burrows during the day. In the arid zone, bilbies construct and maintain multiple burrows within a home range, and the stable temperatures and humidity within burrows indicates that sheltering in burrows likely

reduces the energetic costs of thermoregulation (Lavery and Kirkpatrick 1997; Moseby and O'Donnell 2003; Chapman 2013). The burrows constructed by bilbies are also important structures for other commensal burrowing species and have been found to be used by a wide variety of other mammal, reptile and bird taxa for shelter (Hofstede and Dziminski 2017; Dawson *et al.* 2019). At night, bilbies forage terrestrially on invertebrates, seeds and bulbs, and are ecologically flexible as their diet can vary by location and season (Gibson 2001; Southgate and Carthew 2006; Navnith *et al.* 2009). Bilbies excavate large amounts of soil during their foraging activities and leave behind foraging pits that act as nutrient sinks and increase native plant germination (James and Eldridge 2007; James e*t al.* 2009; James e*t al.* 2011). Bilbies are ecosystem engineers, and their soil excavation and burrow creation activities likely have a positive effect on landscape processes and biodiversity (Eldridge and James 2009; Dawson *et al.* 2019).

Bilbies have been observed to breed throughout the year in the arid zone, where rainfall is low and unpredictable (Colls and Whitaker 2012), as well as in captive colonies (McCracken 1990; Southgate *et al*. 2000; Moseby and O'Donnell 2003). Bilbies have eight teats in their pouch, yet generally only have one or two pouch young in a litter, and very rarely three (Southgate *et al.* 2000). The minimum time between births can be as short as the period of pouch life meaning females can breed almost continuously under good conditions (McCracken 1986; Southgate *et al.* 2000). Two previous studies of the mating system of bilbies produced contradictory results. The first study in a free-ranging arid zone population indicated a single male could dominate breeding in an area and the mating system was described as polygynous (Moritz *et al.* 1997). A subsequent study in a semi-free ranging captive population found mating was not monopolised by a subset of males and concluded a promiscuous mating system was operating (Miller *et al.* 2010a). Prior to this study, the breeding biology of free-ranging bilbies in the more temperate parts of their former range had not been studied.

1.4.4 Knowledge gaps

Because the bilby declined rapidly following European colonisation, prior to this study all research on the species had been carried out on arid zone or captive populations. Therefore, our knowledge on free-ranging bilby biology pertained only to populations in the arid zone, with no published studies from the temperate parts of its range, from which it was absent for over 100 years (Kemper 1990). In addition to this, our understanding of

the relative importance of different threats to the species is not well established. Hence, it is still unclear whether sites that still have some threats present are suitable as release sites for translocations, and which threats must be completely eliminated for population persistence.

The current study came about through a desire to better understand the biology of bilbies, particularly in the temperate zone where their biology had not been previously studied. This study also sought to obtain more information on the impact of various threats to the species, in order to inform future translocations and increase the likelihood of successful conservation outcomes for the species.

1.5 Project aims

The bilby has now been reintroduced to two sites in temperate Australia. However, there has been no previous research into the biology of this species outside arid regions. It is important to examine whether aspects of the biology of the bilby differ between climatic regions, as the long-term recovery of this species is likely to involve further translocations that may require sourcing individuals or translocating individuals to temperate zone sites. This project used a comparative approach to investigate the population dynamics, breeding biology, and behaviour in two translocated bilby populations in southern South Australia. The information gathered in this study will aid in planning future translocations to the temperate zone, and management of existing translocated populations in southern Australia.

Aim 1 (Chapter 2): To investigate aspects of the breeding biology of bilbies exposed to a temperate climate, to determine whether breeding is seasonal and quantify annual female fecundity;

Aim 2 (Chapter 3): To investigate whether the translocation of bilbies to two 'safe haven' sites has been successful, and determine what factors are driving/limiting population growth;

Aim 3 (Chapters 2, 3, 4, and 5): To gain an understanding of bilby biology in a temperate climate and generate information that could be used to improve the establishment and management of translocated populations.

1.6 Thesis Structure

This thesis has been prepared as a thesis by publication. Empirical results arising from research undertaken during my PhD candidature are presented in Chapters 2, 3, and 4, which have been published in peer-reviewed journals, and Chapter 5, which is not yet published. Details of the authorship of all empirical chapters are presented at the start of each chapter, and quantify the contributions of each author. Chapter 6 discusses the findings of my research and its contribution to the fields of ecology and reintroduction biology, the limitations of my research and potential future research directions that could build upon this work.

There is some repetition in the introduction and methodology sections of the empirical data chapters, which was required for them to be stand-alone articles suitable for publication. Throughout this thesis I have also often shortened the name of my study species to 'bilby', and in all cases this refers to the greater bilby, *Macrotis lagotis*.

Chapter 2: Timing of breeding and female fecundity of the greater bilby (Macrotis lagotis) in the temperate zone of South Australia: implications for translocations of a previously widespread species



A pouch young just over one month old.
Photo by Karleah Berris

"*In the Centre the breeding season is regulated more by the rains and the abundance of food than by the actual period of the year.*"

Frederic Wood Jones, 1924, from 1968 reprint, on the breeding of bilbies.

Statement of Authorship

Title of Paper	Timing of breeding and female fecundity of the greater bilby (*Macrotis lagotis*) in the temperate zone of South Australia: implications for translocations of a previously widespread species
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Contribution to the Paper	Designed project, performed field work and collected data, performed data analysis, interpreted analyses and wrote manuscript.		
Overall percentage (%)	90%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	4/08/2024

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Signature		Date	20/08/2024

Abstract

Context: Many of Australia's threatened mammals were once widespread across the continent and present in both arid, semiarid and temperate habitats. However, data on breeding biology can be lacking for some climatic regions due to local extinctions of populations. The breeding biology of the greater bilby (*Macrotis lagotis*) has previously only been studied in captive colonies or free-ranging arid zone populations. Between 1997 and 2005, captive-bred bilbies were released at two sites in temperate South Australia. These populations provided an opportunity to determine whether bilbies adopt a seasonal reproductive strategy in the temperate zone, where this species has not been present since the early 1900s.

Aims: To determine the season of births, litter size, and female reproductive potential in two free-ranging populations of the bilby in the temperate zone of South Australia.

Methods: Bilbies were regularly cage-trapped at Venus Bay Conservation Park and Thistle Island between August 2007 and April 2010 to obtain data on reproductive activity in both populations.

Key Results: Births of pouch young in both populations occurred in winter (June–August), spring and early summer, but not between February and mid-May. Males had a significantly smaller mean testes size relative to head length in late summer, when breeding activity ceased. Average litter size at pouch emergence was between 1.00 and 1.47 pouch young, with females producing up to three litters per annum. The mean head length of females that successfully raised twins was significantly larger than that of females who raised a single pouch young.

Conclusions: The seasonal breeding pattern observed in the present study differs from that of year-round reproductive activity in captivity and the arid zone. The present study suggests that female body size influences fecundity, and that releasing large females at the start of the breeding season in temperate areas may maximise the initial rate of increase of a translocated bilby population.

Implications: The present study suggests previously widespread species such as the bilby can adapt their reproductive strategy to suit local conditions. Cessation of breeding in

autumn, as observed in this study, has also been observed in other bandicoot species in southern Australia.

Additional keywords: reproduction, seasonality, reintroduction, Marsupialia

2.1 Introduction

The rapid decline of Australia's mammal fauna since European settlement has been well documented. Over 10% of Australia's terrestrial mammals have become extinct since European settlement and a further 21% are currently at risk of extinction (Woinarski *et al*. 2015). Many of the species currently at risk of extinction were abundant and widespread at the time of settlement and had species distributions that encompassed arid, semiarid and temperate habitats, but their populations are now confined to relatively small parts of Australia (Hanna and Cardillo 2013; Woinarski *et al*. 2015). These species have been described as 'ecologically flexible' (Woinarski *et al*. 2015), but our knowledge of the breeding biology of these species often comes from small remnant populations that inhabit relatively few habitats compared with their pre-European distribution. This raises two important questions: (1) will ecologically flexible species adapt their reproductive strategy in different habitats?; and (2) when planning translocations, do we need to factor in changes in fecundity and breeding biology across different habitats?

The greater bilby is an ecologically flexible species with a distribution that previously covered 70% of Australia (Southgate 1990a). It was once the most widely distributed bandicoot species in South Australia (Finlayson 1961; Kemper 1990) and was recorded in abundance in close proximity to Adelaide, South Australia (Wood Jones 1924). Museum specimens, Aboriginal knowledge and published literature indicate bilbies were originally present throughout all regions of South Australia except Kangaroo Island and the south-east of the state (Kemper 1990) (Fig. 2.1). The bilby declined rapidly after 1900 and the last records within South Australia were specimens collected between 1928 and 1935 in the north-west corner of the state (Bradley *et al*. 2015; Atlas of Living Australia 2018) (Fig. 2.1). Further surveys in the 1960s failed to find any evidence of bilbies and the species was presumed extinct in South Australia (Kemper 1990). Since 1984, its distribution has been restricted to the arid regions of the Northern Territory, Western Australia and Queensland (Southgate 1990a; Bradley *et al*. 2015). Southgate (1990a) estimated that the greater bilby now occupies only 20% of its former range pre-European settlement. The climate of the

remaining extant range of the bilby differs greatly from that of the southern parts of its former range. The southern parts of South Australia where the bilby previously occurred has a Mediterranean climate, characterised by cool, wet winters and warm, dry summers (Colls and Whitaker 2012). It supports habitats very different from the arid zone to which this species is now restricted.

Our current understanding of the breeding biology of the bilby comes from studies of populations in the arid zone, where rainfall is low and often unpredictable (Colls and Whitaker 2012), or from captive colonies (McCracken 1990; Southgate *et al*. 2000). In these environments, the greater bilby has been observed to breed throughout the year (McCracken 1990; Southgate *et al*. 2000; Moseby and O'Donnell 2003). There is a lack of published data on the breeding biology of free-ranging populations of the greater bilby in the temperate parts of its original range, due to their long absence from southern Australia.

In response to the major decline of this species, there have been recent efforts to re-establish populations in parts of its former range in South Australia, Western Australia, New South Wales and Queensland (Bradley *et al*. 2015). The lack of data on the reproductive potential of female greater bilbies in the temperate zone makes it difficult to predict the potential growth rate of translocated populations in these environments or plan releases that maximise the initial rate of increase of populations. Two releases occurred within the temperate zone of South Australia between 1997 and 2005 (Bradley *et al*. 2015), and these two sites provided an opportunity to study the breeding biology of this species in an area with regular winter (June–August) rainfall. We hypothesised that in this climatic zone the bilby would adopt a seasonal reproductive strategy similar to that of other related species like the southern brown bandicoot (Stoddart and Braithwaite 1979).

The aim of the present study was to investigate the timing of births and litter size of free-ranging female greater bilbies in the southern temperate zone of South Australia. Data from the present study should give insight in to the reproductive potential of female greater bilbies when translocated to temperate southern regions, and may influence optimum numbers of females released in order to quickly reach a minimum viable population size. The present study seeks to add to our knowledge about the reproductive strategies adopted in different habitats by species that were previously widespread across the Australian continent.

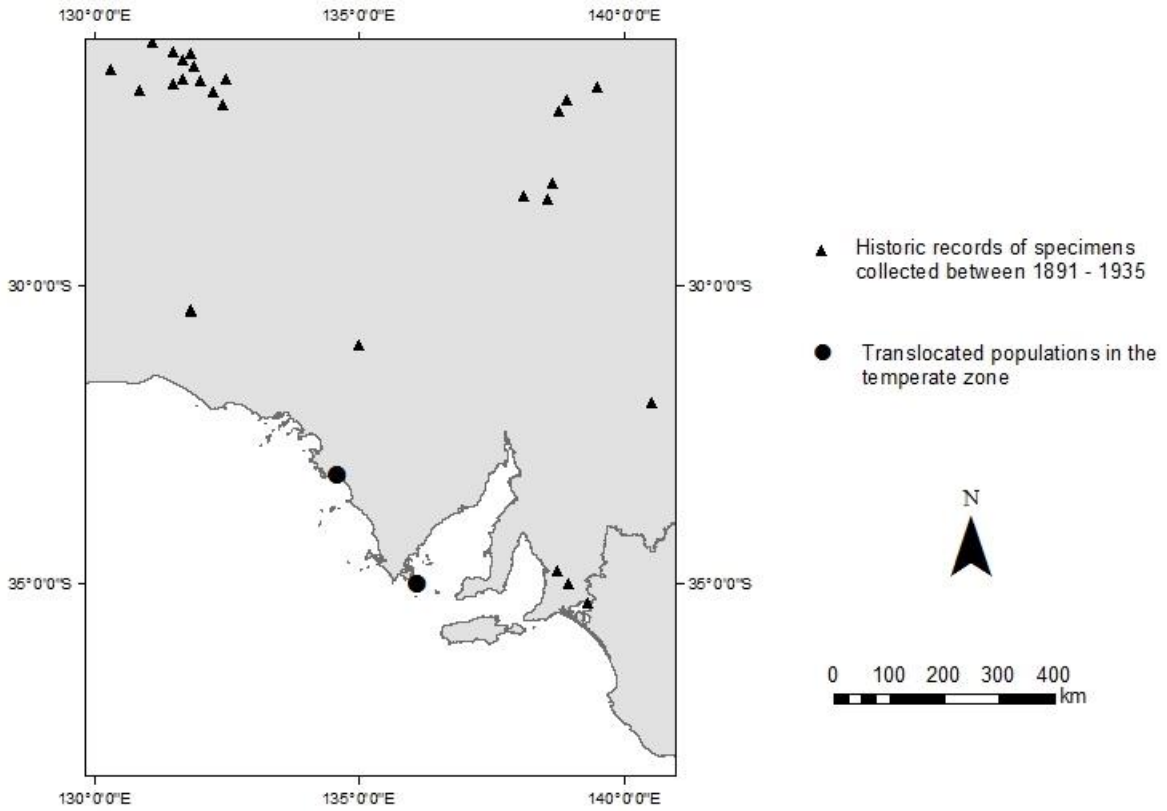


Figure 2.1 The state of South Australia, with the locations of greater bilby (*Macrotis lagotis*) specimens collected between 1891 and 1935 before its extinction in South Australia (▲), and sites of current translocated populations in temperate South Australia (●). Data on the locations of historic specimen collection were downloaded from the Atlas of Living Australia (2018).

2.2 Materials and methods

2.2.1 Study sites

Venus Bay Conservation Park comprises a peninsula of 1460 ha on the west coast of Eyre Peninsula (Fig. 2.1), and is protected by a predator exclusion fence constructed in 1996 that runs from coast to coast across the top of the peninsula (Department for Environment and Heritage 2006). The park contains a mixture of mallee and coastal heath habitats, with some cleared areas of former farmland supporting pasture species. The area where bilbies were mostly found comprised *Eucalyptus oleosa*, *Eucalyptus dumosa* and *Melaleuca lanceolata* open shrubland on sandy calcareous soil (Department for Environment and Heritage 2006). Regeneration of native vegetation in the cleared areas of the reserve adjacent to this open shrubland has been severely limited, due to grazing by abundant

western grey kangaroos (*Macropus fuliginosus*) and European rabbits (*Oryctolagus cuniculus*). Foxes (*Vulpes vulpes*) have been eliminated from the park, but feral cats (*Felis catus*) and rabbits persist. The park has a Mediterranean climate, and receives most of its annual average rainfall of 370 mm in winter (Department for Environment and Heritage 2006).

Twenty-three captive-bred greater bilbies from Monarto Zoo in South Australia were released at Venus Bay Conservation Park between 2001 and 2005. Subsequent trapping and spotlight surveys by park rangers between 2005 and 2008 regularly recorded adult bilbies, and occasionally, smaller juvenile individuals (L. Amey, pers. comm., 2008). Brush-tailed bettongs (*Bettongia penicillata*) have also been successfully translocated to the park (Department for Environment and Heritage 2006).

Thistle Island is 3926 ha in area and is located at the southern end of Spencer Gulf, 7.4 km east of the south-east tip of Eyre Peninsula (Fig. 2.1). Native vegetation was cleared from much of the island and exotic grasses and herbs introduced for domestic livestock as part of its history as a pastoral property (Robinson *et al*. 1996). Remnant vegetation consists of an overstorey of *Eucalyptus diversifolia*, *Allocasuarina verticillata* and *Melaleuca lanceolata*, with extensive sand dunes on parts of the island still supporting a reasonably intact community of coastal shrubland dominated by *Olearia axillaris* and *Leucopogon parviflorus* (Robinson *et al*. 1996). Pastoralism ceased over most of the island in 1986, with the northern half of the island becoming a conservation area (Robinson *et al*. 1996). Many native plant species are now regenerating in stock-free areas, particularly *Acacia paradoxa*. Brushtail possums (*Trichosurus vulpecula*) were translocated to the island in 1950 by pastoral lease holders, and black-footed rock-wallabies (*Petrogale lateralis*) from Pearson Island were translocated in 1974 to establish a second wild population of this species in South Australia (Robinson *et al*. 1996). Both of these translocations have been considered successful (Robinson *et al.* 1996).

Between 1997 and 2000, 21 captive-bred greater bilbies from Monarto Zoo were released on to Thistle Island at the northern end of the island. Although Thistle Island did not historically contain greater bilbies, the island was deemed suitable for the species due to its habitat and lack of exotic predators. Surveys of burrows and diggings conducted between 1997 and 2008 indicated that the population had increased, and that greater bilbies had colonised the entire island (J. van Weenan, unpubl. data, 2008). Although signs

(track, scats, diggings and burrows) have been used to infer abundance, comprehensive population counts have not been undertaken at this site.

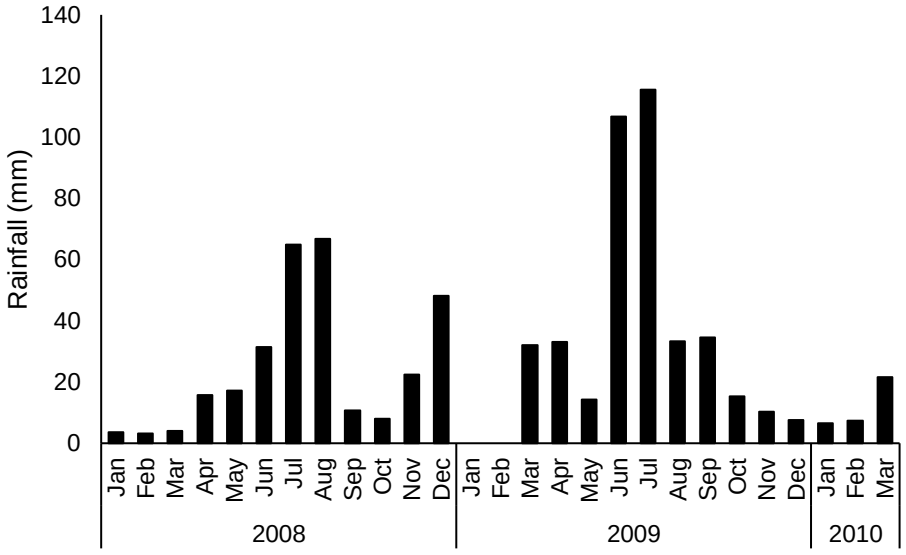
No supplementary food or water was supplied to either population during our study; animals were dependent on the natural resources of the sites.

Data were collected from Venus Bay Conservation Park during 10 field surveys of 10–21 days duration between July 2008 and April 2010. Data for Thistle Island were collected during six field surveys of 7–14 days duration conducted between August 2007 and February 2010.

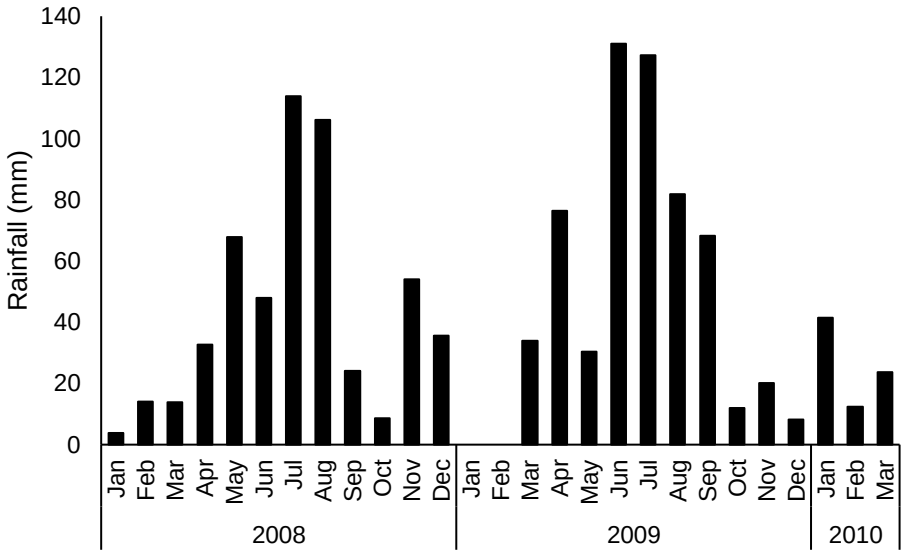
2.2.2 Rainfall at the sites

Rainfall was recorded at the Venus Bay Conservation Park site every month for the entire study period and for the 6 months before the beginning of the study by Department of Environment and Water (DEW) rangers. Annual rainfall at Venus Bay Conservation Park was 296.2 and 402.7 mm in 2008 and 2009 respectively. Rainfall was lowest during January and February in both years. Rainfall records for Thistle Island were not available, so records from the nearest weather station at Port Lincoln (Westmere; ~40 km east of the northern end of Thistle Island) were accessed through the Bureau of Meteorology website (http://www.bom.gov.au/climate/data/index.shtml accessed 28 May 2019). Annual rainfall at Port Lincoln (Westmere) weather station was 522.2 mm in 2008 and 589.4 mm in 2009, and showed a similar pattern of high winter rainfall and little or no rainfall in January and February (Fig. 2.2).

Annual rainfall was much higher on Thistle Island compared with Venus Bay Conservation Park in both 2008 and 2009. However, both sites showed a relatively similar pattern of rainfall over the year, with most rainfall recorded in the winter months at both sites (Fig. 2.2). At Venus Bay Conservation Park, 55.0% and 63.4% of rainfall was in winter in 2008 and 2009 respectively. On Thistle Island, 51.3% and 57.7% of rainfall was in winter in 2008 and 2009 respectively.



a)



b)

Figure 2.2 Rainfall at (*a*) Venus Bay Conservation Park from January 2008 to March 2010 and (*b*) Port Lincoln Westmere weather station 40 km east of Thistle Island from January 2008 to March 2010.

2.2.3 Trapping

Greater bilbies were trapped at both locations using medium treadle-operated cage traps (minimum size 60 cm × 29 cm × 25 cm) baited with peanut butter and oats. Traps were set well into low shrubby native vegetation so that only the trap entrance was accessible. This provided protection from the elements and reduced the chances of traps being set off by animals from outside the trap trying to access the bait.

Traps were set in transects of 5–20 (depending on terrain) at 100 metre intervals. Trap lines were initially chosen to sample the different habitats of each site and to determine the location and density of greater bilbies across the study areas. Some trap lines were then trapped intensively over longer periods during the remainder of the study to recapture females.

Traps were checked once before dawn (between 0200 and 0500 hours) during mild conditions and twice before dawn in cool or wet weather (before midnight, then again between 0200 and 0500 hours). They were closed on nights of severely inclement weather. Traps were closed after the final check before dawn, and the bait was removed and then reopened and rebaited each evening to reduce daytime by-catch. Once caught, animals were placed in a black catch bag to reduce stress. They were kept in the bags when all measurements were taken except for head length. Measurements taken were: head length (mm), pes length (mm), and body mass (g). Bilbies over 600 g in body mass were classified as sexually mature adults based on the previous observations of Southgate *et al.* (2000). Non-target species were encountered frequently at both sites and included brush-tailed bettongs and house mice (*Mus musculus*) at Venus Bay Conservation Park, and brushtail possums and black-footed rock-wallabies on Thistle Island. Non-target species were released immediately at the point of capture when encountered.

Female reproductive condition was classified as: carrying pouch young (where possible, the head and pes lengths of pouch young were measured and their gender determined); lactating (not carrying pouch young, although milk could be expressed from teats); or reproductively inactive (no pouch young or lactation). Because females nurse young bilbies for around 15 days following pouch emergence (Tyndale-Biscoe and Renfree 1987; McCracken 1990), it was assumed lactating females had pouch young that had recently emerged. In adult males length and width of the left testes was measured. Testis size was calculated by multiplying the length and width (to give area in mm^2). To identify individual adults, a subcutaneous passive integrated transponder (PIT tag; Trovan, Douglas, Isle of Man) was inserted under the skin between the shoulder blades. Animals were mostly processed on site, adjacent to the trap and released immediately after taking measurements.

2.2.4 Data analysis

Data were analysed using R version 3.3.1 (R Core Team 2018), an open source software environment and programming language for statistical analysis and graphics creation. Month of birth of pouch young was extrapolated from their head length (following McCracken (1990), who found head length was the most reliable predictor of age). Age was estimated using the equation:

$$X = (\frac{Y}{0.18})^{0.78} - 14$$

where X = age in days and Y = head length in mm (McCracken 1990). This equation was based on observation of captive-bred pouch young. The average litter size at time of birth was estimated from observations of litters <10 days of age. Initial pouch exit has been estimated at anywhere from 67 days of age (Hulbert 1972) to 82 days of age (McCracken 1990). Therefore the average litter size at pouch emergence was estimated from litters observed that were over 60 days in age.

Categorical data, numbers of births and reproductively active females were analysed using the Pearson chi-squared test, with a Yates correction applied for small 2 × 2 contingency tables (Field *et al*. 2012; Gardner 2017). For contingency tables that had expected frequencies below 5 but more than 1, the 'N – 1' Chi-squared test was used (Campbell 2007).

Males had their head length and testes size measured each trapping session to determine whether changes in testes size could be detected in different seasons, resulting in 174 and 92 measurements of these two parameters at Venus Bay Conservation Park and Thistle Island, respectively. Measurements were only included from 2009 and 2010 on Thistle Island because it was only in these years that field trips were regular enough to cover more than one season per year. Correlation between head length and testes size was investigated using all samples from all seasons, and comparisons among seasons were made using the parametric Pearson's product moment correlation. Comparisons between two samples used an independent samples t-test for data with a normal distribution, with the Mann–Whitney U-test used if data were not normally distributed. Comparisons among multiple datasets of testes size for each season were undertaken using a one-way analysis

of variance (ANOVA) if datasets were normally distributed, or the Kruskal‐Wallis rank-based analysis for non-parametric data.

2.3 Results

2.3.1 Cage Trapping

At Venus Bay Conservation Park, 3243 trap nights resulted in 606 captures of 69 independent bilbies. On Thistle Island, 1839 trap nights resulted in 394 captures of 195 individuals. These figures equate to a trap success of 2.1 individuals per 100 trap nights at Venus Bay Conservation Park, and 10.6 individuals per 100 trap nights on Thistle Island. Bilbies were the most frequently trapped species at Venus Bay Conservation Park, with brush-tailed bettongs the second-most frequently trapped species, accounting for 155 captures. Of the 3243 trap nights at Venus Bay Conservation Park, there were 2428 records of unoccupied traps which equates to 74.9% of trap nights. Therefore, at this site, it is likely that the trap success is a reflection of the density of bilbies at the site. On Thistle Island, the most frequently trapped species were brushtail possums, which accounted for 613 captures. Bilbies were the second-most frequently trapped species and unoccupied traps were recorded 802 times, which accounted for 43.6% of all trap nights. At trap sites on the southern end of the island there were some occasions where all traps were occupied by either a brushtail possum or bilby in a night. Therefore, it is possible that the trap success of bilbies on Thistle Island may be higher than recorded here; however, in the present study our results also reflect the high amount of by-catch at the site. The mean number of times individual females were trapped at Venus Bay Conservation Park was 3.53 times ± 2.5 (± s.d., n = 19). The mean number of times individual females were trapped on Thistle Island in 2009 and 2010 was 2.07 times ± 0.98 (n = 30).

At Venus Bay Conservation Park, 53 pouch young were observed with adult females trapped over the course of the present study, and on Thistle Island, 59 pouch young were observed with adult females. Trapping was only carried out regularly enough to detect all breeding events on Thistle Island in 2009 and 2010, and so only the 26 pouch young observed during that period were used to infer the birth month at this site.

At Venus Bay, 59 bilbies were adults (>600 g) at the time of first capture, 10 were subadults (<600 g) and 53 were pouch young, whereas on Thistle Island 160 bilbies were adults at the

time of first capture, 34 were subadults and 59 were pouch young. There was a significant difference between the proportion of subadults and pouch young captured at the two sites ($\chi 2 = 16$, d.f. 21 = 1, P = 0.0003), with lower-than-expected numbers of subadults captured at Venus Bay and higher-than-expected numbers of subadults captured on Thistle Island.

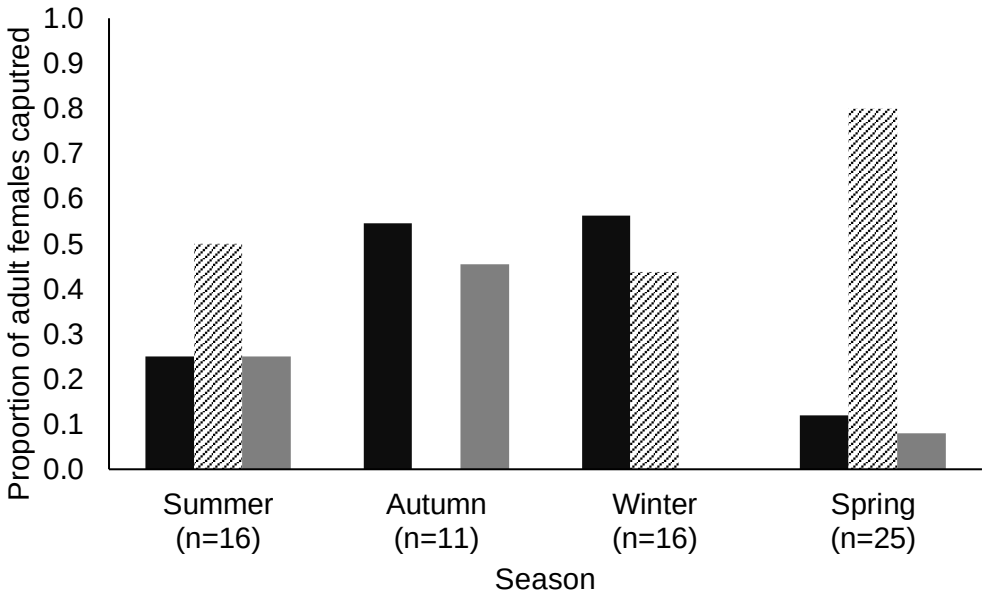
2.3.2 Body mass of adults

The mean body mass of adult females without pouch young was 1014 g ± 175 g (n = 15) at Venus Bay Conservation Park, and 853 g ± 130 g (n = 36) at Thistle Island. Females at Venus Bay Conservation Park had a significantly heavier mean body mass than females on Thistle Island (t-test with unequal variances, t = 3.207, P = 0.004). The mean body mass of adults males at Venus Bay Conservation Park (1512 g ± 317; n = 38) was also significantly heavier than the mean body mass of males on Thistle Island (983.5 ± 240; n = 104) (Mann–Whitney U-test, Z = –7.236, P < 0.001).

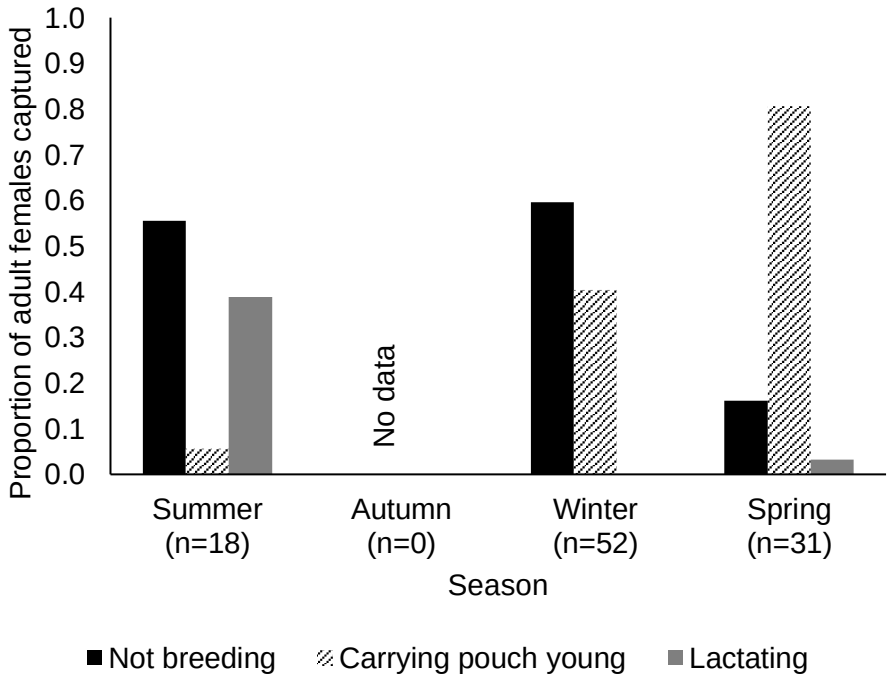
2.3.3 Seasonal changes in female reproductive activity

The presence of pouch young in adult females captured at Venus Bay Conservation Park was seasonal (Pearson chi-square = 75.532, P < 0.001) (Fig. 2.3). Females were observed carrying pouch young in winter (44%), spring (80%) and summer (50%). No females were caught carrying pouch young in autumn. Lactating females were observed in spring (8%), summer (24%) and autumn (45%). The percentage of females carrying pouch young on Thistle Island was 40% and 81% in winter and spring respectively, which was similar to Venus Bay Conservation Park. Comparatively fewer females were carrying pouch young in summer (5%) on Thistle Island compared with Venus Bay Conservation Park. Lactating females were observed in spring (3%) and summer (39%). Although no data were collected from Thistle Island in autumn, neither population had lactating females in winter. Because females lactate for around 90 days with pouch emergence occurring at ~75 days (Tyndale-Biscoe and Renfree 1987), an absence of large pouch young or lactating females in winter indicates there were probably no births over the autumn period.

At Venus Bay Conservation Park, 34 different litters of pouch young from 19 adult females were measured and aged. Births of all litters occurred between late May and January, with most births occurring in winter and spring. Three peaks in births were observed – in June, September and December. No births were recorded between February and mid-May in both 2009 and 2010. Higher-than-average rainfall at Venus Bay Conservation Park in



a)



b)

Figure 2.3 The reproductive state of adult female bilbies captured in each season (*a*) over a 2-year period at Venus Bay Conservation Park (*n* = 68), and (*b*) between August 2007 and February 2010 on Thistle Island (*n* = 101).

November and December 2008 (Fig. 2.3) did not appear to result in a prolonged breeding season in early 2009, with the latest births recorded in both 2009 and 2010 occurring in late January. The number of breeding females (those with pouch young or lactating) also did not differ significantly between February 2009 and February 2010 ('N – 1' chi-square test = 1.25, P = 0.3). A shorter period of breeding was observed on Thistle Island, with

births of pouch young occurring between late July 2009 and early December 2009. No births were recorded between January and June on Thistle Island. Births showed a significant association with season in both populations (Table 2.1). Only one birth was observed in autumn, which was at Venus Bay Conservation Park in late May, shortly before several other births in June of the same year.

Table 2.1 The number of births recorded in each season over a 1-year period on Thistle Island, and a 2-year period at Venus Bay Conservation Park (C.P.) Birth seasons of pouch young were estimated using equation from McCracken (1990)

Site	Number of births				χ^2	*P*-value
	Winter	Spring	Summer	Autumn	(d.f. = 3)	
Venus Bay C.P.	13	13	7	1	11.647	0.008
Thistle Island	15	10	1	0	24.154	0.000

2.3.4 Seasonal changes in testes size

Both Venus Bay Conservation Park and Thistle Island populations showed a significant positive correlation between testes size and head length (Venus Bay Conservation Park r = 0.65, P < 0.001, n = 174; Thistle Island r = 0.67, P < 0.001, n = 92). A ratio of testes size to head length was therefore used to standardise testes size before investigating changes in relative testes size and season. At Venus Bay Conservation Park there was a significant decrease in testes size relative to head length in summer (February) (Kruskal–Wallis chi-square = 18.525, d.f. = 3, P = 0.00034). A similar trend was observed at Thistle Island, with relative testes size in summer (February) being smaller than in winter or spring ($F_{2,85}$ = 5.692, d.f. = 2, P = 0.0048). There was no significant difference in the testes size relative to head length for autumn, winter and spring at Venus Bay Conservation Park, or for winter and spring on Thistle Island.

2.3.5 Litter size

Litters in both populations consisted of one or two (twin) pouch young (Table 2.2). At Venus Bay Conservation Park seven females were observed carrying twin pouch young of >60 days of age. One female was carrying twins estimated to be 86 days of age. On Thistle Island twin litters were observed at <10 days of age, but no twins were observed in litters >60

days of age. The oldest litter containing more than one pouch young on Thistle Island was estimated to be 43 days old.

Table 2.2 Occurrence of single and twin litters close to birth (i.e. <10 days old) and at weaning (i.e. >60 days old) at Venus Bay Conservation Park (C.P.) and Thistle Island

Site	Litters at <10 days old		Litters at >60 days old	
	Single	Twin	Single	Twin
Venus Bay C.P.	3	5	8	7
Thistle Island	2	5	8	0

The average litter size at birth (estimated from litters observed at <10 days of age) was 1.57 (s.e. = 0.2, n = 7) pouch young at Venus Bay Conservation Park, and 1.71 (s.e. = 0.18, n = 7) at Thistle Island. The average litter size at pouch emergence (estimated from litters observed at >60 days of age) was 1.47 (s.e. = 0.13, n = 15) at Venus Bay Conservation Park, and 1.0 (s.e. = 0, n = 8) on Thistle Island.

The proportion of single and twin litters at birth (<10 days of age) was not significantly different between the two sites ('N – 1' $\chi 2$ test = 0.29, P > 0.5; Table 2.2). On Thistle Island, the proportion of twin litters at the time of pouch emergence (>60 days of age) was significantly lower when compared with both the litter size at the same site at <10 days of age ('N – 1' $\chi 2$ test = 8, P = 0.005) and the litter size at Venus Bay Conservation Park for litters of the same age ('N – 1' $\chi 2$ test = 5.13, P = 0.02; Table 2).

Loss of pouch young was observed in both populations. At Venus Bay Conservation Park one pouch young from a litter of twins was found dead in the pouch and estimated to be ~8 days old when it died. On Thistle Island three suspected losses of one pouch young from a litter of twins were observed. A female carrying twins ~4 days old was recaptured 73 days later with only one pouch young and only one lactating teat. On two other occasions, females carrying twins <10 days old were recaptured around the time that young emerge from the pouch and were found to have only one actively lactating teat, indicating the loss of one of the twins some time earlier.

2.3.6 Female body size and litter size

The mean body mass of females together with their twin pouch young ≤10 days old at Venus Bay Conservation Park was 997 g (n = 3), and those with a single pouch young of the same age range was 1060 g (±95 g, n = 4). On Thistle Island, the mean body masses were 838 g (n = 2) for twin pouch young and 783 g (±149 g, n = 5) for single pouch young. These body masses all included the weight of the pouch young.

Mean body masses of females with pouch young >60 days of age were not used for comparison because the weight of pouch young they were carrying could not be determined and would have been significant. Instead, head length was compared between these females. At Venus Bay Conservation Park, females who raised twin pouch young to >60 days of age were found to have significantly larger mean head length (106.2 mm ± 1.5 mm, n = 7) than females who only raised a single pouch young to >60 days (103.6 mm ± 2.6 mm, n = 8) (independent two-sample t-test, t = –2.2953, d.f. = 13. P = 0.04). No females trapped on Thistle Island were observed carrying twin pouch young >60 days of age.

2.3.7 Litters produced per annum

Of the adult females trapped at the two sites, eight at Venus Bay Conservation Park were trapped regularly enough over a year to determine the number and outcome of their litters. Only six females on Thistle Island were able to be trapped regularly enough to determine the outcome of all of their litters over a breeding season, due to the lower recapture rate on Thistle Island and fewer field trips. Three females at Venus Bay Conservation Park produced two litters of pouch young per year and five females produced three litters per year. Of the six females on Thistle Island, one did not produce any litters in a year and five produced two litters per year (Table 2.3). This equated to 2.6 litters per female per annum produced at Venus Bay Conservation Park, and 1.8 litters per female per annum on Thistle Island.

The smallest female observed carrying a single pouch young was from Thistle Island, with a body mass of 590 g (head length 98 mm). The smallest female carrying two pouch young was also from Thistle Island, with a body mass of 675 g (head length 99 mm). One female trapped on Thistle Island, with a body mass of 590 g and considered to be a subadult in July 2009, was then re-trapped in September weighing 710 g. She was trapped regularly throughout 2009 and 2010 and was not observed to reproduce during that breeding season

despite being of adequate body mass and age. This was the only adult female observed not to breed at all during the breeding season at either site.

Table 2.3 Number of litters produced by females followed over an entire year where the number and outcome of all their litters could be determined at Venus Bay Conservation Park (C.P.) and Thistle Island

Site	Total number of females followed	Number of litter produced in a year			
		0	1	2	3
Venus Bay C.P.	8	0	0	3	5
Thistle Island	6	1	0	5	0

2.3.8 Sex ratio of pouch young

Of the 52 pouch young who were able to have their gender determined at Venus Bay Conservation Park, 26 were male and 26 were female. Of the 44 pouch young able to have their gender determined on Thistle Island, 19 were male and 25 were female. Neither of these sex ratios were significantly different from parity (Venus Bay Conservation Park: Pearson $\chi^2 = 1.00$, P = 1.000, n = 52; Thistle Island: Pearson $\chi^2 = 0.818$, P = 0.366, n = 44).

2.4 Discussion

The present study investigated the timing of reproduction in two southern populations of the greater bilby translocated into temperate areas of South Australia. We also assessed the fecundity of females in these free-ranging populations to determine what reproductive output might be expected of females translocated into similar areas. We assumed that the main drivers of breeding activity in the two populations are the habitat and climatic conditions that influence resource availability. The main findings of the present study have been summarised in Table 2.4.

The onset of breeding in both populations coincided with months of high rainfall during winter. The present study also detected a shorter breeding season on Thistle Island in comparison with Venus Bay Conservation Park. A much higher trap success and significantly smaller mean bodyweight of adults on Thistle Island indicates that high population density

Table 2.4 Summary table of rainfall, trap success, bodyweight and reproductive parameters of greater bilbies at Venus Bay Conservation Park (C.P.) and Thistle Island

	Site	
	Venus Bay C.P.	Thistle Island
Annual rainfall	296.2 mm (2008) 402.7 mm (2009)	522.2 mm (2008) 589.4 mm (2009)
Trap Success	2.1 individuals per 100 trap nights	10.6 individuals per 100 trap nights
Male mean body mass (mean ± s.d.)	1512 g ± 317 g	983.5 g ± 240 g
Female mean body mass (mean ± s.d.)	1014 g ± 175 g	853 g ± 130 g
Duration of births	Late May to January	Late July to December
Litter size at weaning	1.47 (s.e. = 0.13)	1.0 (s.e. = 0)
Litters per annum per female	2.6	1.8

and/or resource competition could also be affecting fecundity at this site. Births of pouch young were not recorded in autumn in both populations. Early accounts by Wood Jones (1924) stated the breeding season of greater bilbies was from March to May in South Australia. However, he also proposed that in arid environments rain and food resources would drive breeding rather than time of year. The results of our study differ from observations of breeding in captive colonies. Captive greater bilbies at the Arid Zone Research Institute at Alice Springs, Northern Territory, were found by McCracken (1990) to breed throughout the year, and Southgate *et al.* (2000) reported breeding in all months in captive colonies at the Arid Zone Research Institute and the Western Plains Zoo in Dubbo, New South Wales. In both studies the greater bilbies kept under captive conditions were unlikely to have experienced nutritional or water stress. Therefore, observations of breeding in captive colonies are unlikely to reflect breeding in the natural environment where seasonal variations in rainfall and resource abundance occur.

In a translocated greater bilby population at the Arid Recovery Reserve in northern South Australia, Moseby and O'Donnell (2003) observed females with pouch young and/or lactating females in all months except July and September. Their results suggested breeding can occur at any time of the year in the arid environment where their study took place. The study by Moseby and O'Donnell (2003) was carried out on a population during the first 17 months following release, where the original release population consisted of only nine individual bilbies. Year-round breeding may have occurred at this time because there were so few bilbies in the reserve following the initial release, and because the area received average or above-average rainfall in the 2 years following release (Moseby and O'Donnell 2003). Furthermore, female bilbies had relatively small home-range sizes (mean 0.18 km2) at Arid Recovery Reserve in the 17 months following release (Moseby and O'Donnell 2003). In contrast, the home range of wild greater bilbies from the Northern Territory was estimated at between 1.1 and 3 km2 (Southgate and Paltridge 1998). It is therefore plausible that the small home-range size recorded at the Arid Recovery Reserve during the study by Moseby and O'Donnell (2003) reflected relatively abundant food resources, which thus supported the year-round breeding.

Our results indicate the bilby can alter its reproductive strategy in different habitat types, and that in a temperate environment it adopts a seasonal breeding strategy, with the non-breeding season centred around autumn. Seasonal breeding has been observed in other bandicoot species in southern Australia, including eastern barred bandicoots (*Perameles gunnii*; Heinsohn 1966) and southern brown bandicoots (*Isoodon obesulus*; Stoddart and Braithwaite 1979). Like the bilbies in the present study, both the eastern barred bandicoot and southern brown bandicoot populations were found to have periods of reproductive inactivity around autumn in temperate areas (Heinsohn 1966; Stoddart and Braithwaite 1979), and breeding was found to take place during times of peak food availability in the southern brown bandicoot (Lobert and Lee 1990). Both the southern brown bandicoot and eastern barred bandicoot have been described as opportunistic omnivores, whereby their diet varies seasonally according to what is available in the environment (Quin 1985a, 1985b). The greater bilby also feeds opportunistically on seeds, bulbs and invertebrates in desert areas (Southgate 1990b; Southgate and Carthew 2006), and their diet can vary significantly depending on season in desert climates (Gibson 2001).

There has not been any published research to date on the diet of the greater bilby in southern temperate zones. However, Gibson and Hume (2000) proposed that the low body-fat stores of wild greater bilbies indicate they are vulnerable to nutritional stress, based on studies conducted in the arid zone in far south-western Queensland. It is plausible that food availability could influence breeding in the greater bilby in southern temperate areas, as demonstrated for the southern brown bandicoot by Lobert and Lee (1990). Investigation of the primary food resources of the greater bilby in southern Australia is required to determine what foods are limiting or abundant at different times of the year. It may be that even generalist omnivores such as bandicoots and bilbies face significant resource challenges in autumn, which could relate to the usually low rainfall in the proceeding months and hence lower landscape productivity in autumn.

The maximum number of litters produced by female bilbies in the two populations we studied was three litters per annum. Females were observed to breed continuously during the breeding season at both sites, and then cease reproduction in late summer or autumn. During the breeding season, some females were observed giving birth ~14 days after a previous litter emerged from the pouch. This concurs with findings of Southgate *et al.* (2000), where the minimum time between births in greater bilbies could be as short as the time of pouch life. McCracken (1986) determined that female greater bilbies display a lactational anoestrus that terminates around the time of young's departure from the pouch. With a short gestation period of 14 days, this means females are able to breed almost continuously, and new pouch young can be born soon after the previous litter has left the pouch (McCracken 1986). The average litter sizes of female bilbies in the two temperate populations examined in the present study were similar to that found by Southgate *et al*. (2000), with the exception that no triplet litters were recorded in our study.

We found that female greater bilbies that successfully carried twin litters to pouch emergence had a larger mean head length than females that raised a single pouch young. Southgate *et al.* (2000) also found female greater bilbies that raised twins were significantly heavier than females that produced single or no pouch young in a captive environment, although they did not record any statistically significant differences for the wild population examined. Body mass has also been correlated with fecundity in northern brown bandicoots (*Isoodon macrourus*; Price-Rees *et al.* 2012). However, body mass is difficult to record accurately when females are carrying large pouch young, and in the present study

pouch young were not removed from the pouch when they were measured so that minimal stress occurred during handling. Female head length was thus used as an indicator of body size. Although larger head length does not indicate condition, a female that is able to grow larger may be more effective at exploiting food resources, resulting in higher fecundity. A larger female may also simply be more physically capable of carrying twins.

In the present study, both male and female greater bilbies displayed a significantly smaller mean body mass on Thistle Island compared with those at Venus Bay Conservation Park. The smaller body size of females on Thistle Island may offer some explanation as to why no sibling litters >60 days of age were observed at this site. The present study showed that despite the significant difference in body mass between the sites, females at both sites had a similar likelihood of giving birth to twins, although the smaller females at Thistle Island were not observed carrying twins to >60 days of age. Our data and observations of losses of one pouch young of a set of twins between trapping periods indicates this is likely due to the death of one twin during pouch life, sometime before pouch young reaching 60 days of age.

Although we do not yet know why females were smaller on Thistle Island, there was a much higher trap success on the island than at Venus Bay Conservation Park, suggesting a higher population density. Bilbies here were recorded across the island, while at Venus Bay Conservation Park they appeared absent from some areas of the park. It is possible that resource competition is more pronounced in the Thistle Island population, where the population has increased to occupy the entire available habitat. It could also be that different selection pressures are operating in the two populations, which has been proposed as a possible cause of body size differences over time between populations of other species (Lomolino 2005). The presence of feral cats at Venus Bay Conservation Park may be exerting increased predation pressure on small bilbies, leading to a population bias towards larger bilbies at that site and the reduced occurrence of subadults (<600 g) observed. Feral cats at Venus Bay Conservation Park is also likely the cause of the lower trap success of bilbies and other by-catch within the park.

The present study demonstrates that the bilby, a species that previously occurred over both arid and temperate environments, can adapt its reproductive strategy to suit local conditions. Our results showed seasonal breeding of bilbies in temperate South Australia with a non-breeding season centred around autumn, similar to other bandicoots in

southern Australia. Timing releases to the start of the breeding season in winter in temperate Australia should therefore increase the likelihood of rapid onset of reproduction. Releasing large females, who are more likely to raise two pouch young per litter, could also be a cost effective measure in a translocation to maximise the rate of increase of a release population. High population density and thus resource competition may explain the reduced female fecundity observed on Thistle Island. Further research is needed on the diet of bilbies in a temperate environment to determine whether availability of food resources play a significant role in the seasonal breeding observed in our study. Changes in fecundity due to local conditions needs to be factored into translocation planning, because it could affect both the timing of releases and the long-term population growth observed post-release.

Chapter 3: A comparative study of survival, recruitment and population growth in two translocated populations of the threatened greater bilby (Macrotis lagotis)



A greater bilby released after being trapped. Photo by Karleah Berris

"*By the early colonists the Bilby was not only regarded as an animal against which the methods of the exterminator need not be employed; it was even accorded a certain amount of protection, and was at times kept as a pet about the house…..Unfortunately this regard for the Bilby seems to have been forgotten by a later generation, and in more recent days but little mercy has been shown to them by any section of the community*."

Frederic Wood Jones, 1924. From 1968 reprint.

Statement of Authorship

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Overall percentage (%)	90%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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By signing the Statement of Authorship, each author certifies that:

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ii. permission is granted for the candidate in include the publication in the thesis; and
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Abstract

Context: Translocations have been widely used to re-establish populations of threatened Australian mammalian species. However, they are limited by the availability of sites where key threats can be effectively minimised or eliminated. Outside of 'safe havens', threats such as exotic predators, introduced herbivores and habitat degradation are often unable to be completely eliminated. Understanding how different threats affect Australian mammal populations can assist in prioritising threat-management actions outside of safe havens.

Aims: We sought to determine whether translocations of the greater bilby to two sites in the temperate zone of South Australia could be successful when human-induced threats, such as prior habitat clearance, historic grazing, the presence of feral cats and European rabbits, could not be completely eliminated.

Methods: Greater bilbies were regularly cage trapped at two translocation sites and a capture–mark–recapture study was used to determine survival, recruitment and population growth at both sites.

Key results: Our study showed that bilbies were successfully translocated to an offshore island with a previous history of grazing and habitat clearance, but which was free of exotic predators. At a second site, a mainland exclosure with feral cats and European rabbits present, the bilby population declined over time. Adult bilbies had similar survival rates in both populations; however, the mainland bilby population had low recruitment rates and low numbers of subadults despite high adult female fecundity.

Conclusions: The results indicated that past grazing and habitat clearance did not prevent the bilby population on the offshore island establishing and reaching a high population density. In the mainland exclosure, the low recruitment is probably due to feral cats predating on subadult bilbies following pouch emergence.

Implications: The results demonstrated that the bilby, an ecologically flexible Australian marsupial, can be successfully translocated to sites with a history of habitat degradation if exotic predators are absent. At the mainland exclosure site, threat mitigation for bilbies should focus on control or eradication of the feral cats. The control of European rabbits

without control of feral cats could lead to prey-switching by feral cats, further increasing predation pressure on the small bilby population.

Additional keywords: conservation, mark–recapture, modelling, population dynamics, threatened species.

3.1 Introduction

Human-induced biodiversity loss is a global phenomenon, with mammalian species, in particular, declining at a rapid rate (Hoffmann *et al*. 2011). Extinction of terrestrial mammals has been particularly acute in Australia, with endemic species representing ~35% of the world's mammalian extinctions since 1500 (Woinarski *et al*. 2015). Some of the Australian threatened mammal species that are now rare and have greatly reduced distributions were once abundant and occupied huge areas of the Australian continent because of their ecological flexibility (Ford 2014; Woinarski *et al*. 2015). As part of conservation efforts for these species, translocations have been undertaken at sites across Australia in an attempt to re-establish populations in areas of their former ranges, and to create insurance populations in protected areas (Short 2009; Morris *et al*. 2015; Legge *et al.* 2018). It is widely accepted that the key threats that led to the original decline of a species must be managed or eliminated for translocations to be successful (IUCN 1987; Kleiman 1989; Perez *et al*. 2012).

Extinction risk in Australian mammals has been linked to body size, with primarily ground-dwelling species between the 'critical weight range' of 35 g and 5.5 kg being at most risk of extinction (Burbidge and McKenzie 1989; Johnson and Isaac 2009). It has been suggested that species within this weight range are more susceptible to predation by introduced red foxes (*Vulpes vulpes*) and feral cats (*Felis catus*; Johnson and Isaac 2009). Therefore, translocations in recent decades have focussed on establishing populations of threatened mammals on offshore islands or in mainland fenced exclosures, termed safe havens, to enable wildlife managers to intensively manage, or completely eliminate, exotic predators and competitors (Legge *et al.* 2018). The number of Australian safe havens has increased substantially in recent decades (Ringma *et al*. 2018), with 17 fenced exclosures and 101 islands representing an area of 2498 km2, in which 32 species of mammals at risk of extinction from feral predators are now conserved (Legge *et al*. 2018). However, threats cannot be managed as intensively as in safe havens across most of Australia, and, therefore,

threat prioritisation is still important for management of remnant populations or for translocations outside these safe havens.

The greater bilby (*Macrotis lagotis*) is an Australian ground-dwelling critical weight-range mammal whose range once included most of the Australian continent (Southgate 1990a; Johnson 1995; Bradley *et al*. 2015). The range of this species has declined by ~80% since European settlement (Southgate 1990a; Bradley *et al*. 2015). Key threats to the bilby are similar to those to other critical weight-range species and include predation by introduced red foxes (Finlayson 1961; Southgate 1990a), introduced grazing livestock (Southgate 1990a; McDonald *et al.* 2015), grazing by European rabbits (*Oryctolagus cuniculus*; Southgate 1990a; McDonald *et al*. 2015), predation by feral cats (Lollback *et al*. 2015) and inappropriate fire regimes (Southgate and Carthew 2006). However, the relative importance of these threats is unknown and there may be complex interactions that act differently on bilby populations in different habitat types (Bradley *et al.* 2015). It has been suggested that, in southern Australia, rabbits and foxes may have played a bigger role in the decline of the bilby than have feral cats (Finlayson 1961; Southgate 1990a), with the available evidence suggesting that feral cats may have had more impact on smaller native mammal species such as rodents (Dickman 1996; Murphy *et al*. 2019).

The bilby is an opportunistic omnivore (Southgate 1990b; Navnith *et al*. 2009), which was known to occur in a wide variety of habitat types before its decline (Kemper 1990; Ford 2014) and breeds well in captivity (Southgate *et al.* 2000). Given its ecological flexibility (Southgate 1990b), it is potentially a good candidate for captive breeding and translocation programs. There have been 12 translocations of bilby documented as part of a national recovery program, four of which failed because of predation (Bradley *et al*. 2015) and eight that occurred within safe havens (Bradley *et al*. 2015; Legge *et al*. 2018). Two temperate-zone safe havens are in South Australia. They are both free of red foxes, a major threat to bilbies in the southern parts of Australia (Southgate 1990a), yet still contain other threats including the presence of feral cats, European rabbits, as well as historic grazing and clearance (Robinson *et al*. 1996; Department for Environment and Heritage 2006; Legge *et al.* 2018). No formal assessments have previously been conducted to determine whether these two translocations have been successful. It is currently not known whether the small numbers of feral cats and rabbits at the one site, or previous vegetation clearance and grazing activities at the other, have affected the translocated populations of bilby in the

absence of other key threats such as red foxes, cattle grazing and inappropriate fire regimes.

The present study had two aims. They were to determine (1) whether the translocation of bilbies to two sites in South Australia with managed threats had been successful, and (2) what impacts historical grazing, and feral cat and rabbit presence have had on the long-term population security of bilbies at the two sites. The results could have implications for future translocations of this species, and provide further information on population-level impacts of key threats on Australian critical weight-range mammals with flexible habitat and resource requirements.

3.2 Materials and methods

3.2.1 Release sites

In the mid-1990s, Zoos South Australia established a greater bilby captive-breeding program at Monarto Zoological Park, South Australia, so that animals could be supplied for release into parts of their former range (Bradley *et al*. 2015). Four release sites were selected within South Australia, including Yookamurra Sanctuary (34.5091°S, 139.433°E), Thistle Island (35.0096°S, 136.1704°E), Arid Recovery Reserve near Roxby Downs (30.3705°S, 136.8887°E), and Venus Bay Conservation Park (33.1796°S, 134.5572°E; Bradley et al. 2015). Our study compared the resultant populations at Venus Bay Conservation Park (herein referred to as Venus Bay) and Thistle Island. This project was undertaken under a University of Adelaide Animal Ethics Committee permit, project number S-023-2007 and a Wildlife Research Permit, approval number 33/2007.

Venus Bay is a 1460-ha coastal peninsula on the western coast of Eyre Peninsula, South Australia. In 1996, a predator-exclusion fence was constructed across the narrow northern end of the peninsula, separating it from the mainland (Department for Environment and Heritage 2006). Introduced red foxes were eliminated from the park following fence construction; however, feral cats and European rabbits persisted within the exclosure (Department for Environment and Heritage 2006). Feral cats were reduced markedly in abundance before the translocation of bilbies (L. Amey, pers. comm.) and rabbits were regularly controlled by park rangers (Department for Environment and Heritage 2006). Between 2001 and 2005, 23 captive-bred bilbies were released into the park (five in 2001,

eight in 2002, six in 2003 and four in 2005; Bradley *et al*. 2015). Bilbies were chosen for translocation because subfossil evidence suggested that bilbies were present in the area in recent times (Department for Environment and Heritage 2006). Subsequent spotlighting and trapping between 2003 and 2008 by park rangers regularly recorded adult bilbies, with smaller subadults occasionally being recorded (L. Amey, pers. comm.). Feral cats were confirmed to be present during the study through the observation of feral cat prints on sand-track transects, and one small subadult cat was caught in a cage trap set for bilbies in January 2010. European rabbits were observed in the northern part of the exclosure on all trips during spotlight searches for bilbies. Feral cat and rabbit abundance were not estimated during the study. However, from our field observations, both species were considered to be present on all surveys.

The second study site, Thistle Island, is a large (3926 ha) island free from red foxes, feral cats and European rabbits, located off the south-eastern coast of Eyre Peninsula in South Australia. Bilbies were not recorded on Thistle Island at the time of European settlement. It was primarily used for grazing from the 1850s up until 1986, which resulted in much of the island vegetation being cleared and sown to pasture (Robinson *et al*. 1996). Following the removal of stock, native vegetation has regenerated over parts of the island (Robinson *et al*. 1996). The northern half is now a conservation reserve and 50 holiday house allotments have been created on the southern part of the island, which still retains some sheep-grazing areas (Robinson *et al*. 1996). Between 1997 and 2000, 21 bilbies were released onto Thistle Island (five in 1997, six in 1998 and 10 in 2000; Bradley *et al*. 2015). Surveys of burrows and diggings between 1997 and 2008 showed that bilbies had progressively spread across the island (J. van Weenan, unpubl. data). Although the location of bilbies on Thistle Island has been monitored by indirect evidence (tracks, scats, diggings and burrows), the current study is the first one where comprehensive trapping surveys have been undertaken at this site. In August 2008, 32 bilbies were translocated from within trapping sites on Thistle Island to Lorna Glen Conservation Reserve in Western Australia for a Rangelands Restoration program, and data from these individuals were excluded from the mark–recapture study, but were included in all other analyses.

For further information on the vegetation communities, other mammal species translocated to the two sites and by-catch, see Berris *et al.* (2019).

For the present study, we undertook 10 trapping surveys of 10–21 days of duration at Venus Bay between July 2008 and April 2010, and six trapping surveys of 7–14 days of duration on Thistle Island between August 2007 and February 2010.

3.2.2 Trapping protocol

At both sites, bilbies were captured using cage traps (minimum size 60 cm × 29 cm × 25 cm) baited with peanut butter and oats. Traps were set under low shrubby vegetation, with only the trap entrance being visible. This minimised traps being set off by animals outside the traps trying to access bait and provided protection from the elements for captured individuals.

Transect lines of 5–20 traps (depending on terrain) 100 m apart were established, with an average of 51 traps set per night. Between 168 and 581 trap-nights were conducted during field trips, the exception to this being September 2008 when only 71 trap-nights were able to be conducted at Venus Bay. On the first trapping survey at each location, trap lines were set across Venus Bay and Thistle Island to determine where bilbies occurred. On subsequent trips, some of these trap lines were re-trapped. Traps were set and baited in the late afternoon and evening, and then checked between 0200 hours and 0500 hours during mild conditions, or twice overnight in cool or wet weather. Traps were closed during the day and the baits removed to reduce daytime by-catch and to discourage ants from frequenting the traps.

Captured bilbies were placed in fabric catch bags, processed on site adjacent to the trap and then released. They were kept in bags while measurements were recorded. Independent bilbies captured had a subcutaneous passive integrated transponder (PIT) tag (Trovan, Douglas, Isle of Man) inserted under the skin between the shoulder blades to allow individual recognition on subsequent trips. Measurements taken were head length (mm), pes length (mm) and body mass (g). Bilbies weighing >600 g were deemed to be sexually mature adults and independent bilbies <600 g were classified as subadults (Southgate *et al.* 2000; Berris *et al*. 2019). Females had their pouches examined at each capture and the presence and size of pouch young were recorded.

3.2.3 Statistical analysis

All data were analysed using R (version 3.3.1; R Core Team 2018), unless otherwise indicated. Trap success was estimated as the number of individuals trapped per 100 trap-nights. To test for differences between two means, such as for trap success and body condition between sites, independent samples Student's t-tests were performed for normally distributed data, and the Wilcoxon rank-sum test was used for non-parametric or skewed datasets. Testing for associations was performed using the Pearson chi-square test. To determine whether observed sex ratios significantly differed from parity, a goodness-of-fit test was performed using a chi-square test with an anticipated ratio of 1 : 1. Female reproductive output was estimated from previously reported results (see Berris *et al*. 2019) by multiplying the average number of litters produced per annum by the average number of young at pouch emergence. This estimated the number of young produced per annum per female in both populations.

The density of bilbies at trap sites was estimated using spatially explicit capture–recapture models through the R-package 'secr' version 3.1.8 (Borchers and Efford 2008; Efford 2018). Density was estimated at a site where a minimum of 20 traps were operational at the same time for periods of five or more nights (one site at Venus Bay and one site on Thistle Island). Buffer width in spatially explicit capture–recapture models defines the area around traps where animals caught are likely to reside, and must be wide enough so that individuals at the edge of the buffer area have almost no chance of being caught in traps. Buffer width for bilbies was calculated using a trap-based measure of bilby movement distances by examining graphs of detection probability and distance from trap on the basis of our capture data. Buffer width was set to 1000 m for final density estimates to ensure that the detection probability of individuals outside our buffer area was effectively zero. The variables of detection function (half-normal, exponential and hazard rate) and learned response to traps were modelled. Models were ranked in order of Akaike's information criterion (AIC; Akaike 1973). We used both differences in AIC corrected for small sample sizes (Hurvich and Tsai 1989; Burnham and Anderson 2002; ΔAICC) and Akaike weights (ω) to rank models in order of support (Nichols 2005). Density estimates were obtained using model-averaged estimates if no one model had more than 95% support.

For bodyweight differences between the two populations, see Berris *et al*. (2019). Body condition for all adult male bilbies caught during each trapping survey was estimated using

the scaled mass index (Peig and Green 2009). Females were excluded because they were often carrying pouch young, so their weight could not be accurately determined. The scaled mass index for an individual bilby was estimated using the following equation (Peig and Green 2009):

$$\widehat{M}_i = M_i \left[\frac{L_0}{L_i}\right]^{b_{SMA}}$$

where $\widehat{M}_i$ gives the predicted body mass for individual i when a linear body measurement (in this case, head length) is standardised to L_0 (the mean head length for the study population). M_i and L_i are the body mass and head length respectively, for Individual i, and b_{SMA} is a scaling exponent, which is derived from the slope of a standardised major axis regression of the log-transformed weight and head lengths of all individuals. The mean scaled mass index of individuals from both sites was then compared using the Wilcoxon rank-sum test for non-parametric data.

Demographic parameters were estimated using capture–mark–recapture data and program MARK v9.0 (White and Burnham 1999) through the R interface using the package RMark (Laake 2013). An encounter history for each adult individual was compiled by defining whether an individual was encountered during each trapping survey. We then used Pradel temporal-symmetry open-population models (Pradel 1996), implemented in program MARK, to estimate apparent survival (φ), recruitment rate (f), population growth rate (λ) and capture probabilities (p; Pradel 1996; Nichols 2005). Finite population growth rate (λ) at Time t was used to assess whether populations were either growing ($\lambda > 1$) or decreasing ($\lambda < 1$), with the following relationship assumed (from Franklin 2001):

$$\lambda_t = \varphi_t + f_t = \frac{N_{t+1}}{N_t}$$

where Nt is the population size at Time t. Encounter histories from the two sites were modelled separately because of different trapping frequencies and an unequal number of trapping sessions (encounters) at each site.

We used a survival-modelling approach as described by Lebreton et al. (1992). Survival (φ) and capture probability (p) were modelled with time dependency and gender as covariates. To obtain estimates of recruitment rate (f) and population growth rate (λ) that were comparable between the sites, both these parameters were constrained as recommended

by Franklin (2001). Because no specific goodness-of-fit tests are available for temporal-symmetry Pradel models, goodness-of-fit was tested using a global Cormack–Jolly–Seber model and using Test 2 and Test 3 in the program RELEASE (Burnham *et al*. 1987). The results of Test 2 and Test 3 indicated that there was evidence of a slight over-dispersion in the data from Venus Bay; therefore, a variance inflation factor ($\hat{c}$) was adjusted to 1.09 for data from this site to correct for this lack of fit (Nichols 2005). There was no evidence of over-dispersion in the Thistle Island data. Model selection followed the same methods as for density estimation, with both AICC differences (ΔAIC_C) and Akaike weights (ω) being used to rank models in order of support. We then created a 95% confidence set of models based on Akaike weights (Burnham and Anderson 2002; Johnson and Omland 2004; Symonds and Moussalli 2011) and calculated model average estimates from this model set.

3.3 Results

3.3.1 Trap success and density over time

At Venus Bay, bilbies were able to be trapped only in the northern area of the park, which was ~640 ha in size (~44% of the total area of the exclosure). Bilby sign was observed in the southern areas of the park on three occasions (two sets of prints, one picture on a remote camera); however, bilbies seemed to be in numbers too low to trap in these areas. Therefore, data on bilby captures at Venus Bay pertains to bilbies caught in the northern 640-ha area only. On Thistle Island, bilbies were trapped and their sign observed across the island. Data on bilby captures on Thistle Island are from two areas at either end of the island, ~7 km apart, where bilbies were trapped consistently throughout the study.

At Venus Bay, trap success declined over the 2-year study period from 16.1 individuals per 100 trap-nights in November 2008 to 4.2 individuals per 100 trap-nights in April 2010 (Fig. 3.1). Density estimates of bilbies at Venus Bay followed a trend similar to that of trap success, and had a negative relationship with time (months elapsed) that was approaching significance (Pearson correlation t = –2.4979, d.f. = 5, r = –0.75, P = 0.055; Fig. 3.2). The best-fitting models indicated that there was a positive learned response to traps in April 2009, June 2009 and September 2009 and that there was no learned response to traps in all other surveys. By contrast, trap success remained fairly stable on Thistle Island, and ranged from 9.26 to 18.26 individuals per 100 trap-nights (Fig. 3.1).

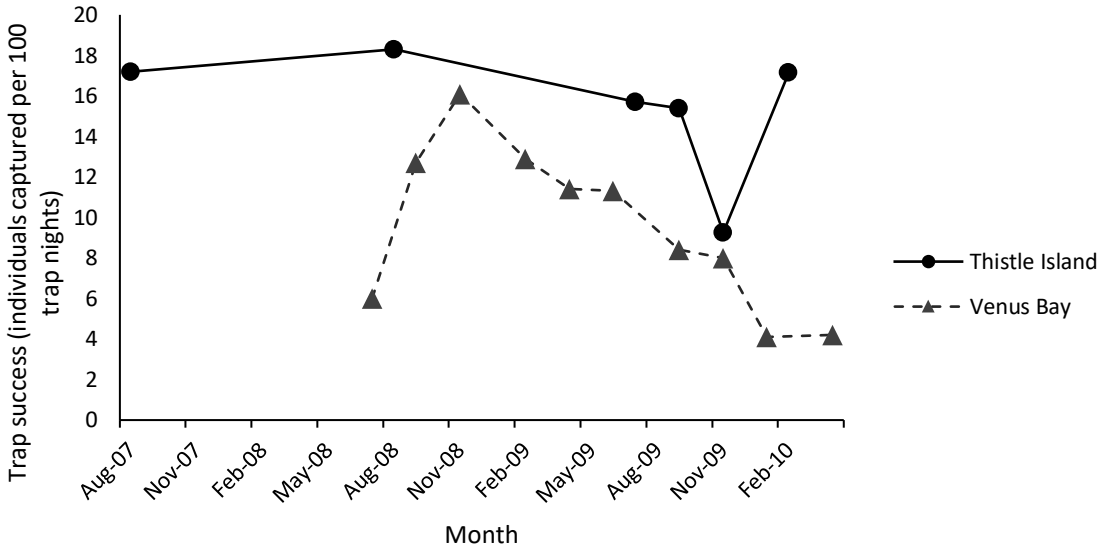


Figure 3.1 Trap success between August 2007 and April 2010, estimated as the number of bilbies captured per 100 trap nights, for Venus Bay (▲) and Thistle Island (●).

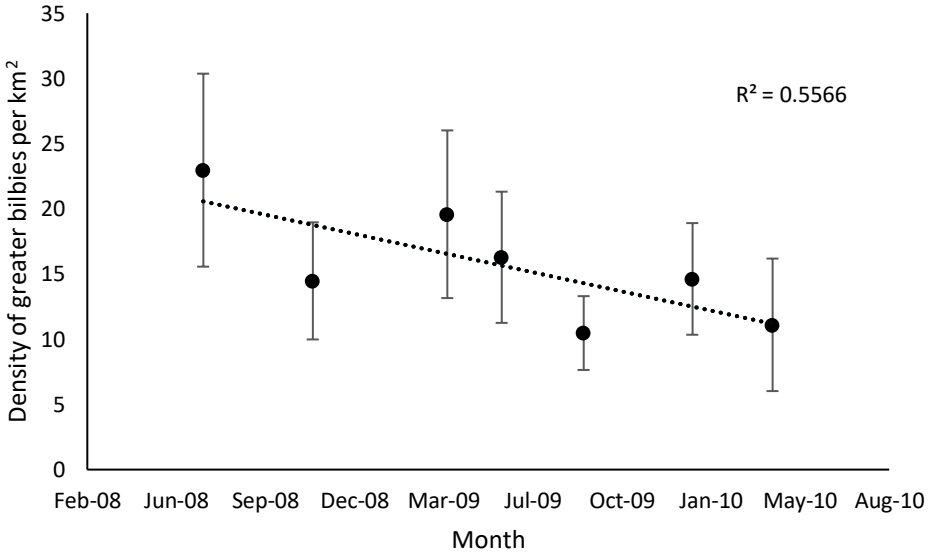


Figure 3.2 Estimated density of bilbies per km^2 at Venus Bay between July 2008 and April 2010 (●) (± S.E.), dotted line represents a linear regression fitted to the density data.

Recaptures of individual bilbies within surveys was low on Thistle Island, and there were high numbers of by-catch (which is detailed in Berris *et al*. 2019). Consequently, density estimates could be estimated with any confidence only for two of the six surveys, and were 75.4 (s.e. = 45.7) and 101 (s.e. = 38.0) bilbies km^{-2} during the July 2009 and September 2009 surveys respectively. The best-fitting models indicated that there was no evidence of a learned response to traps in either of these two surveys. Mean trap success at Venus Bay

was significantly lower than that on Thistle Island (Wilcoxon rank-sum test, W = 53, P = 0.01).

3.3.2 Population composition

At Venus Bay, of the 121 animals captured in total, 59 bilbies were adults (>600 g) at the time of first capture, 10 were subadults (independent bilbies of <600 g) and 52 were pouch young. On Thistle Island, of the 238 individuals caught, 160 bilbies were adults at the time of first capture, 34 were subadults, and 44 were pouch young. There was a significant difference in the proportion of subadults between the two sites (χ^2 = 10.85, d.f. = 1, P = 0.001), with lower subadult numbers occurring at Venus Bay.

3.3.3 Sex ratio of adult population

A significant male bias was evident for adults in both populations. At Venus Bay, 40 adult males and 19 females were trapped (χ^2 = 7.47, d.f. = 1, P = 0.0060), and, on Thistle Island, 105 adult males and 55 females were trapped (χ^2 = 15.63, d.f. = 1, P = 0.0001). A non-significant male bias was also observed in subadults at Venus Bay (males = 8, females = 2, χ^2 = 3.6, d.f. = 1, P = 0.06), but not in the subadult population on Thistle Island (males = 14, females = 20, χ^2 = 1.06, d.f. = 1, P = 0.30).

3.3.4 Body condition

Body condition (mean ± s.d.) as determined by the scaled mass index was significantly greater for males at Venus Bay (1352 ± 211) than on Thistle Island (1232 ± 196; Wilcoxon rank-sum test, *W* = 8354.5, P < 0.001).

3.3.5 Female reproductive output

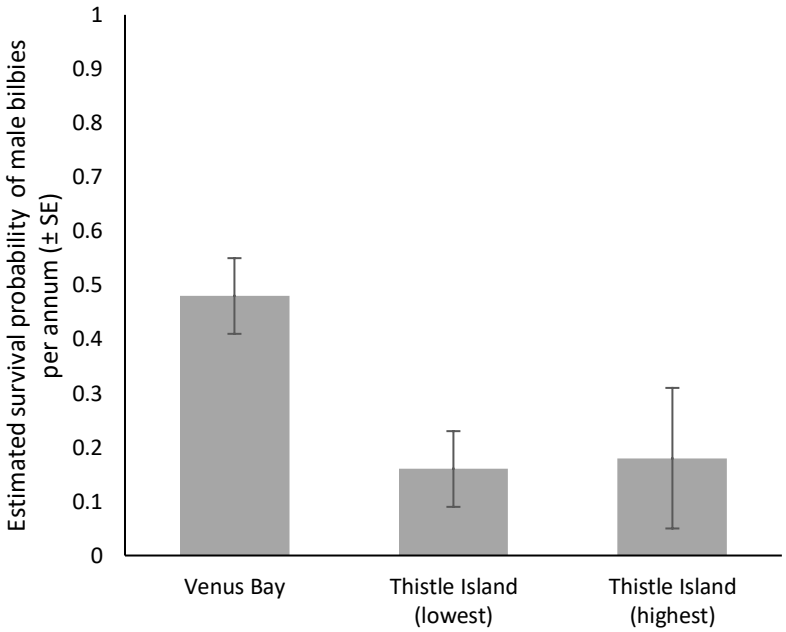
The average number of litters a female raised to pouch emergence was 2.6 per annum in the Venus Bay population, and 1.8 litters per annum in the Thistle Island population (Berris *et al*. 2019). The average litter size at pouch emergence (estimated from all litters observed at over 60 days of age) was 1.47 ± 0.13 (mean ± s.e., n = 15) at Venus Bay and 1.00 ± 0.00 (mean ± s.e., n = 8) on Thistle Island (Berris *et al*. 2019). From these means, we estimated that the approximate number of young a female raised to pouch emergence per annum was 3.82 at Venus Bay and 1.47 on Thistle Island.

3.3.6 Apparent survival, recruitment and capture probability

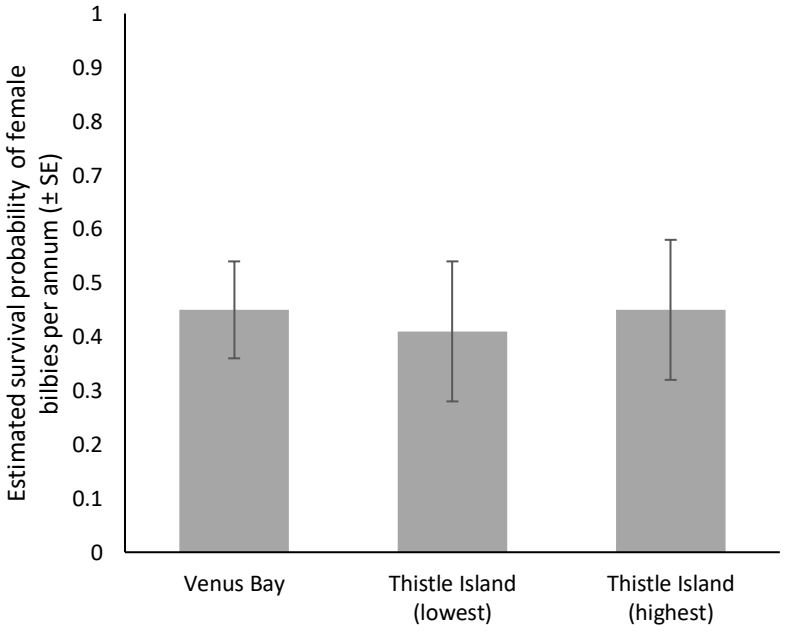
Survival and capture probability were modelled with gender and time as covariates, and with fecundity being constrained. In the Venus Bay population, none of the four models in the 95% confidence set indicated an effect of time on survival, and two of the top four models, representing 30% of the model weights, suggested that gender had an effect on survival probability (Table 3.1). For the Thistle Island population, seven of the nine models in the 95% confidence set suggested an effect of gender on survival and three models indicated an influence of time on survival (Table 3.1). Model-averaged estimates of survival probability for female bilbies in the Venus Bay and Thistle Island populations and males in the Venus Bay population were very similar (between 0.41 and 0.48 per annum). However, male bilbies on Thistle Island had a lower estimated apparent survival (0.16–0.18 per annum; Fig. 3.3). Over the study period, estimated apparent recruitment rate per annum was much lower at Venus Bay than on Thistle Island (Fig. 3.4).

Table 3.1 Summary of the 95% confidence set of models for adult greater bilby apparent survival (φ), capture probability (p) and recruitment (f) with recruitment constrained (Pradel Design) based on the capture history of bilbies at Venus Bay (N = 57) and Thistle Island (N = 130).

Venus Bay models	N	$QAIC_C$	$\Delta QAIC_C$	w_t
$\varphi_{(.)}\, p_{(time)}\, f_{(.)}$	12	566.96	0	0.52
$\varphi_{(Sex)}\, p_{(time)}\, f_{(.)}$	13	568.62	1.65	0.23
$\varphi_{(.)}\, p_{(time+Sex)}\, f_{(.)}$	13	569.17	2.21	0.17
$\varphi_{(Sex)}\, p_{(time+Sex)}\, f_{(.)}$	14	570.88	3.92	0.08
Thistle Island models	N	AIC_c	ΔAIC_C	w_t
$\varphi_{(Sex)}\, p_{(.)}\, f_{(.)}$	4	738.40	0	0.47
$\varphi_{(Sex)}\, p_{(Sex)}\, f_{(.)}$	5	740.40	2.00	0.17
$\varphi_{(Sex)}\, p_{(time)}\, f_{(.)}$	9	740.90	2.50	0.14
$\varphi_{(Sex)}\, p_{(time+Sex)}\, f_{(.)}$	10	742.90	4.50	0.05
$\varphi_{(time+Sex)}\, p_{(.)}\, f_{(.)}$	8	743.19	4.80	0.04
$\varphi_{(.)}\, p_{(Sex)}\, f_{(.)}$	4	744.66	6.27	0.02
$\varphi_{(.)}\, p_{(.)}\, f_{(.)}$	3	744.68	6.29	0.02
$\varphi_{(time+Sex)}\, p_{(time)}\, f_{(.)}$	13	744.90	6.50	0.02
$\varphi_{(time+Sex)}\, p_{(Sex)}\, f_{(.)}$	9	744.99	6.60	0.02



a)



b)

Figure 3.3 Estimated apparent survival probability (± S.E.) of (a) male and (b) female bilbies per annum at Venus Bay between July 2008 and April 2010 and Thistle Island between August 2007 and February 2010. Thistle island models showed an influence of time on survival probability, so the lowest and highest survival estimates for Thistle Island are shown.

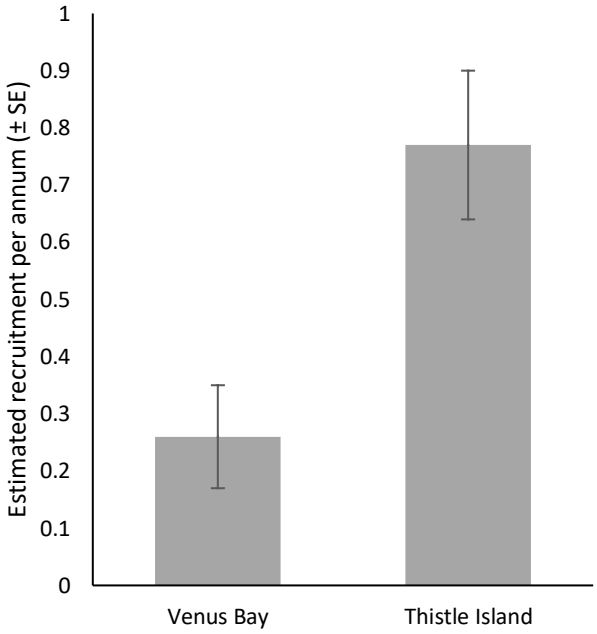


Figure 3.4 Estimated apparent recruitment rate (± S.E.) of adult bilbies per annum at Venus Bay between July 2008 and April 2010 and Thistle Island between August 2007 and February 2010.

In the Venus Bay population, all four models in the 95% confidence set indicated that time (trapping session) influenced capture probability (Table 3.1). Capture probability varied between 0.55 (95% CI 0.36–0.73) and 0.92 (95% CI 0.22–1.00), with September 2009 recording an unusually low capture probability of 0.25 (95% CI 0.12–0.46). In the Thistle Island population, capture probability was more consistent across the study, varying between 0.61 (95% CI 0.32–0.84) and 0.71 (0.43–0.89).

3.3.7 Population growth rate

Six of the 25 models run for population growth rate (λ) made up the 95% confidence set of models for the Venus Bay population (Table 3.2) and seven models of 25 represented the 95% confidence set of models for Thistle Island (Table 3.2). The model-averaged estimate of annual population growth rate for bilbies was higher for the Thistle Island population than for the Venus Bay population, with the Thistle Island population growth rate close to being stable, whereas the Venus Bay population declined over the study period (Fig. 3.5).

Table 3.2 Summary of the 95% confidence set of models for adult greater bilby apparent survival probability (φ), capture probability (p) and population growth (λ) with population growth constrained (Pradel Design) based on the capture history if bilbies at Venus Bay (N = 57) and Thistle Island (N = 130).

Venus Bay models	N	$QAIC_C$	$\Delta QAIC_C$	w_t
$\varphi_{(.)} p_{(time)} \lambda_{(.)}$	12	566.96	0	0.37
$\varphi_{(Sex)} p_{(time)} \lambda_{(.)}$	13	567.75	0.79	0.25
$\varphi_{(.)} p_{(time+Sex)} \lambda_{(.)}$	13	569.17	2.21	0.12
$\varphi_{(time)} p_{(time)} \lambda_{(.)}$	20	569.80	2.84	0.09
$\varphi_{(Sex)} p_{(time+Sex)} \lambda_{(.)}$	14	570.00	3.04	0.08
$\varphi_{(time+Sex)} p_{(time)} \lambda_{(.)}$	21	571.59	4.63	0.04
Thistle Island models	N	AIC_c	ΔAIC_C	w_t
$\varphi_{(Sex)} p_{(.)} \lambda_{(.)}$	4	734.99	0	0.47
$\varphi_{(Sex)} p_{(Sex)} \lambda_{(.)}$	5	737.02	2.02	0.17
$\varphi_{(Sex)} p_{(time)} \lambda_{(.)}$	9	737.29	2.30	0.15
$\varphi_{(time+Sex)} p_{(time)} \lambda_{(.)}$	13	739.03	4.04	0.06
$\varphi_{(Sex)} p_{(time+Sex)} \lambda_{(.)}$	10	739.17	4.17	0.06
$\varphi_{(time+Sex)} p_{(.)} \lambda_{(.)}$	8	740.05	5.06	0.04
$\varphi_{(time+Sex)} p_{(time+Sex)} \lambda_{(.)}$	14	741.14	6.15	0.02

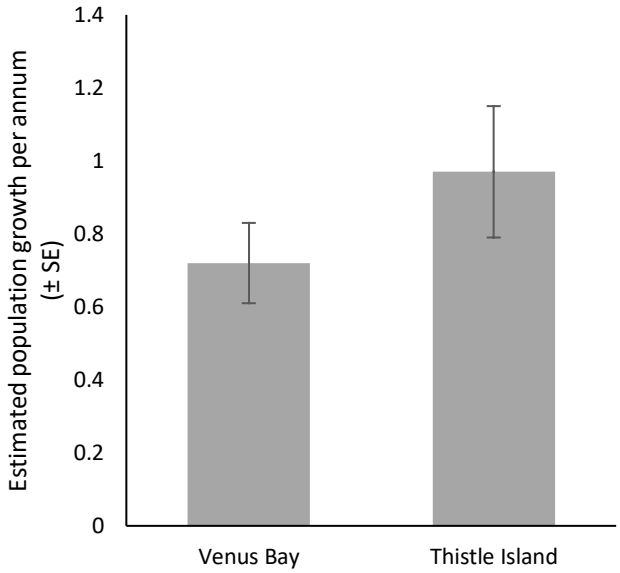


Figure 3.5 Estimated population growth rate (± S.E.) of the adult bilby population per annum at Venus Bay between July 2008 and April 2010 and Thistle Island between August 2007 and February 2010.

3.4 Discussion

Our study evaluated whether two translocations of the greater bilby to safe havens in South Australia had been successful. At Venus Bay, a small population located close to the original release site was present 7–9 years after translocation. Both trap-success rates and the population growth-rate estimate of 0.72 (95% CI 0.53–0.98) per annum indicated that the population declined over the 2-year study period. At this time, this bilby population was apparently not self-sustaining, and, hence, the long-term viability of this population was in doubt. In contrast, at the second translocation site, Thistle Island, bilbies were captured and observed across all areas of the island, with both trap success and the population growth-rate estimate of 0.97 (95% CI 0.66–1.42) suggesting that this population was at least stable over our study period. The Thistle Island population, unlike the one at Venus Bay, appeared to be self-sustaining 10–13 years after translocation, indicating that the translocation had been successful up to the present time. These findings suggest that it is possible to establish a viable population of greater bilbies at a site where there has been historic grazing and habitat clearance, provided that no exotic predators are present. Our results also indicated that the small, declining bilby population at Venus Bay is being affected by a lack of recruitment of adult bilbies.

Our results indicated that high female fecundity did not result in high recruitment to the adult population at Venus Bay. Because the study at Venus Bay occurred over a 2-year period, it is hard to say whether these observed trends were short term or whether they had been occurring over a longer period. Rainfall at Venus Bay was 296.2 mm in 2008 and 402.7 mm in 2009 (Berris *et al*. 2019), and the long-term average at the site is 370 mm per annum (Department for Environment and Heritage 2006). Therefore, in one year of the study, rainfall was somewhat below the long-term average, which may have affected available resources at the site. The relatively high reproductive output of adult female bilbies at Venus Bay compared with Thistle Island, and the higher body condition of adult males at Venus Bay, indicated that it is unlikely that food-resource shortages owing to competition, habitat suitability or climatic factors had limited recruitment in this population. There was also no evidence that lower than average rainfall affected the survival of adult bilbies, which at Venus Bay was similar to, or even higher than, that of bilbies on Thistle Island. However, the population at Venus Bay did show a low rate of recruitment of new adults and significantly fewer than expected subadults were present (individuals with body mass of <600 g). We contend that the decline of this bilby population was most likely a result of the presence of feral cats within the fenced area of the park (Department for Environment and Heritage 2006; Legge *et al*. 2018). Moreover, data suggested that feral cats may have been primarily preying on the smaller, subadult, individuals but were having little impact on the survival of adults.

Feral cats have been shown to prey on bilbies, with remains of individuals previously found in the stomach contents or scats of feral cats collected in South Australia, Queensland and the Northern Territory (Paltridge 2002; Bradley *et al.* 2015; Moseby *et al*. 2015). Amplified feral cat predation of subadults, as suggested in the present study at Venus Bay, has previously been observed at Astrebla Downs National Park in Queensland where over 100 predominantly subadult bilbies were found in the stomachs of feral cats in 2012 (Bradley *et al.* 2015). Predation of subadults has also been observed to occur in a reintroduced population of a related species, the western barred bandicoot (*Perameles bougainville*), at Shark Bay in Western Australia (Richards and Short 2003), with that population later having been driven to extinction because of predation by feral cats (Short 2016). Our results suggest that, in the absence of red foxes, feral cat predation alone may result in population declines in the bilby through predation of subadults and, hence, subsequent low recruitment into the adult population.

The presence of feral cats and foxes has resulted in unsuccessful bilby translocations elsewhere is Australia (Moseby *et al.* 2011a; Bradley *et al.* 2015). Feral cats, in the absence of red foxes, resulted in a population crash of translocated bilbies at Currawinya National Park, Queensland (Bradley *et al*. 2015). This population was estimated to have initially been ~300 individuals but then declined to fewer than 10 bilbies following a breach in the fence that allowed feral cats to access the park (Bradley *et al.* 2015). The six sites in Australia where translocated bilbies are currently being maintained (including Thistle Island) are all free of both foxes and feral cats (Bradley *et al.* 2015). Recent research has indicated that controlled exposure to feral cats, either through predator-avoidance training or co-existence with low-density feral cat populations, can result in behavioural changes in bilbies that may help them avoid predators (Moseby *et al*. 2012; Steindler *et al.* 2018; Ross *et al.* 2019) and could increase their survival in the presence of feral cats (Blumstein *et al*. 2019). Our results showed that adult bilbies co-existing with a feral cat population can have survival rates similar to those living in a feral cat-free population. However, our research has highlighted that adult survival alone may not be sufficient for population persistence, and suggested that subadult bilbies may be more at risk of predation either owing to their size or predator naiveté. Our results, thus, support the concept that safe havens for bilbies must be free of all exotic predators, including red foxes and feral cats, to be effective at safe-guarding populations of this critical weight-range mammal species at risk of extinction. Improving the survival and predator awareness of subadult bilbies following pouch emergence in populations that co-exist with low density feral cat populations could be an area of future research, which is required to determine whether, at a population level, bilbies could co-exist long-term with feral cats.

The abundance of feral cats at Venus Bay during the present study was not known; however, feral cats and their prints were occasionally seen. Other studies have shown that there does not need to be a high population of feral cats to have a detrimental impact on small populations of prey species such as bilbies. A recent study by Moseby *et al.* (2019) recorded a bilby population persisting, and even increasing in size, with cat densities of up to 0.46 feral cats km^{-2} at an arid-zone site. However, large (over 3.5 kg) male cats have reportedly been responsible for 'catastrophic' rates of predation of medium-sized marsupials in some studies, including bilbies, even when the overall feral cat density is low (Moseby *et al.* 2015b). At our Venus Bay study site, one large 5.75 kg male cat was found to be responsible for killing 14 reintroduced brush-tailed bettongs in ~1 month (*Bettongia*

pencillata; Department for Environment and Heritage 2006; Moseby *et al*. 2015b). Once this large male cat was killed, no further bettong deaths were recorded despite other cats persisting in the park (Department for Environment and Heritage 2006; Moseby *et al*. 2015b). A translocation of 102 greater stick-nest rats (*Leporillus conditor*) in 1995 and 1996 was also considered unsuccessful at Venus Bay because of cat predation; however, almost 10 years post-release, a set of prints believed to be from a stick-nest rat were found, indicating that a small number of individuals may have persisted (Department for Environment and Heritage 2006). The results of the present study, thus, support the conclusion that feral cat predation could be a major threat to bilby populations (Pavey 2006), particularly to a small translocated population during the mid- to long-term establishment phase.

Because feral cats are known to feed on a wide variety of prey (Woinarski *et al*. 2017, 2018; Murphy *et al*. 2019), the magnitude of feral cat predation on a medium-sized marsupial such as the bilby may vary in relation to the presence/abundance of other suitable prey species. Bilbies were the most frequently trapped species at Venus Bay during the study period, with about four times more bilby captures being recorded than captures of brush-tailed bettongs, which were the next most frequently trapped species (Berris *et al*. 2019). Rabbit abundance was not measured during the present study; however, they were being regularly controlled by park rangers during the study (K. Berris, pers. obs.). European rabbits are known to be a major food source for feral cats in many parts of Australia (Molsher *et al*. 1999; Read and Bowen 2001; Doherty 2015), and reducing rabbits without controlling feral cats can lead to prey-switching to native mammals immediately after rabbit populations decline (Doherty *et al*. 2015; McGregor *et al*. 2020). To preserve the native species within the Venus Bay exclosure, careful consideration should be given to the order by which exotic species are removed from the park. Ideally, feral cats should be eradicated from the exclosure before eradicating rabbits. European rabbit control in the absence of feral cat control could cause feral cats feeding on rabbits to prey-switch to native mammals when rabbits decline, thereby increasing their predation pressure on the small bilby population.

The high trap success rates, high-density estimates and observation of bilbies across Thistle Island indicated that bilbies have successfully increased their population size and distribution across the island since their release. The lower body condition, lower

reproductive output and a near-stable population growth rate at Thistle Island suggest that this population may be close to its maximum size, with intraspecific resource competition possibly occurring among individuals. The high trap success and high estimated densities of bilbies at Thistle Island indicate that previous grazing and vegetation clearance has not impeded initial population growth at this site. Grazing ceased across most of Thistle Island in 1986 (Robinson *et al*. 1996), and bilbies were first released at the site 11 years later. It appears that the habitat had either sufficiently recovered to a state suitable for bilbies within that time or that bilbies were reasonably tolerant to the past disturbances and habitat clearance at this location. Bilbies have previously been associated with open habitats that contain only sparse groundcover (Southgate 1990b), and, hence, vegetation structure may be of secondary importance to food availability in this species. Bilbies are opportunistic omnivores, and feed on a range of insect, seed and plant material (Southgate 1990b; Navnith *et al*. 2009). No dietary analysis was undertaken during the present study to examine what bilbies were feeding on at the two sites. Research on the diet of these two populations could provide useful information on the primary food resources that are relied on in these environments, and could help inform future site selection for translocations of this species.

Adult male bilby survival was low on Thistle Island, between 0.16 (95% CI 0.06–0.34) and 0.18 (95% CI 0.04–0.56) per annum over the study period. This was lower than that estimated for adult bilbies at Venus Bay, even though feral cats were present at the latter site. Thistle Island also recorded a much higher recruitment rate than did Venus Bay and a significant male bias was observed in both adult populations. The sex ratio of pouch young has been found to be close to parity at Thistle Island (Berris *et al.* 2019); therefore, it is unlikely that male bias is due to a male bias in pouch young or decreased female survival. Moseby and O'Donnell (2003) found that male bilbies in the arid zone had home ranges up to 17.5 times greater than did females, and that, on average, 80% of a male's home range overlapped with that of other males. Aside from being significantly smaller, a female home range had only an 18% overlap with that of another female (Moseby and O'Donnell 2003). Although bilbies were not radio-tracked during the present study, it is plausible that, in the temperate environment, males still maintain larger home ranges than do females, especially if competition for access to females is driving male home-range size. A previous study by Miller *et al.* (2010a) indicated that bilbies have a promiscuous mating system, and the strategy of males in this species is likely to look for receptive females by roaming the

landscape. If males do roam larger areas that overlap with the ranges of other males on Thistle Island, it is possible that a randomly placed trap line could intersect more male home ranges than it would female home ranges. Apparent survival estimates include the influence of both mortality and emigration (Nichols 2005); therefore, males moving over larger areas or emigrating out of trapping areas could also be reasons for the low apparent survival estimates. No published research has estimated home-range size in bilbies in temperate environments, and this could be an area of future research.

3.4.1 Conclusions

The small and apparently declining bilby population at Venus Bay provides further evidence for the adverse impact of feral cats on successful translocations of medium-sized marsupials such as the bilby. In contrast, the stable population on Thistle Island indicates that this ecologically flexible species can survive quite successfully at a site with a long history of human disturbance, provided that exotic predators are absent. The results suggest that future translocations of the bilby should aim to take place at sites free of both red foxes and feral cats, but that this species may be tolerant of prior habitat degradation such as historic grazing. It is recommended that, at the Venus Bay site, feral cats are eradicated from the exclosure before the removal of rabbits, so as to prevent a situation where feral cats in the reserve prey-switch from rabbits to native mammals and further increase the predation pressure on the bilby population. Further research on the diet of bilbies in these populations could help our understanding of what food resources are driving the population dynamics of bilbies in southern temperate environments and assist in future translocation planning.

Chapter 4: Burrow use by bilbies in temperate South Australia



A greater bilby at the entrance to its burrow.
Photo by Karleah Berris

"The members of the Genus Thalacomys (bilbies) *differ from the rest of the Peramelidae in their truly fossorial mode of life. Isoodon and Perameles will scratch out shallow runways, but none of the species with which I am acquainted ever excavates real burrows in which to live. Thylacomys, on the other hand, passes most of its time in the depth of a burrow of its own making."*

Frederic Wood Jones, 1924. From 1968 reprint.

Statement of Authorship

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Contribution to the Paper	Designed project, performed field work and collected data, performed data analysis, interpreted analyses and wrote manuscript.		
Overall percentage (%)	90 %		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	11/08/2024

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Abstract

Remnant natural populations of greater bilbies (*Macrotis lagotis*) are confined to the Australian arid zone, where bilbies construct and shelter in multiple burrows within their home range. We investigated burrow use behaviour of bilbies in a translocated population in temperate southern Australia to determine if behaviour differed in this climatic zone. Over a 12 month period, 43 adult bilbies at Venus Bay Conservation Park were spool-and-line tracked to 118 burrows. Active burrow density was 0.55 per ha, and bilbies at the site used multiple burrows (up to 13 for males and 8 for females) and regularly moved between diurnal burrows. Male bilbies had significantly larger burrow ranges (10.2 ± 5.8 ha, MCP100, mean ± s.d.) than females (2.6 ± 1.8 ha), and were similar in size to those recorded in an arid zone population. Males' burrow ranges tended to overlap with the burrow range of multiple other males and females. The density of burrows in the current study suggests that bilby burrows were likely to have been a common landscape feature within the southern parts of the species range prior to their local extinction. Further research is needed to determine the impact of the loss of bilby burrows from southern Australia on other burrow commensal species.

Additional keywords: behaviour, marsupial, threatened species, Thylacomyidae

4.1 Introduction

Semi-fossorial mammals are species that forage terrestrially and construct subterranean burrows (White 2005). Burrows can provide shelter from harsh environmental conditions (Roper *et al.* 2001; Burda *et al.* 2007), act as a refuge from predators and fire (Harper and Batzli 1996; Blumstein 1998; Camp *et al.* 2012), provide a place to raise young (Reichman and Smith 1990), and are used for food storage (Begall and Gallardo 2000). The energetic costs of burrow construction for semi-fossorial mammals can be high (Ebensperger and Bozinovic 2000; White *et al.* 2006), therefore the microclimatic and refuge benefits burrows provide should outweigh the construction costs. Burrows are not only beneficial to the individuals that construct them, but also provide refuge sites for co-existing non-burrowing species (Machicote *et al.* 2004; Gálvez Bravo *et al.* 2009; Whittington-Jones *et al.* 2011). Species that alter the environment through the creation of new habitats or microhabitats, such as burrows, are termed ecosystem engineers and can have benefits for species richness (Romero *et al.* 2015).

The greater bilby (*Macrotis lagotis*) is a semi-fossorial nocturnal Australian marsupial which digs burrows up to three metres deep for shelter during the day (Johnson 1995; McRae 2004). In the 1800's, the bilby was abundant in many arid, semi-arid and temperate regions of southern Australia and was distributed across approximately 70% of the Australian continent (Wood Jones 1924; Kemper 1990; Southgate 1990a). The range of the species contracted rapidly between the late 1800's and 1935, and it now occupies about 20% of its former range (Southgate 1990a; Pavey 2006; Bradley *et al.* 2015). Due to the long absence of bilbies from the southern parts of their former range (Kemper 1990), the burrow use behaviour of bilbies exposed to a temperate climate has not yet been investigated. Since the bilby once occurred across such a wide variety of landscapes and habitat types, it is necessary to determine whether differences in biology and behaviour occur between individuals in different regions so that this species can be effectively repatriated and managed.

Burrow use by bilbies has previously been studied in the arid zone, where they have been found to construct, and use, multiple burrows within their home range (Lavery and Kirkpatrick 1997; Moseby and O'Donnell 2003). Temperature and humidity are relatively stable in relic bilby burrows in the arid zone (Chapman 2013), indicating that sheltering in burrows may reduce the energetic costs of thermoregulation. Bilby burrows were observed "in some numbers" in parts of South Australia, and were also observed in south-west Western Australia prior to their local extinction (Wood Jones 1924; Abbott 2001, 2008). Although bilbies were recorded constructing burrows throughout their historic range, how individuals used burrows in different habitats was not documented. Recent research has highlighted the importance of both active and disused bilby burrows as a refuge for other species in the arid zone (Read *et al.* 2008; Hofstede and Dziminski 2017; Dawson *et al.* 2019). The extinction of bilbies and the loss of their burrows from over half of the Australian continent may also have had a negative effect on other species in temperate environments. However our current lack of knowledge on the prevalence of bilby burrows in southern landscapes makes this difficult to quantify.

As part of a national recovery program, at least eight sites across Australia currently support translocated bilby populations (Bradley *et al.* 2015). One of these sites is Venus Bay Conservation Park in South Australia, where 23 captive-bred bilbies were released between

2001 and 2005 in four release events (Bradley *et al.* 2015). The site has recorded densities of up to 23 bilbies per km^2 in parts of the park close to the release site three to five years after the last release (Berris *et al.* 2020). Bilby burrows were encountered infrequently during searches in areas of remnant vegetation where bilbies were regularly trapped (K. Berris, personal observation). It is not clear whether this was due to bilbies using few burrows per individual at the site, bilbies foraging in different areas to where they located their burrows, or due to difficulty detecting burrows in vegetated landscapes.

The aim of this study was to describe the burrow use behaviour, the distribution of burrows and burrow characteristics in a bilby population exposed to a temperate climate. The study site at Venus Bay Conservation Park experiences a Mediterranean climate with regular winter rainfall (Department for Environment and Heritage 2006). It has similar climatic conditions and habitat features to other semi-arid and temperate sites in southern South Australia where bilbies were historically thought to have occurred (Kemper 1990). We anticipated that higher annual rainfall at this site would lead to more concentrated resources compared to sites in the arid zone. Therefore, we hypothesise that bilbies at Venus Bay Conservation Park construct and use multiple burrows, but that individuals would have smaller burrow ranges than those in the arid zone.

4.2 Materials and methods

4.2.1 Site details

Greater bilbies were trapped and spool-and-line tracked during six field trips of 10-21 days in duration between April 2009 and April 2010 at Venus Bay Conservation Park (-33.1796°N, 134.5572°E), a 1460 hectare coastal peninsula in South Australia (Fig. 4.1). A predator-exclusion fence constructed at the northern end of the peninsula separates it from the mainland and excludes red foxes (*Vulpes vulpes*); however feral cats (*Felis catus*) were still present in the park at the time of the study (Department for Environment and Heritage 2006, Berris *et al.* 2020). This site is in a temperate climatic zone (Colls and Whitaker 2012) and the dominant vegetation at the study site where trapping was conducted consisted of *Eucalyptus rugosa* and *E. gracilis* open woodland with an understory of *Melaleuca lanceolata* (Department for Environment and Heritage 2006). Small mammals recorded at the site include the little long-tailed dunnart (*Sminthopsis dolichura*), fat-tailed dunnart (*Sminthopsis crassicaudata*), western pygmy possum (*Cercartetus concinnus*) and

Mitchell's hopping mouse (*Notomys mitchellii*) (Department for Environment and Heritage 2006). Brush-tailed bettongs (*Bettongia penicillata*), greater stick-nest rats (*Leporillus conditor*) and greater bilbies have all been translocated to this park as part of an ecosystem restoration program (Department for Environment and Heritage 2006), with greater bilbies being released between 2001 and 2005 (Bradley e*t al.* 2015).

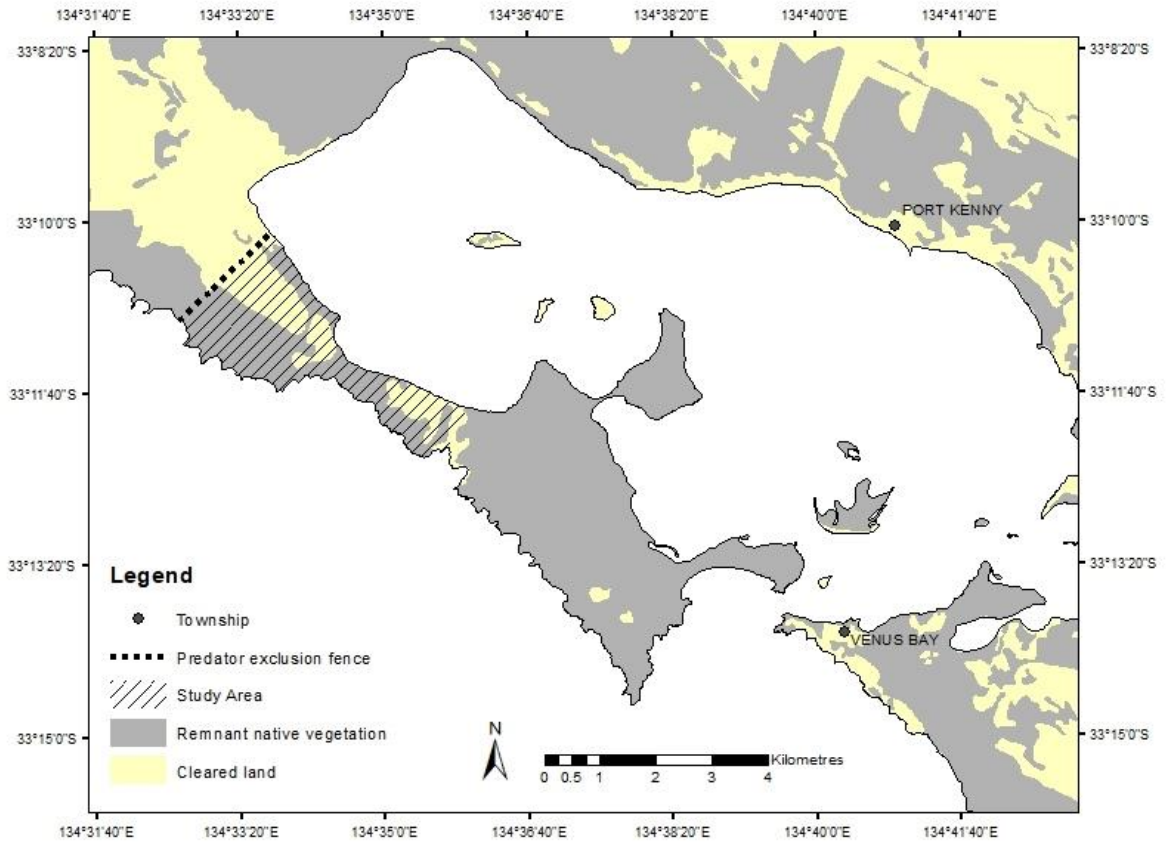


Figure 4.1 Map of the study site at Venus Bay Conservation Park in South Australia.

4.2.2 Trapping protocol

Bilbies were captured using medium sized cage traps (minimum size 60cm x 29cm x 25cm) baited with a mixture of oats and peanut butter. Trapping occurred in a 640 hectare area of the park where trap rate was greatest (Fig. 4.1). Traps were placed under low shrubby vegetation for both protection from the elements and to minimise animals trying to access the bait from the sides of traps. Traps were placed 100 metres apart in transects of 5-20 traps, depending on terrain. Traps were opened at dusk and checked between 2 am and 5am each day so bilbies could be processed and released prior to dawn. Traps were closed during the day and the bait removed to reduce by-catch. Trapped bilbies were placed in black catch bags and processed at the trap site. Adult bilbies had a subcutaneous passive

integrated transponder (PIT tag) (Trovan, UK) inserted under the skin between the shoulder blades for individual recognition.

4.2.3 Spool-and-line tracking

Spool-and-line tracking was used to track bilbies to their diurnal burrows (Miles *et al.* 1981; Steinwald *et al.* 2006; Dawson *et al*. 2018). Immediately following processing, we attached a reverse-spun bobbinless cocoon made of 265m of 70-2 nylon quilting thread (Penguin Threads PTY LTD, Victoria, Australia). Cocoons were wrapped in electrical tape and the spool attached to the fur between the shoulder blades of bilbies using a super glue contact adhesive, with care taken to ensure the spool device was not glued directly to the skin. The interior thread of the spool was tied to the trap the bilby was captured in, after which the bilby was released. As the bilby moved around the site, the thread spooled out leaving a trail of where the bilby had travelled. Once the bilby reached the end of the spool line, the thread detached from the electrical tape which then fell off later. Individuals were released around 1-2 hours prior to sunrise.

Spool lines were followed at least two hours after sunrise on the day of release. It was assumed that a bilby was sheltering in a burrow that day when the spool line entered a burrow entrance and did not re-emerge. Characteristics recorded for each burrow used were: GPS location, aspect of main entrance the bilby went in to, number of burrow entrances (within a 5 metre radius of the burrow the bilby had entered), whether or not burrow entrances were under vegetation and whether the entrance had clearly visible tracks or signs of use. Burrows were assumed to be constructed by bilbies if the entrances were similar in size and shape to those described by Smyth and Philpott (1968) and Thompson and Thompson (2008), with an oval shaped entrance with a flattened base. If the spool line ended in the open, it was assumed that the line had run out prior to the bilby reaching a burrow and these observations were excluded from analyses.

4.2.4 Data analysis

Statistical analysis was performed using R version 3.3.1 (R Core Team 2018) and mean values are given in the format mean ± standard deviation, except in Table 1 where standard error is used for comparison with other studies. To determine patterns of burrow usage, a tracking history was compiled for each bilby tracked to a burrow during the study, consisting of the dates an individual was successfully spooled and the burrow it was found

to be sheltering in during the day. Burrows were given an identification number, and the date(s) each burrow was used and which individual bilbies used it were recorded. Kendall's Tau rank test for non-parametric data was used to test for a correlation between the number of nights a bilby was tracked over the study and the number of burrows used.

The spatial distribution of burrows that individuals used for daytime shelter over the entire study was investigated using two methods. These were (1) calculation of the standard distance of each bilby's burrow set, which is a measure of dispersion of the burrows used by an individual around the mean centre of distribution (Bachi 1963; Jensen and Jensen 2013), and (2) calculation of a minimum convex polygon using the burrow locations of each bilby. The R-package "*aspace"* version 3.2 (Buliung and Remmel 2008; Bui e*t al.* 2012) was used to calculate the standard distance for each individual's burrow set. Bilbies were each tracked to between one and 13 burrows during the study and data were used from bilbies that were tracked to burrows on a minimum of four separate occasions during the study. The standard distance of burrow sets was compared between male and female bilbies using the Welch two sample t-test for unequal variances.

Burrow range, defined as the area over which an individual bilby's burrows occurred, was calculated by generating 100 % Minimum Convex Polygons (MCPs) from spatial data on burrows used by each bilby. MCPs were calculated using the R-package '*adehabitatHR*' version 0.4.18 (Calenge 2006). The burrow ranges of males and females were compared using the Wilcoxon Rank Sum Test for non-parametric data.

The mean, median and angular variance of burrow entrance direction was calculated using the R-package 'circular' version 0.4-93 (Agostinelli and Lund 2017), which performs statistical techniques for circular data. In this case, the direction of burrow entrance was treated as circular data (directional) rather than linear. To test for departures from uniformity of the circular directional data, both the Rayleigh test was used in package 'circular' and an adaptation of Watson's U^2 test for grouped data was used, as described by Choulakian *et al.* (1994), with the method used to perform this in R described in Pewsey *et al.* (2013). For both of these tests, the null hypothesis was that the direction of burrow entrances was uniformly distributed in all directions, and a p-value of less than 0.05 indicated that a significant departure from a uniform distribution.

4.3 Results

4.3.1 Burrow use by individuals

Over a 12 month period, 43 adult greater bilbies at Venus Bay Conservation Park were tracked to 118 different burrows on 231 occasions. A further 100 spool and line tracking attempts failed because the spool line terminated before reaching a burrow entrance. This mainly occurred when bilbies were released more than one and a half hours before dawn, in which case the animals often spent time foraging prior to returning to their burrow.

Twenty-five bilbies (58%) were tracked to burrows on four or more occasions, and the maximum number of times bilbies were tracked over the study was 19 occasions. The largest number of different burrows a single bilby used was 13, which was by a male bilby tracked 19 times. A female bilby, also tracked 19 times, used eight different burrows. A statistically significant positive correlation occurred between the number of nights a bilby was tracked and the number of burrows used by both males (Kendall's tau = 0.89, n = 30, $P < 0.001$) and females (Kendall's tau = 0.89, n = 13 $P < 0.001$) (Fig. 4.2).

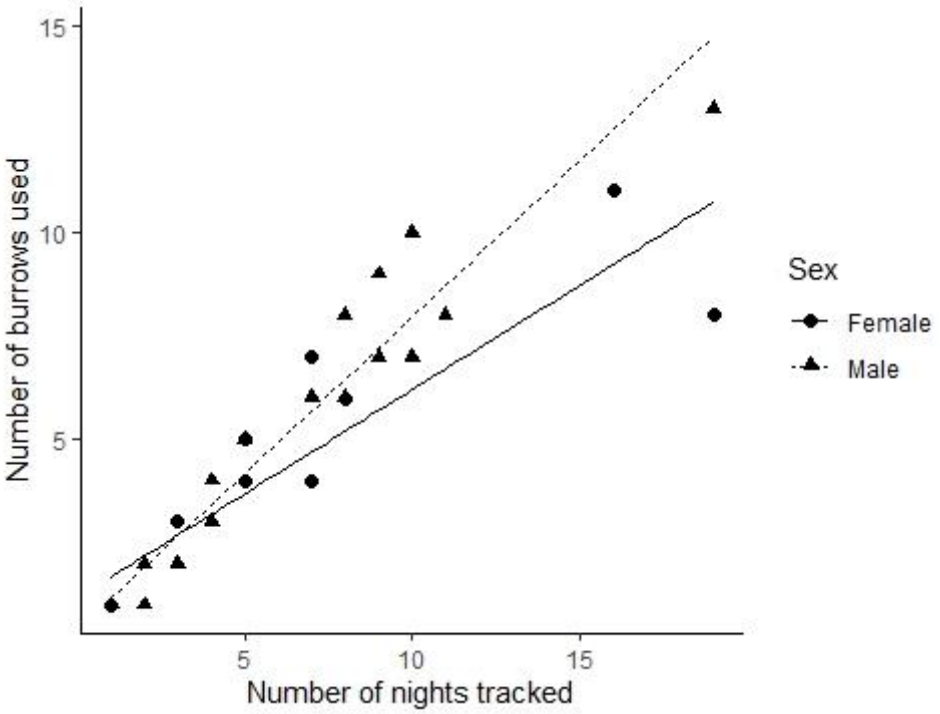


Figure 4.2 The relationship between the number of nights tracked and the number of burrows used by male and female bilbies.

On 36 occasions, bilbies were trapped and successfully tracked to their burrows on two consecutive days. In 34 instances (94%), the individual used two different burrows. Using data from the 34 occasions when bilbies used two different burrows on two consecutive nights, the mean distance between the two burrows was significantly greater for males (277 m ± 209 m, n = 24) than for females (97 m ± 84 m, n = 10) (Wilcoxon rank sum test, W = 40, P = 0.003) (Table 4.1). Over the entire study period, bilbies were recorded re-using the same burrows. Of the 33 bilbies tracked to their burrow on a minimum of two occasions, 19 (58%) were tracked to the same burrow more than once, and 21% of successful tracking attempts were to burrows that had been previously used by the individual being tracked.

4.3.2 Occurrence of burrow sharing

Tracking of bilbies revealed that individuals sometimes shared burrows on the same day and also used the same burrows as one another on different days. Sharing of a burrow by two bilbies on the same day was observed on seven occasions; in four of which two males were spooled to the same burrow, and on three occasions a male and a female were spooled to the same burrow. No records were obtained of two females sharing the same burrow on the same day.

Of the 118 burrows used by bilbies, 43 (36 %) had more than one bilby tracked to the same burrow over the study period (but on different nights). Twenty six burrows (22 %) had two or more males utilising them over the study period, but only two burrows (1.7 %) recorded use by more than one female.

4.3.3 Spatial distribution of burrows

The standard distance of burrow sets were calculated for seven female (140 ± 40 m) and 18 male bilbies (316 ± 147 m) which were tracked to their burrows on four or more occasions. Males had a significantly larger mean standard distance for burrow sets than did females (t = 4.65, d.f. = 21.81, P < 0.001). Data from five female and 12 male bilbies that were tracked to their burrows on five or more occasions were used to generate minimum convex polygons of burrow range. Male bilbies had a significantly greater mean burrow range (10.2 ± 5.8 ha) that was almost four times larger than that of females (2.6 ± 1.8 ha) (Wilcoxon Rank Sum Test, W = 9, P = 0.03) (Table 4.1). The relationship between burrow range and number of nights tracked was weak and not statistically significant for either

males (Kendall's tau = 0.32, n = 12, P = 0.16) or females (Kendall's tau = 0.2, n = 5, P = 0.82). The burrow range of male individuals generally overlapped with the burrow range of other males (Fig. 4.3). The burrow range of females tended to overlap with the burrow range of males, however there was very little overlap between the five females that burrow range data were generated for (Fig. 4.3).

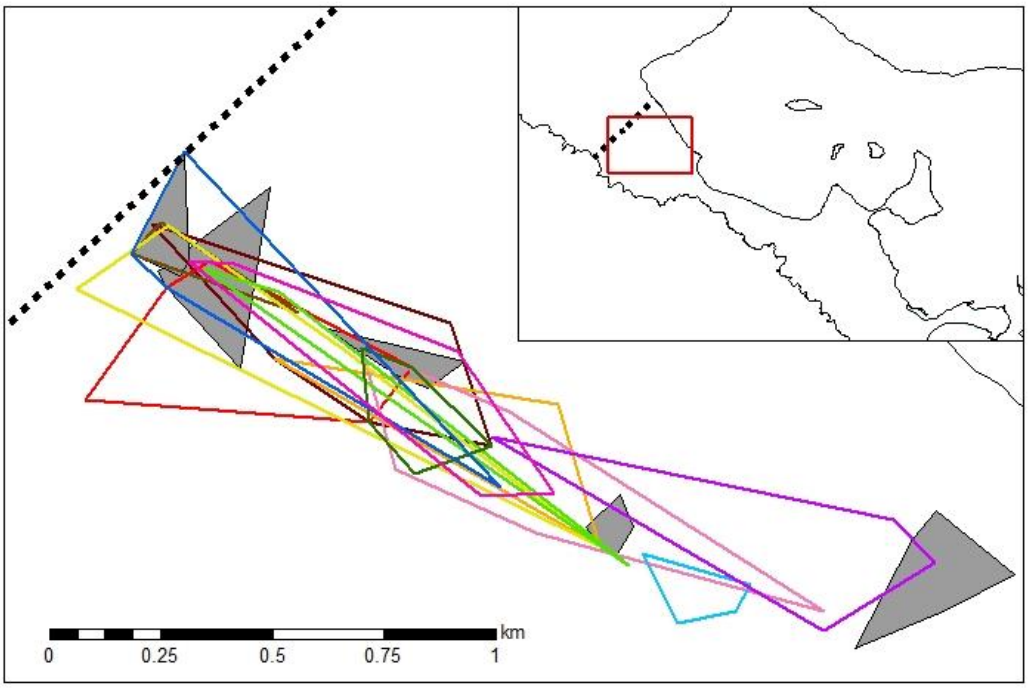


Figure 4.3 The burrow ranges for female (grey shaded polygons, N = 5) and male bilbies (coloured outline polygons, N = 12). Burrow range was calculated using minimum convex polygons. Dotted black line represents the predator exclusion fence. A further 26 bilbies that were trapped were unable to be tracked to burrows enough times to generate burrow ranges.

The 118 burrows recorded via spool-and-line tracking occurred over a total area of 204 ha. Based on the used burrows found through spool-and-line tracking alone, the density of bilby burrows presumed to be constructed by bilbies (excluding five that were thought to be old rabbit warrens) was 0.55 burrows per ha over the area where the burrows occurred. This is 2.75 to 5 times the density of bilbies recorded at the site during the study period.

Table 4.1 The mean (± standard error) burrow range for bilbies at Venus Bay Conservation Park and distances between diurnal burrows used on successive nights for this study and other recent studies. Home range size of bilbies in other areas of Australia is also included. Burrow range and home range was calculated using minimum convex polygons in all studies.

Site	Average annual rainfall (mm)	Burrow range (ha)		Home range (ha)		Distance between successive burrows (m)		Source
		Male	Female	Male	Female	Distance	Group	
Venus Bay	370	10.2 ± 1.7	2.6 ± 0.8	-	-	277 ± 43	Males	This study
						97 ± 27	Females	
SW Qld	200	15.3 ± 4.6	3.0 ± 0.8	51 ± 1	20 ± 1	435 ± 41	Males	McRae 2004
						188 ± 28	Females	
Northern SA	166	-	-	316 ± 128	18 ± 4	-	-	Moseby and O'Donnell 2003
Northern SA	166	-	-	-	-	235 ± 49	Mixed sex control	Moseby *et al.* 2012
						172 ± 27	Mixed sex predator trained	

4.3.4 Burrow characteristics

Most burrows (65 %) used by bilbies had either one or two entrances (Fig. 4.4). Five burrows, all with five or more entrances, appeared to be old rabbit warrens since old rabbit scats were evident around the burrows and they also had a warren-like structure. Burrow entrances were located under low shrubby vegetation (mostly *Melaleuca lanceolata*) in 86 % of burrows, with those under vegetation often difficult to observe except by crawling under the vegetation (Fig. 4.5). All burrows suspected of being originally constructed by rabbits (N = 5) had entrances in the open, and a further 11 burrows that were assumed to be constructed by bilbies had all entrances in the open (not under vegetation). Most (72 %) burrow entrances at Venus Bay used by bilbies faced in a north, north-east or east direction (Fig. 4.6). Tests of uniformity rejected the hypothesis that burrow entrance direction was randomly distributed in all directions (Rayleigh test statistic = 0.45, P < 0.001; Omnibus test for grouped data, test statistic U_G^2 = 1.17, P < 0.001).

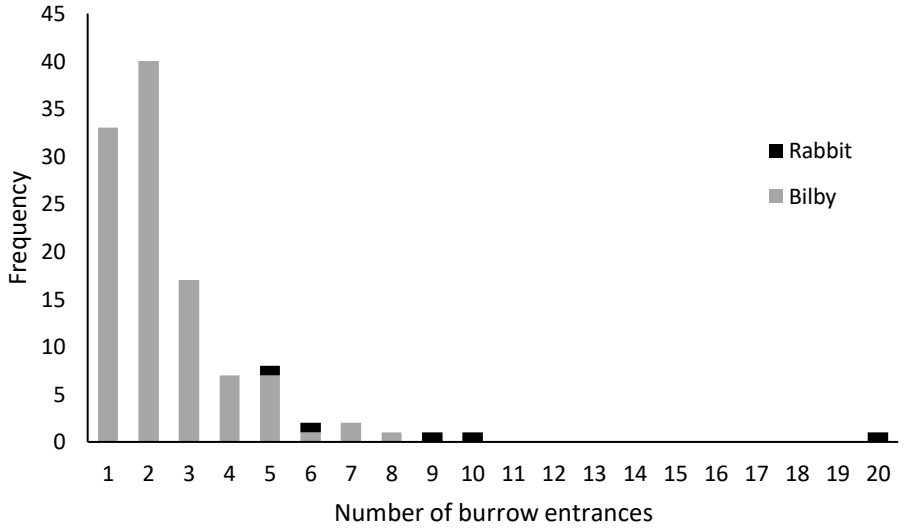


Figure 4.4 The number of entrances detected for bilby burrows at Venus Bay Conservation Park (n = 113). Burrows that only had evidence of bilby occupation are grey, and burrows with old rabbit scats in the entrances that were suspected of being old rabbit warrens are black.



Figure 4.5 The entrance to a bilby burrow at Venus Bay Conservation Park circled in red. Note the mound of fresh sand outside the entrance to the burrow.

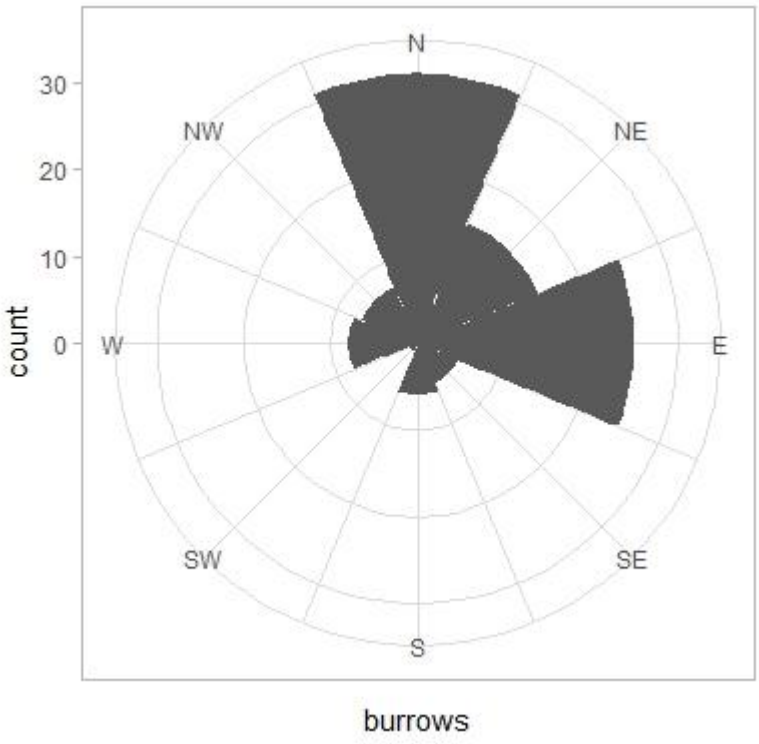


Figure 4.6 Rose diagram of the direction burrow entrances were facing, based on the entrance the bilby was observed using through spool-and-line tracking.

All burrows had mounds of sand (the dominant soil substrate at the site) outside one entrance, usually the one that was entered by the bilby, and all burrows except one had visible bilby tracks at the entrance (Fig. 4.5). The one burrow that did not was probably an old rabbit warren, and old rabbit scat and grass debris present at the entrance to the burrow made it difficult to see tracks.

4.4 Discussion

The present study is the first investigation on burrow use behaviour of bilbies when exposed to a temperate climate. Due to the extinction of bilbies from much of their southern range around 100 years ago, very little is known about their behaviour in temperate habitats. Our study demonstrates that translocated populations provide an opportunity to generate basic knowledge of species biology and behaviour, and to determine whether variation exists across climatic zones.

At Venus Bay Conservation Park, bilbies used multiple diurnal burrows within their home range. There was a significant positive relationship between the number of times bilbies were tracked and the number of burrows they were recorded using. In contrast, burrow

range did not significantly increase with the number of times each individual bilby was tracked. This result indicates that over time bilbies may use or construct new burrows, but burrow range remains relatively stable. Our results also indicate that bilbies are mostly solitary, but will occasionally shelter in a burrow with another bilby. Burrows were often used by more than one bilby, but individuals tended to use the same burrows at different times. These results are consistent with behaviour that has been observed in arid zone populations in Northern Territory, Queensland and/or northern South Australia (Southgate and Possingham 1995; Lavery and Kirkpatrick 1997; Moseby and O'Donnell 2003; McRae 2004). The burrow range stability observed at our site and in northern South Australia (Moseby e*t al.* 2003) could indicate that both sites had adequate food resources over time to support continuous occupation. It could also have been a consequence of limited dispersal opportunities, given that both our study as well as the one in northern South Australia occurred in fenced conservation reserves.

Our results show that the mean burrow range of male bilbies was significantly larger than that of females. Males also travelled further between consecutive diurnal burrows than did females, and males' burrow ranges tended to overlap with the burrow range of multiple other males and females. Burrow range and distances travelled between consecutive diurnal burrows did not differ greatly from what has been reported in arid zone populations (Table 4.1). However in our study, the burrow ranges of males overlapped considerably with other males and females. In the arid zone, home ranges of males were found to overlap in another fenced population (Moseby and O'Donnell 2003), however, in a wild population in south-west Queensland burrow ranges had minimal overlap (McRae 2004). Our results suggest that in a temperate environment where resources are more concentrated, bilbies do not necessarily reduce their individual range, but rather share their range with more individuals in higher density situations.

Male bilbies have been recorded moving larger distances than females in the arid zone in both wild-born and reintroduced populations (Southgate and Possingham 1995; Moseby and O'Donnell 2003; McRae 2004). This behaviour could be a strategy that males adopt to find mates, as males with larger home ranges are more likely to intercept more females. Previous studies have suggested the mating system of the bilby is likely to be either polygynous or promiscuous (Moritz *et al.* 1997; Miller *et al.* 2010a), thus males can mate with several females in a breeding season. In order to access multiple females, males need

home ranges that encompass those of several individual females. Therefore, the larger burrow range of males at our site indicates a similar mating system could be operating at the Venus Bay site to that of the other populations that have been studied.

Given that there are high energetic costs associated with the construction of burrows (Gibson and Hume 2004), there are likely to be benefits to creating and using multiple burrows. Bilbies in our study were observed moving between diurnal burrows over two consecutive days. It is possible that bilbies were moving burrows in response to being handled, and/or due to our presence close to their burrow the next day when following spool-lines. However, McRae (2004) also recorded bilbies moving burrows regularly with radio-tracked bilbies that were not captured regularly. Other possible reasons for frequently moving burrows could be thermal differences between burrows, reducing parasite load, access to food resources and mates, or to avoid predation. Moseby and O'Donnell (2003) postulated that female bilbies may also move burrows when weaning juveniles.

Bilbies have been recorded frequently moving burrows, modifying their burrow emergence behaviour and seeking the shelter of shrubs more often in response to predator exposure (Moseby e*t al*. 2012; Steindler e*t al.* 2018; Ross *et al.* 2019). Early observations of bilbies in South Australia suggest they rapidly retreated to their burrows to avoid predation (Wood Jones 1924). Feral cats were present at Venus Bay Conservation Park during our study, and were likely to be a predation risk to bilbies (Paltridge 2002; Moseby *et al.* 2015; Berris *et al.* 2020). For prey animals like bilbies, there is a trade-off between avoiding predation and meeting other needs such as obtaining ample food (Lima and Dill 1990). Remaining in one place for a long period of time leads to an accumulation of waste scent, and an increase in predation risk by predators that use scent to detect prey (Banks *et al.* 2002). However, staying close to refuges while foraging has also been associated with decreased predation risk (Blumstein 1998). The tendency of bilbies at our site to position burrows under shrubs, maintain multiple burrows and to regularly move diurnal burrows could be a retained predator avoidance behaviour and/or a response to the presence of feral cats at the site.

Our results suggest that in places where the bilby was once abundant, bilby burrows were a common landscape feature. The density of bilbies at our study site was between 11 and 20 bilbies per km^2 during the study period (Berris e*t al*. 2020) and the density of burrows

was 2.75 to 5 times the density of bilbies. The loss of the bilby and other burrowing species from many locations in Australia may have had implications for other species that co-existed with burrowing species (Fleming e*t al.* 2014). In a tropical monsoonal climate, 45 species of mammal, bird and reptile have been recorded as interacting with bilby burrows (Dawson *et al.* 2019). Similarly, in both the arid zone and temperate environments of Australia, a wide variety of mammal, bird and reptile species have been recorded using the burrows of other species (Wood Jones 1924; Copley 1999; Haby *et al.* 2013; Ostendorf *et al.* 2016; Hofstede and Dziminski 2017; Thornett *et al.* 2017). Bilby burrows provide a refuge from environmental extremes, and have less variable temperature and humidity fluctuations than control microclimates outside burrows (Chapman 2013). It is also possible that in fire-prone environments such as southern Australia, burrows were an important refuge during bushfires (Newsome *et al.* 1975; Long 2009). The loss of bilby burrows from temperate Australia may have reduced other species access to shelter from extreme temperatures, predation and bushfires, making them more vulnerable to these events. An area of future research could be to conduct camera trap studies on bilby burrows at this site to determine what other species are using bilby burrows. If bilby burrows are an important refuge for other temperate zone species, translocations of bilbies to southern Australia could be both beneficial for the conservation of bilbies as well as for that of other native species.

All but one burrow that bilbies sheltered in during the day were easy to identify as bilby burrows, as they had loose soil and fresh bilby tracks just outside the entrance to the burrow. However, the majority of burrows that bilbies were spooled to at Venus Bay were located near the trunk of shrubby vegetation, making them difficult to see as the burrow entrance and sand mound outside the front of the burrow were often under the vegetation. This is likely the reason that so few burrows were observed in areas of remnant vegetation at this site prior to this study (K. Berris, personal observation). Bilby burrows at the Arid Recovery Reserve have also been recorded mostly beneath trees and shrubs (Moseby and O'Donnell 2003). Numerous studies have used "active" burrows, defined as those with fresh soil and bilby tracks at the entrance, to indicate bilby occupancy (McRae 2004; Thompson and Thompson 2008; Burrows *et al.* 2012). Our study suggests that ground or aerial surveys for bilby burrows may not be possible at sites with thick bushy vegetation such as Venus Bay Conservation Park, and could lead to false negatives if solely relied upon. Surveys in open sandy areas for tracks are likely to be a more suitable detection method

for bilbies than burrow surveys in these environments, as detailed by Southgate *et al.* (2019).

It is not known why bilbies at Venus Bay tended to construct their burrows so that the main entrance was facing north, north-east or east. In an arid zone study, burrow orientation was found to be random, even in different seasons (Moseby and O'Donnell 2003). The Venus Bay study site generally slopes gently to the south. Strong winds during the summer months often come from the south-west in southern South Australia, however, prevailing winds are from the west, north-west, north and north-east in winter (Colls and Whitaker 2012). In winter, burrows with a northerly aspect would also receive more sunlight at the entrance to the burrow. Possible reasons for facing burrow entrances in a certain direction could be to take advantage of the natural wind patterns or sunlight to maximise microclimatic benefits in burrows, or possibly to maximise scent detection of predators when bilbies emerge from burrows. Bilbies have been found to alter their burrow emergence behaviour in response to predator odour near the entrance of burrows (Steindler *et al.* 2018), therefore scent detection of predators is likely an important predator avoidance strategy. An area of interesting future research could be to determine the effect of wind direction and burrow orientation on both burrow microclimatic conditions and scent detection in bilbies emerging from burrows.

A limitation of this study is that data are based on a translocated bilby population, the founders of which were from a captive population of bilbies originally derived from Western Australia and Northern Territory (Bradley *et al.* 2015). Therefore caution is needed in the interpretation of the results, as it is not known whether bilbies that historically occurred at the site differed behaviourally or genetically to those of the current population. There were also two main limitations of using the spool-and-line tracking technique, the first being that bilbies could only be tracked on days when they were captured the previous night, limiting the amount of data we could collect. The second was that spools were only 265 metres long, and bilbies are known to move further than 265 metres from diurnal burrows while foraging at night (Moseby and O'Donnell 2003). Therefore the burrows we found are likely to only be a subset of all burrows used by individuals at the site, and burrow ranges reported are likely to be underestimates of the true burrow range of individuals. We were unable to calculate a burrow range for approximately 60 % of the individuals we trapped, because those individuals were not spooled to burrows on enough occasions.

Therefore burrow range overlap at the site is also likely higher than we reported. Our methods detected burrows actively being used by bilbies during the study period, but did not include unoccupied or disused bilby burrows, which are a common feature where bilbies occur in the arid zone (Smyth and Philpott 1968; Lavery and Kirkpatrick 1997; Burrows *et al.* 2012). Despite these limitations, our study supports the notion that burrows were likely to have been a common landscape feature across the southern parts of the bilbies range prior to their local extinction.

4.4.1 Conclusions

The translocated population of bilbies at Venus Bay Conservation Park used multiple burrows within their home range and regularly moved between diurnal burrows. Male bilbies had a significantly larger burrow range than did females, and burrow range was similar to what has been reported in the arid zone. The density of burrows was 2.75 to 5.0 times the density of bilbies recorded at the site and there was significant overlap in the burrow ranges of males. Our results suggest burrows were a common landscape feature in the southern parts of the bilbies range prior to their decline. Further research is needed to determine the impact of the loss of bilby burrows from southern Australia on other burrow commensal species.

Chapter 5: The mating system of the greater bilby (Macrotis lagotis) in two translocated populations and implications for ongoing management



Two bilbies captured occupying the same burrow.
Photo by Karleah Berris

"Not more than thirty years since it was usual for rabbit trappers, even in the immediate neighbourhood of Adelaide, to take more Bilbies … than rabbits in their traps."

Frederic Wood Jones, 1924, from 1968 reprint. A description of how common bilbies were in the Adelaide region prior to their extinction.

Statement of Authorship

Title of Paper	The mating system of the greater bilby (*Macrotis lagotis*) in two translocated populations and implications for ongoing management
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☑ Unpublished and Unsubmitted work written in manuscript style

Principal Author

Name of Principal Author (Candidate)	Karleah K. Berris		
Contribution to the Paper	Designed project, performed field work and collected data, performed data analysis, interpreted analyses and wrote and edited manuscript.		
Overall percentage (%)	80 %		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	11/08/2024

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Steven J. B. Cooper		
Contribution to the Paper	Advised on design of project, advised on methodology, advised on data analysis, advised on data analysis methodology, advised on interpretation of analyses, assisted with manuscript preparation, commented on manuscript drafts.		
Signature		Date	25/7/2024

Name of Co-Author	Amanda McLean		
Contribution to the Paper	Performed laboratory work, interpreted output from laboratory work and compiled raw genetic data.		
Signature		Date	31/08/2024

Name of Co-Author	William G. Breed		
Contribution to the Paper	Advised on design of project, advised on interpretation of analyses, assisted with manuscript preparation, commented on manuscript drafts.		
Signature		Date	25/7/2024

Name of Co-Author	Susan M. Carthew		
Contribution to the Paper	Advised on design of project, advised on interpretation of analyses, assisted with manuscript preparation, commented on manuscript drafts.		
Signature		Date	20/08/2024

Abstract

Knowledge of the mating system of a species, and the factors that influence individual reproductive success, are important for captive breeding, translocation and genetic rescue programs. In this study we investigated the genetic diversity and mating system of the greater bilby (*Macrotis lagotis*) in two free-ranging translocated populations in the temperate zone of South Australia, one at Venus Bay Conservation Park and the other on Thistle Island. In both locations, male and female bilbies bred with multiple partners in a breeding season, however twin litters probably had single paternity. In both populations, nearly all females bred, whereas only a maximum of 20% of the males trapped had sired offspring. A small number of males in the two populations dominated the breeding in the areas they were trapped. Body mass and relative testes size of males were poor predictors of reproductive success. Relatedness values of breeding pairs was lower than expected by chance in the Venus Bay population. Fewer alleles per locus were recorded in the Venus Bay population compared to other studies and the expected heterozygosity was significantly lower in the Venus Bay and Thistle Island populations compared to published data from a wild Northern Territory population. However, further investigation of the genetic diversity within both remnant and translocated populations using contemporary samples is recommended. Our results suggest that female-biased release groups would increase the initial rate of increase in a translocated population and that translocated males should come from genetically dissimilar populations to increase their likelihood of breeding. Both the Venus Bay Conservation Park and Thistle Island bilby populations will likely require supplementation with individuals from other sites as part of their long-term management to improve genetic diversity.

5.1 Introduction

Mammalian mating systems may be either monogamous, polygynous, polyandrous or promiscuous/polygynandrous (Emlen and Oring 1977; Clutton-Brock 1989). In Australian marsupials, polygynous, polyandrous and polygynandrous mating systems are common, in which one or both of the sexes have an opportunity to increase their reproductive success through mating with multiple partners in a breeding season (Emlen and Orring 1977; Croft 2001). Female marsupials generally provide most, or all, of the parental care, by raising their pouch young on a diet consisting exclusively of maternal milk (Croft 2001). In species where females assume most, or all, of the responsibility for caring for young, they often

have fewer opportunities to reproduce than males, which may cause them to be selective in their choice of mates. This can result in variation in reproductive success amongst males (Kokko and Jennions 2003). In recent decades, advances in genetic techniques have facilitated a growing body of research on marsupials into the factors that influence male reproductive success and female mate choice (Miller *et al.* 2010b; Pope *et al*. 2012; Gill *et al.* 2023).

Knowledge of the mating system of a species and the factors that influence individual reproductive success are important in captive breeding and translocation situations. Translocations, particularly to islands and fenced reserves, are important in mammal conservation programs in Australia (Moseby *et al.* 2011a; Taggart *et al.* 2016; Thavornkanlapachai *et al.* 2021), but the number of individuals in founding groups is often small and sourced from captive populations (Short 2009, Legge *et al.* 2018). Captive breeding success can be enhanced by taking female mate choice into consideration (Hartnett *et al.* 2018; Parrott *et al.* 2019). The mating system of a species and the species traits that influence male reproductive success may be taken into consideration when planning translocation release groups to maximise the reproductive success of the founder population and hence increase initial effective population size (Sigg *et al.* 2005). Since populations within fenced reserves and on offshore islands are isolated, they may require supplementation with individuals from other populations to maintain or enhance the genetic diversity of the population (White *et al.* 2020b; Pacioni *et al.* 2020). It is possible that the efficacy of genetic rescue programs could also be increased if individuals for translocation are selected based on traits known to maximise breeding success.

The greater bilby (*Macrotis lagotis*) is a medium sized marsupial species that underwent a significant range contraction following European settlement (Southgate 1990a). It is now classified as vulnerable to extinction and, in recent decades, captive populations have been established at zoos and wildlife sanctuaries throughout Australia, with free-ranging populations re-established through translocations to at least seven fenced reserves and an offshore island (Bradley *et al.* 2015). However, the current management of bilby populations has led to the occurrence of significant genetic structuring between some of the captive populations (Lott *et al.* 2020). This has led to genetic clusters in captive and translocated populations over time, with some alleles that are rare in one population being fixed at high frequencies in another despite founding individuals being sourced from the

same populations (Lott e*t al.* 2020). Some populations of bilbies reintroduced to fenced reserves have also experienced a loss of genetic diversity over time (Lott *et al.* 2020). This situation can be improved through admixing individuals from other populations, and the genetic diversity of several captive bilby populations has been maintained over time through such an approach (Miller *et al*. 2015). However, it is possible that admixing could potentially be more successful if female mate preferences are taken into account.

Bilbies have been translocated to two sites in the temperate zone of South Australia (Berris *et al.* 2020). In contrast to arid zone populations studied to date, these populations exist at high densities and breed seasonally (Berris *et al.* 2019, 2020). The mating systems of bilbies at these two temperate zone sites have not yet been investigated. Given differences between the timing of breeding and spatial organisation of bilbies at these sites compared to those in more arid regions, there may be differences in the mating system between populations.

Currently, there is very little known about the mating system of bilbies and the factors that influence reproductive success. Results from a study using samples from 27 bilbies from a wild population in south-west Queensland suggested that the species has a polygynous mating system with a single male dominating the breeding with females in an area (Moritz *et al.* 1997). A subsequent study in a captive population in south-west Western Australia that lived within a 20 hectare enclosure concluded that a promiscuous mating system was operating, and that paternity was not monopolised by a small number of males (Miller *et al*. 2010a). However, the captive population studied was confined to a small area, was provided with supplementary food, and large males had been periodically removed from the population for reintroduction into the wild (Miller *et al*. 2010a). In both studies, the mating system was investigated by assigning maternity and paternity to young individuals that had left the pouch (Moritz *et al.* 1997; Miller *et al*. 2010a). However, where maternal identity can be definitively assigned (such as when marsupial pouch young are sampled when still with their mother), the types of analyses available are more varied and parentage analysis can be more powerful (Jones *et al*. 2010).

Male reproductive success in some other species of Australian marsupials has been linked to factors such as male body mass (Holleley *et al.* 2006; Miller *et al.* 2010b), genetic relatedness between pairs (Glen *et al.* 2009; Parrott *et al.* 2015) and home range overlap

of males with females (Brown *et al.* 2007). Male bilbies have a relatively small testes mass compared to body mass (Johnston *et al*. 2010), and in other marsupial species a small relative testis mass has been associated with single male breeding systems such as monogamy or polygyny (Rose *et al*. 1997). Although a previous study on a captive bilby population found no evidence of increased reproductive success in larger males (Miller *et al.* 2010a), the mating system warrants investigation in a free-ranging continuous population. Moreover, social organisation in the wild can be variable; the sole mating system study on wild bilbies occurred at an arid zone site where bilbies were clustered in the landscape in colonies (Moritz *et al*. 1997), while studies of bilby ecology in southern Australia have recorded more continuous populations with large overlap in male ranges (Berris *et al.* 2020, 2022). Female mate choice, and relatedness of male partners, has not yet been investigated in bilbies. However this could be important given the social organisation of populations differs between sites. Additional studies within free-ranging populations and in different environments are required to resolve the mating system of this species and to effectively manage captive and translocated populations.

The aim of this study was to elucidate the mating system within two free-ranging translocated bilby populations in the temperate zone, and to determine what factors influence the reproductive success of males. We set out to answer the following questions:

i. How does genetic diversity, based on microsatellite markers, in the two translocated populations compare to published results of wild and captive populations?
ii. What is the mating system of bilbies in these two temperate zone translocated populations? Is there support for polygynous or polygynandrous mating systems in these populations?
iii. Does annual reproductive success vary between males and females?
iv. Do male physical traits that are easily measured in the field, such as body size and testes size, influence male reproductive success?
v. Is there any evidence for multiple paternity in twin litters of bilbies?
vi. Does male - female relatedness influence female mate choice?

We further discuss whether information obtained through this study of the mating system of bilbies has implications for translocations.

5.2 Materials and Methods

Tissue samples were obtained from 160 adult bilbies and 84 pouch young from two South Australian populations, one at Venus Bay Conservation Park (33.1796°S, 134.5572°E) (hereafter referred to as Venus Bay) and the second on Thistle Island (35.0096°S, 136.1704°E). Both these sites occur in a temperate climatic zone (Colls and Whitaker 2012) and bilbies were observed breeding seasonally at both sites, with most births occurring in winter, spring or early summer (Berris *et al.* 2019). Female bilbies have eight teats in their pouch and commonly have one or two young in a litter, with occasional litters of three young recorded (Southgate *et al.* 2000; Berris *et al.* 2019). Studies of wild and translocated populations of the greater bilby have shown that individuals are mostly solitary, males generally have a greater body mass than females and that males have much larger home ranges than females (Moseby and O'Donnell 2003; McRae 2004; Berris *et al*. 2022). At Venus Bay, 23 captive-bred bilbies were released between 2001 and 2005 to a 1460 hectare part of the park protected by a predator exclusion fence (Department for Environment and Heritage 2006; Bradley *et al.* 2015). At the time of sampling, bilbies were only able to be trapped in the 640 hectare northern part of the park, and a declining population trend was found to occur, which likely reflected the impact of feral cat predation on the population (Berris *et al.* 2020). Samples were obtained from this free-ranging population during 10 field surveys of 10-21 days duration carried out between June 2008 and April 2010. On Thistle Island, which is 3926 hectares in size and free of exotic predators, 21 bilbies were released between 1997 and 2003 (Bradley *et al*. 2015). Since then, the population of bilbies increased, with densities of up to 101 bilbies per km^2 recorded and bilbies present across the entire island (Berris *et al.* 2020). Here, samples were obtained during four field surveys of 7-14 days duration carried out between June 2009 and January 2010.

5.2.1 Trapping and sampling protocol

Bilbies were captured using medium - sized cage traps (minimum size 60cm x 29cm x 25cm) baited with peanut butter and oats. Traps were placed in transects of 5-20 traps spaced 100 metres apart. The same trap lines were sampled during each trip with the aim of recapturing females to sample multiple pouch young litters, and to sample as many of the potential fathers of pouch young in the area as possible. Traps were placed under shrubby vegetation so that only the entrance to the trap was visible. They were opened in the late

afternoon and checked between 0200 hours and 0500 hours when weather conditions were mild. In cool or wet weather, traps were also checked prior to midnight so bilbies were not left in traps for long periods of time in inclement weather. Traps were closed during the day and the bait removed to reduce by-catch and prevent ants frequenting traps.

When a bilby was captured, it was placed in a black fabric catch bag, processed adjacent to the trap, and then released. Independent bilbies had their head length (mm), pes length (mm), body mass (g) and testes size (mm) measured and had a subcutaneous passive integrated transponder (PIT) tag (Trovan, Douglas, Isle of Man) inserted between the shoulder blades so they could be individually identified on subsequent trips. Adults had a 2mm by 4mm ear tissue biopsy taken from the edge of their ear with surgical scissors, that was then stored in a vial of 100% ethanol. Females were checked for the presence of pouch young, and if present, pouch young also had a 1-2 mm^2 skin biopsy taken either from the outer edge of their ear or from the very tip of their tail. If pouch young were less than 5 days of age and/or too small to obtain a skin tissue sample, the mother and pouch young were released without the sample being taken. Efforts were made to recapture the mother at a later date for sampling when the pouch young was slightly larger and a tissue sample of adequate size could be obtained.

5.2.2 Genotyping and Microsatellite screening

Genomic DNA was extracted from tissue samples using the Gentra Puregene extraction kit with the procedures specified by the manufacturer (Gentra Systems Inc., USA). Each individual bilby was screened at 11 polymorphic microsatellite loci (Bil02, Bil16, Bil17, Bil22, Bil41, Bil55, Bil56, Bil63, Bil66, ML1 and ML2). All the microsatellite loci with the prefix “Bil” have been described by Moritz *et al.* (1997). ML1 and ML2 were characterised by Amanda McLean using methods identical to those given in McLean *et al.* (2014) and Gardner *et al.* (2011). Primers were designed spanning microsatellite markers which were identified from partial 454 shot gun sequence data generated from a single bilby on a Titanium GS-FLX platform at the Australian Genome Research Facility (Brisbane, Australia). For a description of the loci and the primers used see Appendix 1-3.

PCR amplifications were conducted in a final volume of 15 µl containing approximately 10 ng of template DNA, a fluorescently labelled forward locus-specific primer (VIC, FAM, NED or PET) an unlabelled reverse locus-specific primer (see Appendix 1 for primer sequences, fluorescent tags and amounts used in each PCR multiplex), 0.15 U Immolase DNA polymerase (Bioline, Luckenwalde, Germany) and 1 X MRT buffer (Hayden *et al*. 2008). PCR amplifications were performed using the Multiplex Ready Technology (MRT) method (Hayden *et al*. 2008) with three separate multiplex PCR panels, with Panel 1 comprising the loci Bil16 (NED), Bil17(FAM), Bil22 (VIC), Bil41 (PET), Bil55 (FAM), Panel 2 comprising Bil02 (FAM), Bil56 (VIC), Bil63 (NED), Bil66 (PET) and Panel 3 comprising ML1 (VIC) and ML2 (FAM). All loci were amplified using a PCR program with an initial 10 minute denaturation at 95°C, then 5 cycles with 94°C for 15 sec, an annealing temperature starting at 58°C and decreasing by 1°C each cycle for 30 sec and 72°C for 45 seconds. The second phase consisted of 35 cycles of the following sequence: 94°C for 15 seconds, 50°C for 30 seconds, 72°C for 45 seconds. This was followed by a final extension of 30 minutes at 72°C. PCR panels were genotyped on an ABI 3730 DNA Analyser (Australian Genome Resource Facility), alleles were scored using GeneMapper® v 3.7 (Applied Biosystems), and 10 % of randomly selected samples were repeated to assess errors in scoring.

The typing error rates per locus and per allele were determined according to methods outlined by Pompanon *et al.* (2005), to derive the mean error rate across all loci and alleles. These error rates were used as a parameter in the paternity analysis. The program Micro-Checker (Van Oosterhout *et al.* 2004) was used to check loci for evidence of null alleles, scoring error due to stuttering and for allelic dropout. The program CERVUS version 3.0.7 (Kalinowski *et al.* 2007) was used to assess deviations from Hardy-Weinberg equilibrium and to perform allele frequency analysis using only adult individuals. Locus Bil66 deviated from Hardy-Weinberg equilibrium in both populations, therefore was excluded from calculations of genetic diversity and parentage analysis, reducing the total number of loci used to 10. The mean number of alleles per locus, observed heterozygosity (H_O) and expected heterozygosity (H_E) were then calculated for both populations. The program FSTAT version 2.9.4 (Goudet 1995) was then used to calculate the genetic variation in populations relative to the total genetic variance (F_{st}), the inbreeding coefficient for each population (F_{is}), the allelic richness (N_{AR}), and to perform tests for linkage disequilibrium between pairs of loci. The Holm-Bonferroni Sequential Correction was then used to generate corrected P-values for multiple hypothesis tests.

5.2.3 Genetic diversity

Allelic richness was compared between the Venus Bay and Thistle Island populations using a t-test. The number of alleles per locus, H_O and H_E was recalculated using just the nine "Bil" loci described by Moritz *et al.* (1997) for the Venus Bay and Thistle Island populations and compared to published data on these genetic diversity measures from other populations for the same loci. Data were obtained from Moritz *et al.* (1997), including summary information on samples from a wild Northern Territory (NT) bilby population and wild Queensland (QLD) population. Further data were obtained from Miller *et al.* (2015), which provided genetic diversity measures using the same loci for two Western Australian (WA) captive populations. Data for the nine "Bil" loci from all six populations were compared using an analysis of variance for H_O, and a Kruskal-Wallis rank sum test with a post-hoc Dunn's test to identify pairwise differences for H_E (nonparametric tests). Loci were excluded for a population if they deviated from Hardy-Weinberg equilibrium for that population: BIL66 for Venus Bay, Thistle Island, WA colony 1, WA captive colony 2; BIL63 for QLD population; BIL17 failed to amplify for both WA captive colonies (Moritz *et al.* 1997; Miller *et al.* 2015).

5.2.4 Parentage analysis

CERVUS version 3.0.7 (Kalinowski *et al.* 2007) and COLONY version 2.0.6.5 (Jones and Wang 2010) were both used to assign paternity to offspring. CERVUS determines the most likely sire of each pouch young by calculating a likelihood ratio for each candidate sire, whereas COLONY implements full-pedigree likelihood methods to infer sibship and parentage of sampled young (Jones and Wang 2010). We used both programs to increase confidence of our assigned paternities. For both analyses, it was assumed that mothers were known, as young were sampled while in the pouch. For the input parameters, it was also assumed that the probability of the true father of a pouch young being one of the candidate fathers was 0.9 at Venus Bay and 0.7 on Thistle Island, which was estimated based on capture probabilities and the number of new captures recorded each trapping session (Berris *et al.* 2020). We also assumed that males and females could breed with multiple partners. All males captured at the same site where females were captured with pouch young were included as a candidate sire in the analysis. CERVUS calculates LOD scores, which are the natural log of the overall likelihood ratio, for each candidate sire and the known mother of each pouch young. The candidate sire with the largest LOD score then had the confidence

of that score evaluated. Any assigned fathers with 2 or more allelic mismatches were excluded as potential fathers, due to the low likelihood of this occurring. Paternity results from CERVUS were then compared to assigned paternities by COLONY.

Paternity was assigned to pouch young if it was assigned with ≥ 95 % confidence in CERVUS or COLONY, or if it was assigned as the most likely candidate father by both programs with a likelihood of ≥ 80 % confidence by at least one program. To determine which males we could confidently assume had not sired any of the pouch young with unassigned paternities, we generated confidence tables of LOD scores in CERVUS for exclusion of males. We used a confidence level of 80% for assignment of non-sires. Using the confidence table we determined that when 10 loci were used, any males with LOD score of < 1.5 at Venus Bay and < 3.3 at Thistle Island could be eliminated as a potential sire with 80% confidence. When six loci were used, LOD scores of < 0.8 at Venus Bay and < 1.4 at Thistle Island were used to eliminate potential sires. Males with two or more mismatches at the trio level were also excluded as potential sires due to the low likelihood of two allelic mismatches via new mutations. All other males with LOD scores in excess of those values were assumed to be potential sires of those unassigned pouch young. Males trapped at each site were then all classified as sires, potential sires or non-sires.

5.2.5 Mating system

Statistical analyses were performed using R (version 4.0.0) (R Core Team 2020) unless otherwise stated. Mean values are given as the mean ± standard deviation. The parents of each litter, as determined by direct observation for females and parentage analysis for males, were compared. The number of females each male bred with and the number of litters sired by each male were determined for two breeding seasons at Venus Bay and one breeding season on Thistle Island. We calculated the percentage of males that were confirmed breeding in a given breeding season through parentage analysis and the percentage of males that were potential sires and non-sires. To determine the percentage of females that bred in a breeding season, we used the number of females recorded with pouch young or lactating and the total number of females trapped regularly throughout that breeding season.

5.2.6 Male reproductive success

To determine if any easily measurable traits of males influenced breeding success, we only compared males that were assigned paternity (sires) with those that were not a potential sire of any of the unassigned pouch young (non-sires) in the 10 loci analysis. The relationship between male body traits (body mass and relative testes size) and reproductive success of males (dependent variable, sired young: 1, did not sire young: 0) was analysed using binomial generalised linear mixed models for data from Venus Bay (GLMM; family = binomial) with year as a random effect, and generalised linear models for data from Thistle Island (GLM; family = binomial). Both analyses were conducted using the approach described by Grueber *et al.* (2011). Input variables were standardised using the r-package *arm* version 1.12-2 (Gelman and Su 2021) as recommended by Gelman (2008). A model set was generated that represented all possible combinations of predictor variables included in the global model using the r-package *MuMIn* version 1.43.17 (Barton 2020). We ranked models using the Akaike Information Criterion corrected for small sample sizes (AICc) and Akaike weights (ω). Models with a ΔAICC < 2 (top model set) were used to calculate model-averaged estimates for the fixed effects.

5.2.7 Relatedness between individuals

To determine whether female mate choice was influenced by relatedness to males, and to determine the relatedness between sibling pouch young, the pairwise relatedness of individuals were estimated using the R-package 'related' (Pew *et al.* 2015). Four estimators, which are described by Li *et al.* (1993), Lynch and Ritland (1999), Queller and Goodnight (1989) and Wang (2002), were compared using a simulated dataset of genotypes of known relatedness with the same locus characteristics as our dataset. A simulated dataset was generated for each population to take into account different allele frequencies between the sites. Simulated datasets comprised 100 simulated pairs for each of four different relatedness classifications; parent-offspring (PO), full-sibling (FS), half-sibling (HS) and unrelated (UR). Relatedness values were then calculated in 'related' using each of the four estimators and a correlation coefficient was obtained by comparing these values to expected relatedness values (PO = 0.5, FS = 0.5, HS = 0.25 and UR = 0.0). The Wang (2002) relatedness estimator had the highest correlation coefficient, and hence was used for subsequent pairwise relatedness analysis. Pairwise relatedness of all possible adult male and female pairs was calculated based on our genotype dataset for each site. To determine

if females preferentially bred with unrelated males, the relatedness of male-female breeding pairs was compared to the relatedness of randomly selected male-female pairs using a t-test. Random selection was ensured by giving each possible pair a number, then using a random number generator to select pairs for the analysis to compare to breeding pairs.

To determine the relatedness of siblings within the same litters, and whether there was evidence for multiple paternity in twin bilby litters, 25 simulated relatedness values were generated for each relatedness category for each site (as described above). The relatedness values of simulated datasets based on allele frequencies at each site were compared to the relatedness values of twin siblings from our samples at each site to determine whether our sample values were most similar to simulated full-sibling or half-sibling values. We compared relatedness values amongst groups using an analysis of variance (ANOVA) and Tukey post hoc testing for the Venus Bay site, and using a Kruskal-Wallis test with a Dunn's test of multiple comparisons for Thistle Island (non-parametric). We also compared the relatedness values of pairs of pouch young siblings to paternity results generated in CERVUS and COLONY.

5.3 Results

At the Venus Bay site, 10 field surveys of 10-21 days in length were conducted between July 2008 and April 2010. Over this period, genetic samples were collected from 53 pouch young (15 single pouch young, 19 sets of twins) from 13 adult female bilbies. Females had samples collected from between 1-5 of their litters over two breeding seasons. In the same trapping area, 40 adult male bilbies were captured, and were therefore considered to be potential fathers. At the Thistle Island site, four field surveys of 7 -14 days in length were conducted between July 2009 and February 2010. At this site, genetic samples were collected from 31 pouch young (17 single litters, 7 sets of twins) from 18 adult females, with six of those females having samples collected from two consecutive litters. A further 57 adult male bilbies were trapped in the same areas as these females, and were considered potential fathers.

5.3.1 Genetic diversity measures

The mean error rate per locus was calculated as 0.045 and the mean error rate per allele was 0.0325. The data from locus Bil66 deviated from Hardy-Weinberg equilibrium and had

a high expected frequency of null alleles in both the Venus Bay and Thistle Island populations, therefore it was excluded from further analyses. There was no evidence of null alleles, scoring error due to stuttering or allelic dropout in the remaining 10 loci. In the Venus Bay population, the 10 remaining loci had between three and seven alleles and an expected heterozygosity (H_E) of between 0.452 and 0.783 (Appendix 2). In the Thistle Island population, the 10 loci had between three and eight alleles and an expected heterozygosity (H_E) of between 0.393 and 0.818 (Appendix 3).

Mean alleles per locus (N_A), allelic richness (N_{AR}), H_E and observed heterozygosity (H_O) were all higher in the Thistle Island population than the Venus Bay population (Table 5.1). Both populations had a negative F_{IS} value, therefore there was no evidence of inbreeding relative to expectations based on random mating with the observed allele frequencies in either population (Table 5.1). The genetic distance (F_{ST}) between the two populations was 0.1081 (P = 0.05), indicating moderate genetic differentiation between the sites. Six of the 45 pairwise tests for linkage disequilibrium had significant P-values. It was assumed for parentage analysis that loci were independent, however linkage disequilibrium can be heavily influenced by past population history, such as population bottlenecks, periods of small population size and range expansion (Slatkin 2008; Lucek and Willi 2021). Our detection of linkage disequilibrium may have been influenced by the small translocation founder groups at the sites, and linked loci have not been reported in previous studies using these loci (Miller *et al.* 2010b). Therefore, we performed parentage analysis using both the full set of 10 loci, and with only six loci so no pairs of loci used had significant values for linkage disequilibrium (excluding loci Bil16, Bil17, Bil22 and Bil63).

Table 5.1 Mean genetic diversity measures for greater bilby populations at Venus Bay Conservation Park and Thistle Island derived from all 10 loci used for parentage analysis. Includes the number of alleles (N_A), allelic richness (N_{AR}), observed heterozygosity (H_O), expected heterozygosity (H_E) and inbreeding coefficient (F_{IS}).

Population	No. of individuals	N_A	N_{AR}	H_O	H_E	F_{IS}
Venus Bay	69	4.9	5.98	0.690	0.645	-0.072
Thistle Island	91	6.1	6.45	0.749	0.725	-0.034

When genetic diversity measures were recalculated using just the "Bil" loci to enable comparison with other studies (see Appendix 4 for values), both Venus Bay and Thistle Island had a significantly lower H_E than the wild Northern Territory population (Fig. 5.1; Venus Bay z = 3.32, P < 0.001; Thistle Island z = 1.98, P < 0.02). Although N_{AR}, H_O and H_E were lower in the Venus Bay population than the Thistle Island population, this difference was not significant (N_{AR}: t = -0.77, df = 17.87, P = 0.45; H_O: $F_{5,41}$ = 1.33, P = 0.27; H_E: z = 1.30, P = 0.10). Allelic richness was not able to be calculated for the other studies based on the available published data, however the mean number of alleles per locus recorded was lower at Venus Bay and Thistle Island compared to the wild Northern Territory and Queensland populations, despite the relatively small number of individuals sampled in those wild populations (Appendix 4).

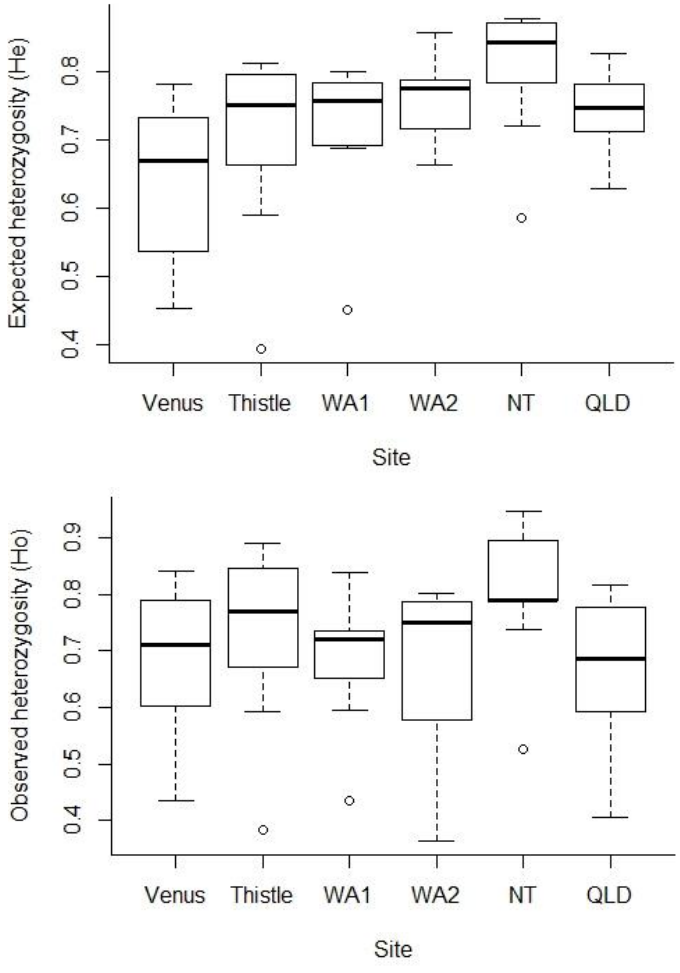


Figure 5.1 Mean genetic diversity measures of bilbies using just the "Bil" loci used in previous studies for Venus Bay (Venus, n = 69) and Thistle Island (Thistle, n = 91). Comparison data were obtained for two Western Australian captive colonies (WA1, n = 216; WA2, n = 266; data from Miller et al. 2015) and samples from a wild Northern Territory population (NT, n = 19; data from Moritz *et al.* 1997) and a wild Queensland

population (QLD, n = 27; data from Moritz *et al.* 1997). Genetic diversity measures are observed heterozygosity (*Ho*) and expected heterozygosity (*He*).

5.3.2 Parentage analysis

For pouch young sampled at Venus Bay, paternity could be assigned to 40 pouch young (75% of those sampled) from 27 different litters when all 10 loci were used for analysis. For pouch young sampled on Thistle Island, 22 pouch young (71% of those sampled) from 17 different litters were assigned paternity using 10 loci. When the parentage analysis was run with six loci, 24 pouch young (45%) from 19 litters at Venus Bay were assigned paternity, and 10 pouch young (32%) from 9 litters at Thistle Island were assigned paternity. The probability of non-exclusion of an unrelated candidate sire in CERVUS when the mother was known was 0.005 for the Venus Bay population and 0.0006 for the Thistle Island population when all 10 loci were used. When six loci were used, the probability of non-exclusion was 0.02 for the Venus Bay population and 0.008 for the Thistle Island population. Parentage assignments are given for each pouch young in Appendix 7 (six loci) and Appendix 8 (10 loci).

5.3.3 Mating system

Of the 40 candidate fathers trapped at Venus Bay, 13 were assigned paternity of pouch young when 10 loci were used for analysis, and seven of those males were still assigned paternity of pouch young when six loci were used. One male was assigned paternity in two breeding seasons, but only with the 10 loci analysis, and all other males were assigned paternity in only one of the two breeding seasons (Table 5.2). One male was recorded fathering pouch young from six different litters with five different females within one breeding season (M18, Table 5.2). A further 15 males were excluded from fathering any of the sampled pouch young from two breeding seasons in both analyses, and 12 males were potential sires of unassigned pouch young (Appendix 7 and Appendix 8). Five out of seven males that were assigned paternity of pouch young in the six loci analysis, and six out of 14 males assigned paternity in the 10 loci analysis were recorded breeding with more than one female in a breeding season (Table 5.2). The 10 loci analysis indicated seven females bred with more than one male within a single breeding season and two females bred with the same male twice in a breeding season (Table 5.2). Seventeen pouch young that had paternity assigned in the 10 loci analysis also had the same sire identified as the most likely

(> 80%) or a potential sire in the 6 loci analysis. One paternity assigned in the 6 loci analysis had that sire discounted in the 10 loci analysis.

Table 5.2 Paternity of greater bilby pouch young litters based on microsatellite markers during two breeding seasons at Venus Bay Conservation Park. Litters from the 2008/09 breeding season are represented by a black dot (●) if assigned in both the six and 10 loci analysis and a red dot (●) if assigned only in the 10 loci analysis. Litters from the 2009/10 breeding season are represented with a black diamond (♦) if assigned in both the six and 10 loci analysis, and a red diamond (♦) if only assigned in the 10 loci analysis.

Female ID	Male ID													Unassigned litters
	M08	M12	M18	M19	M26	M27	M28	M31	M35	M39	M44	M55	M58	
F04	●			●										
F09			♦				♦							●
F10						●			●●	♦	♦			
F11													●●	●
F13		●	♦				♦						●	
F23							●●	●						
F25											♦			●●♦
F32									●					
F37													●	
F41														●
F47			♦♦											
F50			♦		♦						♦			
F57			♦									♦		

At Thistle Island, of the 57 candidate sires captured within the same area as females with pouch young, only three were assigned paternity when analysis was performed with six loci, whereas a further five males were assigned paternity in the 10 loci analysis (Table 5.3).

All three males assigned paternity of pouch young in the 6 loci analysis sired two or more litters of young (Table 5.3). Four females had paternity assigned to more than one of their litters in the 10 loci analysis, two of which had the same male sire both of their litters that season and the other two had different males sire their litters (Table 5.3). Twelve pouch young that had paternity assigned in the 10 loci analysis also had the same sire identified as the most likely (> 80%) or a potential sire in the 6 loci analysis. One pouch young that had paternity assigned in the 6 loci analysis, had that sire discounted and a different sire assigned paternity in the 10 loci analysis.

Table 5.3 Paternity of Thistle Island greater bilby pouch young litters from 2009/10 based on microsatellite markers. Litters are represented by a black dot (●) if assigned in both the six and 10 loci analysis and a red dot (●) if assigned only in the 10 loci analysis.

Female ID	Male ID								Unassigned litters
	M097	M142	M147	M150	M160	M168	M189	M193	
F027								●	
F040									●
F095						●			●
F096									●
F111					●●				
F114						●●			
F120		●							
F122	●		●						
F131				●					
F134									●
F137	●								
F143				●					
F146									●
F156									●●
F164						●	●		
F166					●				
F167	●								
F181	●								

5.3.4 Proportion of the population breeding each season

The proportion of captured males that were confirmed to have sired pouch young litters within a breeding season was between 5% and 20% depending on the site and whether the results were from the six or 10 loci paternity analysis (Table 5.4). This was much lower than the proportion of females trapped that were confirmed breeding within a given breeding season at both sites (Table 5.4). Between 47.5 % and 81 % of males trapped at sites were highly unlikely to have sired any of the pouch young sampled in the area (80% confidence; Table 5.4). There was a significant association between sex and breeding status within a breeding season (confirmed breeding, confirmed not producing pouch young) for both Thistle Island ('N-1' $\chi^2 = 39.67$, $P < 0.0001$) and Venus Bay (2008/09: ('N-1' $\chi^2 = 22.50$, $P < 0.0001$; 2009/10: $\chi^2 = 18.97$, $P < 0.0001$), with females captured more likely to produce young in a breeding season than males captured.

5.3.5 Male breeding success and body traits

In our dataset, the male traits of body mass and relative testes size were not good predictors of male reproductive success. The highest ranked model in GLMM analysis for Venus Bay contained body mass. However it only performed marginally better than the null model. Model averaged estimates of the effect of body mass were not significant (Appendix 5 and Appendix 6). For Thistle Island, three of the four models generated in GLM analysis had a $\Delta AICc < 2$, one of which included the null model. Similarly for this site, although body mass and relative testes size were featured in some of the top models, their estimates of effect size in model average estimates were not significant (Appendix 5 and Appendix 6). However, due to the small number of males that were assigned paternity at both sites, these results must be interpreted with caution.

Table 5.4 The total number of male and female greater bilbies captured at each site and in each breeding season, and the percentage of males and females that bred, had uncertain breeding status (females = not captured regularly enough to ascertain breeding for the whole breeding season; males = potential sire of a pouch young with unassigned paternity) or were confirmed to not be parents of sampled pouch young. Male breeding status is based on paternity assignments and results are provided for both the six loci and 10 loci analysis (in brackets). Females were recorded as breeding if they were observed with pouch young or had lactating teats during the breeding season.

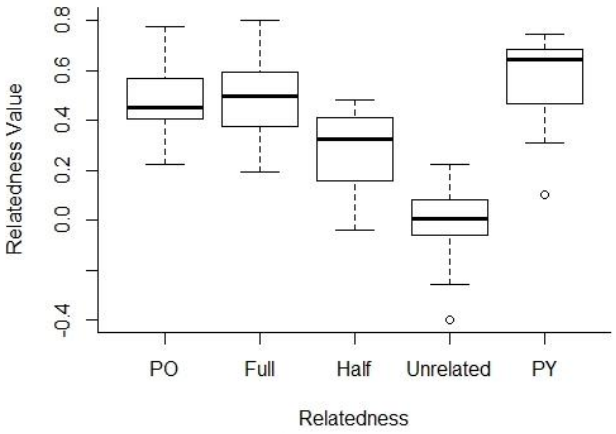
Sex	Site	Breeding season	Individuals trapped	Percentage that produced young	Percentage uncertain	Percentage that did not produce young
Male	Venus Bay C.P.	2008/09	40	10% (20%)	42.5% (33%)	47.5% (60%)
Male	Venus Bay C.P.	2009/10	40	7.5% (15%)	20% (22.5%)	72.5% (62.5 %)
Male	Thistle Island	2009/10	57	5% (14%)	19% (5%)	75% (81%)
Female	Venus Bay C.P.	2008/09	17	88%	12%	0%
Female	Venus Bay C.P.	2009/10	9	89%	11%	0%
Female	Thistle Island	2009/10	29	72%	21%	7%

5.3.6 Paternity of siblings within litters

A one-way analysis of variance and subsequent Tukey post hoc analysis indicated that the mean relatedness values for twin pouch young sampled at Venus Bay were significantly higher than the mean relatedness values for simulated half-sibling and unrelated pairs, but did not differ significantly from full-sibling pairs (Figure 5.2; $F_{(4,94)} = 37.48$, $p = <0.001$). A non-parametric Kruskal-Wallis test and Dunn's test of multiple comparisons similarly showed the mean twin pouch young relatedness values from Thistle Island were not significantly different from simulated full-sibling pairs, but were significantly different from the mean relatedness values of half-sibling and unrelated pairs (Figure 5.2; $H_{(4)} = 52.21$, $p < 0.001$).

Thirteen of the 19 relatedness values for twins from Venus Bay litters were within the interquartile range of full-sibling values simulated using allele frequencies at the site. A further six were within the interquartile range of simulated half-sibling values, however five of these pairs had paternity assigned to both pouch young which indicated the same male sired both. When the relatedness simulation was run to produce 100 pairs per relatedness group for each site, it was ascertained that all relatedness values obtained for our sampled twin litters at Venus Bay were within the range of possible full-sibling simulated values. For Thistle Island twin siblings, relatedness values for five of seven twins were within the interquartile range for full-siblings, one twin pair was within the interquartile range of half-siblings and one pair had relatedness values similar to unrelated individuals and was not within the range of simulated values for full-siblings for the 100 pairs per group simulation (Figure 5.2). However, both the pair with the half-sibling and unrelated relatedness values were assigned paternity to both twins in the litters during parentage analysis, which indicated single paternity of both of these litters. All of the 18 litters from the two sites that had paternity assigned to both twins in a litter were sired by the same father.

a)



b)

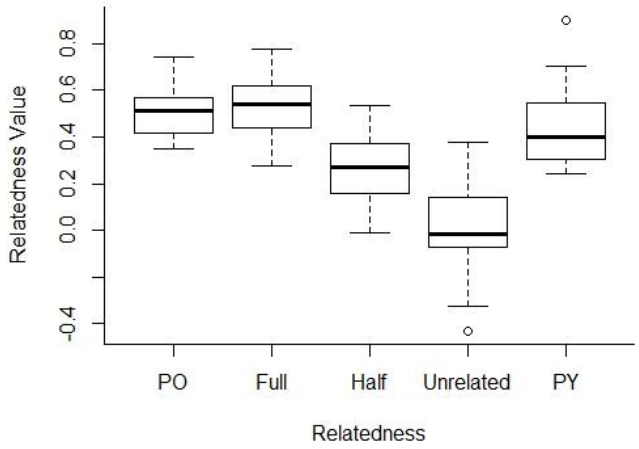


Figure 5.2. Relatedness values of samples from twin pouch young (PY) and simulated values for relatedness based on our data for parent-offspring pairs (PO), full-siblings (Full), half-siblings (Half) and unrelated individuals (Unrelated) from a) Venus Bay, and b) Thistle Island.

5.3.7 Relatedness of known breeding pairs

The mean relatedness values of breeding pairs of bilbies at Venus Bay were significantly lower than the relatedness values of randomly selected male-female pairs (Breeding pairs = 0.03 ± 0.23, n = 24; Random pairs = 0.18 ± 0.24, n = 24; t-test t = -2.10, df = 45.99, p = 0.04). However, the mean relatedness of breeding pairs at Thistle Island did not differ significantly from the mean relatedness of randomly selected male-female pairs (Breeding pairs = -0.02 ± 0.23, n = 15; Random pairs = -0.002 ± 0.22, n = 15; t-test t = -0.20, df = 27.92, p = 0.84). The mean relatedness of the randomly selected pairs differed between sites, and was lower for Thistle Island compared to Venus Bay (t = 2.37, df = 31.31, p-value = 0.02).

5.3 Discussion

The present study is the first to investigate the mating system in free-ranging populations of the greater bilby using genetic samples from both adults and pouch young with known maternity. It is also the first such analysis from temperate zone populations. Our results suggest that in two relatively continuous populations in the temperate zone, the mating system is polygynandrous. There was evidence that males who sired young mated with multiple females in a breeding season, and females who produced multiple litters in a breeding season could have different sires for different litters. Both six and 10 loci analyses concluded that a small number of males were responsible for siring a large proportion of the pouch young at our sites. Interestingly, our study showed that many males we captured did not sire any of the pouch young we sampled in the areas they were trapped, whereas most females in our populations were recorded breeding. These results have important implications for translocation scenarios, particularly when translocating individuals to established populations with the aim of improving genetic diversity.

A single male at our Venus Bay study site was recorded siring six litters with five different females in one breeding season. By contrast between 47.5 % and 81% of males we trapped at our sites did not sire any of the pouch young we sampled that females at those sites were carrying. Our findings are similar to results from a study in an arid zone population of wild bilbies in south-west Queensland, where a single male dominated breeding in the study area (Moritz e*t al.* 1997). This is despite the differences in climate and spatial distribution of bilbies at our sites compared to those in the arid zone. Our study occurred in the temperate zone, where there is regular winter rainfall and reintroduced bilby populations occupied the landscape more-or-less continuously and in high densities (Berris e*t al.* 2020). The south-west Queensland areas where a similar mating system was recorded experiences low and irregular rainfall, with bilby populations showing spatial clumping of individuals in the landscape (Moritz *et al.* 1997; McRae 2004; Colls and Whitaker 2012). Our results suggest that even in high-density continuous bilby populations such as on Thistle Island, small numbers of males are able to dominate the breeding opportunities in an area.

Size was not a reliable predictor of male reproductive success in our study, which is similar to findings in a captive populations of bilbies (Miller et al. 2010a). A dominant breeding male in an arid zone study on wild bilbies was found to roam large distances each night (Moritz *et al.* 1997), however we did not have enough data on the home range of breeding

and non-breeding males to investigate this further. In our study, we detected that breeding pairs at the Venus Bay site had a lower mean relatedness value than a random sample of male-female pairs from the site. Therefore, our results also suggest that female mate choice may, in part, be driven by relatedness when there are many related males in the area. Females breeding with genetically dissimilar males has also been recorded in other Australian marsupial species, including the agile antechinus (*Antechinus agilis*), spotted-tailed quoll (*Dasyurus maculatus*) and mountain brushtail possum (*Trichosurus cunninghami*) (Glen *et al.* 2009; Parrott *et al.* 2015; Blyton e*t al.* 2016). Our observation of females selecting relatively unrelated males in a less genetically diverse population has also been recorded in agile antechinus (Banks *et al.* 2005). Furthermore, in Cunningham's skinks (*Egernia cunninghami*) lower relatedness amongst breeding pairs compared to potential pairs, as in our study, was considered evidence of active avoidance of breeding between related individuals (Stow and Sunnucks 2004). Our results indicate that assessing genetic similarity of populations and sourcing males from unrelated populations for both founder groups and population supplementation may improve males' chances of successfully breeding and contributing to a population. However, to fully understand female mate choice in this species, further work is required to assess the impact of other factors on male reproductive success that we were unable to explore, such as home range size and male home range overlap with females.

In terms of implications for translocations, our results suggest that most females reliably produce young each breeding season. As females can also produce multiple litters per year (Berris *et al.* 2019), it could be more cost-effective to release new females into established populations for the purpose of genetic rescue rather than males. It could also be beneficial for translocation founder groups to have a female-bias, which could increase the initial rate of increase of a population compared to a 50:50 sex ratio. Given that very few males at our study sites sired sampled pouch young in the area, the number of breeding females is more likely to be a limiting factor in initial population growth than the number of males. However, our results also show that if a male does successfully breed, he has the potential to reproduce with multiple females over a breeding season.

The Venus Bay bilby population had fewer alleles per loci recorded compared to two wild populations and a captive colony, and both Venus Bay and Thistle Island had a significantly lower expected heterozygosity compared to the wild Northern Territory population (Moritz

et al. 1997; Miller *et al.* 2015). This occurred despite our dataset containing samples from far more individuals than the wild populations. Our data suggest that the Venus Bay and Thistle Island populations may have lower genetic diversity than their wild counterparts. However, further analysis of allelic richness using genotype data from all the populations would be required to verify this. Venus Bay also had lower genetic diversity measures, including allelic richness, than Thistle Island, but none of the differences were significant. There were several differences between the two populations we studied; the Venus Bay population was confined to a smaller area and had a declining population trend due to impacts from feral cat predation (Berris *et al.* 2020). In contrast, the Thistle Island population recorded high bilby densities, and had expanded post-release to occupy the entire island which was more than twice the size of the Venus Bay fenced area (Berris *et al.* 2020). Small populations are more likely to have lower genetic diversity (Frankham 1996), and the predation pressures that were keeping the Venus Bay population small and restricted could have been impacting the genetic diversity at this site. We could not assess whether genetic diversity had declined over time at our two sites, as samples were not available from founder individuals. However, in bilbies and other Australian marsupials translocated to fenced reserves, supplementation with new individuals has been necessary to maintain genetic diversity (Miller e*t al.* 2015; White *et al.* 2018). Founding individuals for the Monarto Zoo captive colony came from Western Australian and Northern Territory wild populations (Lott e*t al.* 2020), which was the source of the two translocation founding groups at our sites. Given the populations we studied are isolated and our data suggest they may be exhibiting lower genetic diversity than their wild source populations, it is recommended these sites are supplemented with individuals from other sites as part of their ongoing management.

Results from both paternity analysis and analysis of relatedness between twin pouch young indicate that both pouch young in twin litters are most likely full-siblings. However, given our small sample sizes, and the overlapping simulated relatedness values of full-siblings and half-siblings at our sites, these results must be interpreted with caution. Adult bilbies are generally solitary, have a small relative testes size/mass, have sexual size dimorphism and large overlapping male home ranges (Moseby and O'Donnell 2003; McRae 2004; Berris e*t al.* 2019; Berris *et al.* 2022). Sexual size dimorphism and overlapping male home ranges have been associated with polygynous mating systems (Emlen and Oring 1977; Weckerly 1998; McPherson and Chenoweth 2012). Small relative testes mass in marsupial species is

more commonly associated with a mating system where the females only mate with a single male during their fertile period (Rose *et al.* 1997). The species traits of the bilby predict that males would mate with multiple females, but that females would not mate with multiple partners in a single fertile period. Our study generally supports this prediction, given that our results using relatedness values and parentage analysis both suggest the twin litters we sampled probably each had only a single sire.

A limitation of our study was that we used a relatively small number of microsatellite loci for our paternity and genetic diversity analysis. However, by using the same microsatellite loci as previous studies on the bilby, we were able to directly compare these loci at our sites to both wild and captive bilby populations using published data from Moritz *et al.* (1997) and Miller *et al.* (2015). Microsatellite markers are also effective when dealing with very small genetic samples or samples that will yield low levels of DNA, such as those obtained from pouch young or from scat sampling. However, in order to undertake a better comparison of genetic diversity, it would be preferable to have obtained the genotypes of individuals from the studies by Moritz *et al.* (1997) and Miller e*t al.* (2015) so that allelic richness, accounting for sample size using a rarefaction approach, could have been calculated for all sites rather than just our two study sites. Another limitation was that we were comparing genetic measures from populations from different time periods, and in particular, the wild population samples from NT and QLD came from collections that occurred prior to 1997 (Moritz *et al.* 1997). It has been almost 30 years since these samples were collected, and in that time remnant bilby populations may have undergone further changes in their size and distribution. In recent years, next-generation sequencing technologies have enabled researchers to use single nucleotide polymorphisms (SNPs) to sample large numbers of loci for relatively less cost than traditional microsatellite techniques (Schlötterer 2004). Although microsatellites and SNPs appear to be similarly powerful for parentage analysis, SNPs may give more precise genetic diversity estimates compared to microsatellites, particularly for population-level diversity (Flanagan and Jones 2019; Zimmerman *et al.* 2020). Some work on this has commenced; the most recent genetic study on bilbies collected samples mostly from translocated populations however did include samples from some wild populations (Hogg *et al.* 2024). However, for the wild populations sample sizes were small, in some cases less than six individuals, therefore the results must be interpreted with caution (Hogg *et al.* 2024). A renewed sampling effort of current wild populations across the bilbies extant range would be beneficial for

understanding the status of bilbies across Australia and the management needs of the different populations.

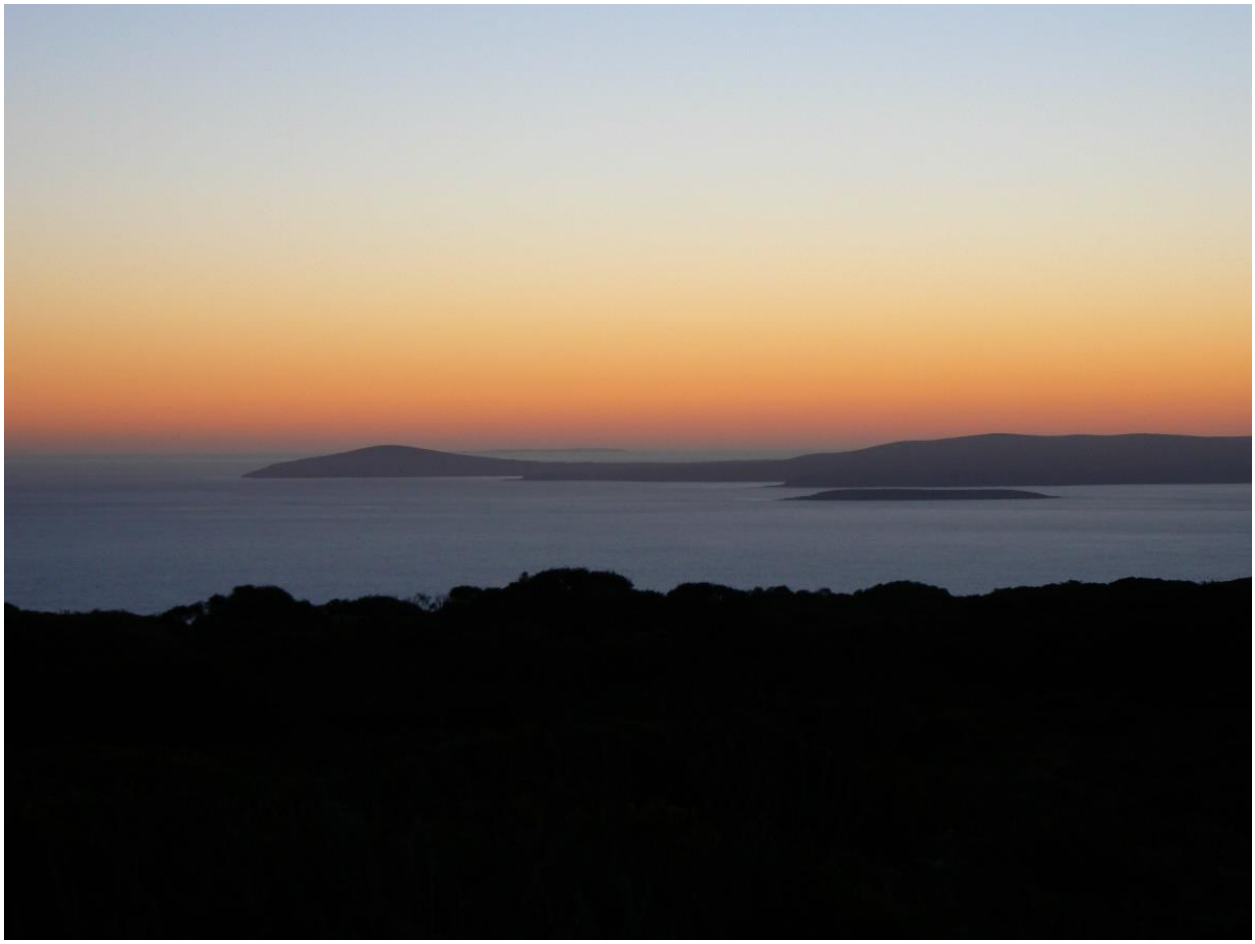
Further limitations of our study are the sample sizes for some of the analyses. Because so few males were recorded breeding, analysis involving male traits such as body mass and relative testes size had only small sample sizes for animals categorised as sires. We also were not able to trap all the bilbies at our sites despite our best efforts. This constrained the number of pouch young that were able to be assigned paternity. There were several pouch young that did not have any males at the 6 or 10 loci analysis that could be assigned paternity at a reasonable level of confidence, and this suggests their actual sire had not been captured. We are also not sure whether the pouch young we sampled were all from single sire litters, although in most cases that appears to be most likely the case. Relatedness values for full-siblings and half-siblings overlapped substantially, making relatedness values somewhat problematic for confirming single or multiple paternity of litters.

5.4.1 Conclusion

Our results provide evidence for a polygynandrous mating system in the two bilby populations we studied that were translocated to temperate zone sites in South Australia. Small numbers of males were found to be dominating breeding in an area, and a large proportion of males were not recorded siring any of the pouch young we sampled. Although the physical traits of body mass and relative testes size were poor predictors of breeding success in males, mean relatedness between breeding pairs was lower than expected at the Venus Bay site. Our results suggest that female-biased founder groups should be considered for new translocations to increase the initial population rate of increase. If males are being translocated to an established population, we recommend sourcing them from genetically dissimilar populations to improve the likelihood of them breeding. Our two translocated study populations recorded fewer alleles per locus and lower expected heterozygosity compared to the small number of samples collected from a wild Northern Territory population. The Venus Bay population had the lowest mean number of alleles and expected heterozygosity of all populations we compared. However, further analysis using the full genotypes from all sites is required to verify differences in genetic diversity. We recommend that further investigation of the genetic diversity of bilbies across their range and translocated sites is conducted, and that the two South

Australian temperate zone bilby populations are supplemented with new individuals from other sites as part of a long-term management plan to improve their genetic diversity.

Chapter 6: General Discussion



Sunset on Thistle Island

Photo by Karleah Berris

"In general it may be said that the Peramelidae are rapidly disappearing over the whole of continental Australia. Although formerly they were very common and familiar animals, they have now disappeared from many of their old haunts, and even though they are still locally abundant in certain spots, their number as a whole have vastly diminished in a comparatively few years."

Frederic Wood Jones, 1924. From 1968 reprint. Even 100 years ago, the dire state of bilby and bandicoot populations within the state was already evident.

6.1 Scope of this research

This discussion chapter reviews the main findings of the research presented in my thesis, and discusses the implications of these findings for conservation of the greater bilby (*Macrotis lagotis*), which is listed as Vulnerable to extinction under the *Environment Protection and Biodiversity Conservation Act 1999.* My research contributes new knowledge on the ecology of the bilby, particularly to the fields of reproductive ecology, population dynamics and behaviour. It also contributes to the field of translocation ecology, and details the response of an ecologically flexible species translocated to habitats which are very different from where remnant populations still occur.

Prior to this study, there was no published research on the ecology or behaviour of bilbies in the temperate areas of its former range. The greater bilby once occurred throughout many parts of temperate South Australia and more broadly across southern Australia. However, its rapid decline led to its extinction in South Australia sometime around the 1930's (Kemper 1990). Translocations of bilbies to two sites in the temperate zone of South Australia have facilitated an investigation into their ecology in an environment in which they were once common. The two study sites at Venus Bay Conservation Park and Thistle Island also enabled a comparison into how different threats may impact on their translocation success at these sites. It has also helped to determine what factors contribute to successful translocations of this species, and added to our knowledge about what threats may have contributed to their original decline.

Initially it was hoped that an arid zone translocated population at Arid Recovery Reserve in South Australia could be included as a comparison site to the temperate sites. Unfortunately, the field work for this project coincided with a long period of very low rainfall in northern South Australia, and a two week field trip to Arid Recovery resulted in no captures of bilbies, only a handful of foot prints were detected, and only a single bilby was observed when spotlighting. Over the following 18 months, staff at the reserve continued to observe few bilbies, making further field work at this site likely to yield few, if any, captures. Footprint monitoring of reintroduced species at the reserve also documented the low bilby abundance over our field data collection period of 2008-2010 (Moseby *et al.* 2011a). Therefore, comparative data could not be obtained on this population. However, in lieu of obtaining comparative data from an arid zone site, effort was made to review the literature on bilby ecology in the arid zone and to compare the

current findings with previous published research, all of which occurred in the arid zone or on captive populations.

My research demonstrates that an ecologically flexible species, such as the bilby, can be translocated to sites where historical habitat disturbance has occurred, and has an ability to adapt their reproductive activity to suit local conditions. My research also supports the view that introduced predators are a key threat to the persistence of bilbies in the wild. In addition, my research demonstrates how valuable translocated populations can be for improving our understanding of the ecology of threatened species, particularly medium-sized marsupials which have declined significantly and have few remnant wild populations left.

In the following discussion sections below, I outline my findings on the ecology of the bilby, and review how this new knowledge may contribute to our understanding of how best to conserve this species and improve translocation success in Australian marsupials. I then suggest some management recommendations for this species based on the findings in this thesis. I also suggest areas for future research by highlighting the knowledge gaps that still exist for this species and in the field of translocation ecology for Australian marsupials.

6.2 The biology of the bilby in a temperate environment

Australia once had multiple species of mammals with broad distributions that encompassed arid, semi-arid and temperate habitats. However, many of these species now only inhabit a small fraction of their former range (Woinarski *et al.* 2015). These species not only had distributions that encompassed large parts of the continent, but were abundant based on records of skins obtained by hunters and were able to exist across different habitat types due to their ecological flexibility (Woinarski *et al.* 2015). It is likely that their diet, habitat use, and behaviour may have varied between different climatic zones. The findings of my research of two populations in a temperate environment suggest this is the case. We found that bilbies could occur in very high densities and were able to adapt their reproductive timing and output in a temperate environment by adopting seasonal breeding patterns (Chapter 2; Chapter 3). By contrast in the arid zone, bilbies can breed throughout the year-in response to favourable environmental conditions (Southgate *et al.* 2000; Moseby and O'Donnell 2003). At our sites where temperate climatic conditions occur, bilbies bred primarily during winter, spring and early summer, with the onset of

breeding coinciding with the months of higher rainfall in southern Australia (Colls and Whitaker 2012). My research detected a shorter breeding season, smaller litter size at weaning and lower adult body condition in the population that occurred at higher density compared to the other temperate site at lower population density (Chapter 2, Chapter 3). These results suggest that resource competition may occur in high density populations, and could lead to a natural reduction in fecundity of females, thereby reducing population growth.

Burrow range was similar in the Venus Bay population that experienced temperate environmental conditions to those recorded in the South-West Queensland population (McRae 2004; Chapter 4). At our temperate zone sites, however, there was evidence of high population density, a spatially continuous population (Chapter 3) and significant burrow range overlap between individuals (Chapter 4). This contrasts with the observations in Queensland, which was small colonies of individuals spatially located in areas of suitable habitat (Moritz e*t al.* 1997). It is possible that in more productive environments, bilbies co-exist at higher densities with overlapping home ranges, rather than reducing their home range size. My observation of significant overlap of burrow ranges in males but less in females suggests that bilby home range size may not only be determined by resources, but may also be influenced by suitable sites/substrate for burrow construction and access to potential mates (Chapter 4). Females also had significantly smaller burrow ranges than males (Chapter 4), and in an arid zone study a dominant breeding male was found to be roaming large distances each night (Moritz *et al.* 1997; McRae 2004). Therefore, it is likely that a female's home range is primarily driven by food resources and burrow substrate suitability, whereas a male's home range is also influenced by access to mates.

Burrow range and recapture results indicated that individuals at our temperate sites were relatively sedentary over the study period and held stable home ranges over that time (Chapter 3; Chapter 4). These observations could reflect the relative stability of food resources in the temperate environment where there was regular winter rainfall compared to arid zone environments. It could also be an artefact of the smaller and more restricted areas in which these populations occurred: a fenced conservation reserve and an offshore island. Home range was previously found to be relatively stable over time in adult bilbies in an arid zone translocated population, although those individuals also occurred within a fenced conservation reserve (Moseby and O'Donnell 2003). Given the abundance of bilbies

in the temperate areas around Adelaide in the late 1800s (Wood Jones 1924), it is likely that in areas with reliable seasonal rainfall, bilbies were reasonably sedentary and occupied stable home ranges prior to their extinction in these locations. However, this could only be confirmed if bilbies were able to be released outside the fenced enclosures and survived long-term.

This thesis further suggests that there are some aspects of bilby ecology that are similar between populations occurring in vastly different habitats and climatic zones. Our findings confirm that male reproductive success is hard to predict in this species, but that a small number of males can dominate breeding even in high density continuous populations in the temperate zone, whereas a large proportion of other males in the same population may sire no young in the area in which they were trapped within a breeding season (Chapter 5). Our findings confirmed that bilbies have a polygynandrous mating system, which is similar to that reported in arid zone and captive populations (Moritz *et al.* 1997; Miller *et al.* 2010a). Our research also supports previous studies of arid zone populations that bilbies are mostly solitary and that they construct and maintain multiple burrows within their home range (Moseby and O'Donnell 2003; McRae 2004; Chapter 4).

Studies of burrow use in the Venus Bay population found bilbies tended to orientate the entrance of their burrows in a non-random fashion (Chapter 4); a finding which differs from the random burrow entrance orientation observed in the arid zone (Moseby and O'Donnell 2003). There is a curious observation in the early literature by Sir Baldwin Spencer that in winter bilbies would sometimes lie "within a foot or so of the entrance of its burrow, and only uses the inner chamber during the summer" (Wood Jones 1924). The thermal properties of occupied bilby burrows is something which was not investigated during the current study, nor has it yet been investigated in other populations of bilbies. However, it could be that the thermal properties of bilby burrows are influenced by aspect and location, and that bilbies orientate their burrows to enhance the thermal suitability of their burrows in cooler climates (Chapter 4).

6.3 Improving translocation success in bilbies

The results of this study indicate there are several ways in which translocation success could be improved during the initial translocation of founder individuals. Firstly, it is recommended that both red foxes (*Vulpes vulpes*) and feral cats (*Felis catus*) are absent, or

at the very least heavily controlled, at translocation sites. Our research has demonstrated that feral cats alone can impact population growth and persistence of bilbies through preying on smaller sub-adults (Chapter 3). Therefore, the sites where translocations are most likely to be successful are sites free of exotic predators. This means that the most appropriate sites continue to be fenced conservation reserves or offshore islands where these predators are fully precluded (Legge e*t al.* 2018). Since this study was conducted, control of feral cats has taken place at Venus Bay Conservation Park. However, in the mid-2020s results from the trials of Felixer grooming traps within the park indicated low numbers of feral cats still persisted within the park (Ryan-Schofield *et al.* 2024).

Our research also demonstrated that sites in the temperate zone can support high densities of bilbies, potentially due to the higher and more reliable rainfall in temperate areas compared to the arid zone (Chapter 3). Therefore, temperate sites could be highly suitable as source populations or to provide security for the persistence of the species, as they are less likely to experience harsh droughts and subsequent population crashes. Temperate populations could also be a source of bilbies for future translocations or for genetic management of other populations. Bilbies have already been obtained from Thistle Island for translocation to Western Australia as well as to the Arid Recovery Reserve in South Australia (Chapter 3; Lohr *et al.* 2021).

My research suggests that the initial rate of increase of a population could be enhanced by releasing larger females that are more likely to raise twin litters (Chapter 2), as well as by releasing female-biased founder groups (Chapter 5). Females in our study had litters of one or two young, could raise up to three litters per annum and females with a larger head size were more likely to raise twins to pouch emergence than smaller females (Chapter 2). Most females in the populations we studied were observed to breed multiple times annually (Chapter 2; Chapter 5), however only between 5-20% of males were detected siring young in our populations (Chapter 5). A small number of males were recorded siring multiple litters with multiple females in a breeding season, suggesting the number of females in a founder group likely has a greater influence on recruitment and population growth than the number of males. Therefore, initial recruitment in a translocated population could be improved by releasing a greater percentage of females than males in founder groups. Most females within a release group are likely to breed, and one male could successfully mate with multiple females. Sourcing males that are from genetically dissimilar populations to

the females could also improve the likelihood of breeding for males in founder and translocation groups, given our findings that breeding pairs in the Venus Bay population were more unrelated than a random sample of male-female pairs (Chapter 5). However, further research is required to identify other traits of males that lead to an increased likelihood of breeding so these males can be selected in founder groups.

A further recommendation for improving translocation success in the temperate zone, is to release the founder group at the start of the breeding season (winter) when rainfall is higher and resources are better able to support breeding (Chapter 2). Releases should avoid late summer and autumn, which coincides with a cessation in breeding in temperate populations and could be a time of year when resources are scarce (Chapter 2). It is likely that the cessation in breeding that occurs at this time is due to limited food resources after the long dry temperate zone summer, which could also affect the survival of founder individuals if released into an unfamiliar environment. Timing releases for the start of the breeding season in temperate areas would also encourage the founder individuals to commence breeding and may result in multiple litters being produced by females in the first year post-release.

6.4 Further conservation recommendations for bilby populations

6.4.1 Protection of remnant wild populations

My research has demonstrated that feral cats are a significant threat to the persistence of bilby populations through the predation of small sub-adult individuals, which limited recruitment into the adult population (Chapter 3). Previous studies have also recognised that introduced red foxes are a significant threat to bilbies (Southgate 1990a). Remnant populations of the greater bilby are now restricted to the arid central and northern parts of Western Australia and Northern Territory, and small areas in arid south-west Queensland (Bradley *et al.* 2015). There is evidence that feral cats occupy these areas and prey on remnant populations of bilbies (Paltridge 2002; Bradley e*t al.* 2015; Moseby *et al.* 2015b). Feral cats have also been implicated in the historic decline of other marsupial species (Moseby *et al.* 2021). Arguably, one of the most important things that could be done for the protection of remnant bilby populations, as well as other medium-sized marsupial species in Australia, is to more effectively manage, or even eradicate, red foxes and feral cats from much of the Australian landmass.

Red foxes tend to be readily controlled using established baiting programs, however baiting has often been less successful in the control of feral cats (Moseby e*t al.* 2011b). Feral cats can also be hard to trap in some areas of Australia (Short e*t al.* 2002) and they have the ability to build up in numbers quickly in response to rainfall, likely due to increased prey availability (Legge *et al.* 2017). In recent years, significant improvements have been made in feral cat control that both reduce the non-target impacts of control and improve the effectiveness of feral cat control techniques. One approach has been to increase the palatability of feral cat baits (Cox *et al.* 2022) and to make baits that are of less risk to endemic wildlife and hence more suitable for use in wilderness areas (Hetherington *et al.* 2007). Another approach has been the development of the Felixer grooming trap, which recognises the shape and gait of cats, and uses feral cat grooming behaviour to administer a toxin to the coat of feral cats, which they then lick off (Read *et al.* 2019). The limitation of this device is that feral cats must walk perpendicular to the front of the device at a distance of 1-4 metres to be targeted, which could still pose problems with neophobic feral cats (Moseby e*t al.* 2020). It also means knowledge on the location and movement paths used by feral cats in an area is needed for this device to be effective. Another potential feral cat management approach is cat hunting by Indigenous tracking experts (Paltridge *et al.* 2020). This is a viable option for areas with open sandy substrates that are managed by Indigenous land managers. A recent study on the effectiveness of this type of hunting found that areas with regular Indigenous cat hunting had fewer cat detections recorded and better threatened species persistence over time than control areas with no hunting (Paltridge e*t al.* 2020). Therefore, further support for feral cat control by First Nations land managers could also help to preserve remnant populations of threatened species, including bilbies.

With these recent developments in feral cat control, Australia may now be in a better position to effectively manage feral cats in areas where remnant populations of threatened mammal species occur. Given our findings on the impact of feral cats on bilby population persistence (Chapter 3), it is likely that the most effective management action for remnant populations of bilbies is to significantly reduce, or even eradicate, feral cats from the areas where bilbies occur. This is already taking place on a small scale in some areas of the bilbies' range (e.g. Paltridge e*t al.* 2020), but ideally exotic predator control should occur across the bilbies extant and historic range in order to reverse the trend of population decline recorded during the last century and encourage the species to expand its current range.

6.4.2 Management of translocated and captive populations

My research demonstrates that translocated bilby populations can successfully become established and exist at high population density in the temperate zone in the absence of exotic predators. Populations such as the one that has established on Thistle Island where bilbies are abundant and occur across the entire island (Chapter 3), provide additional long-term security for the species. The Thistle Island population is isolated from mainland threats, such as exotic predators, and occurs in a location with a relatively predictable climate where it is unlikely that the population will experience harsh droughts like in the arid zone. Given the mild climatic conditions, higher rainfall and increased landscape productivity in the temperate zone, further translocations of bilbies to protected sites in southern Australia could be beneficial for their long-term persistence. Translocation of the bilby to additional sites in temperate Australia could also benefit the landscapes in which they are released into as observed in the arid zone, through the ecosystem services they provide through their foraging pits (James *et al.* 2010), and also by benefiting burrow commensal species that utilise bilby burrows for shelter such as native rodents, dasyurids and small macropods (Hofstede and Dzmininski 2017; Dawson *et al.* 2019). Given bilbies are ecosystem engineers, their translocation should be considered as part of an ecosystem restoration program in any region of Australia in which they used to be present.

One management issue associated with small, fragmented populations, such as those translocated to reserves or islands and within captive breeding colonies, is the risk of inbreeding, loss of genetic diversity and genetic divergence and structuring between populations (Thavornkanlapachai *et al.* 2021; Rick *et al.* 2023). Our study indicated that over a relatively short time period there was already moderate genetic differentiation between the two translocated populations we studied, despite the founders of both populations being sourced from the same captive breeding colony (Chapter 5). Genetic diversity measures at the Venus Bay population, primarily the number of alleles per locus, were also significantly lower than wild remnant mainland populations and two captive populations (Chapter 5). Supplementation to translocated populations with new individuals has been shown to assist in the maintenance of genetic diversity of small populations (Miller et al. 2015; White et al. 2018). Supplementation, or genetic rescue, may also improve the population health of small, isolated populations. Indeed, improvements in fecundity and longevity of 'hybrid' individuals (offspring from the breeding of local

individuals with translocated individuals) compared to non-hybrids has been recorded (Weeks e*t al.* 2017). In order to maintain the value of translocated populations and prevent loss of genetic diversity and genetic load, it is important that supplementation with individuals from other populations is carried out regularly as part of the long-term management of isolated translocated populations.

6.5 Limitations of this study

There were several limitations of this study. The first was that I was unable to collect data from a comparative translocated population in the arid zone at the same time as data collection was carried out in the two temperate translocated populations. As described in section 6.1, a long period of dry conditions in northern South Australia precluded data collection from the translocated population at the Arid Recovery Reserve, and track transects recorded very low numbers of bilby prints during the study period (Moseby *et al.* 2011). Therefore, I was unable to directly compare the timing of breeding and population density with an arid zone translocated population in this study. However, the fact that bilby abundance at Arid Recovery was so low that bilbies were unable to be trapped is a comparison in itself, and demonstrates that populations in the temperate zone may be less at risk of dramatic fluctuations and population "busts" due to unfavourable environmental conditions such as severe drought.

My choice of tracking method to find bilby burrows also limited the data I could collect. Spooling enabled me to track many bilbies to their burrow per day, without the associated cost and ethical issues of using radio telemetry devices. However, each spool only enabled me to track a bilby to its burrow once immediately after release, and only if the burrow was within ~300m of the trap location. In order to track bilbies to their burrows over consecutive nights, I needed to capture bilbies over consecutive nights, which was not always possible. I was able to do this on quite a few occasions due to high recapture rates at Venus Bay, and at this site spooling was a successful method of locating burrows and determining aspects of burrow use of individuals. However, much lower recapture rates at Thistle Island due to high overall capture rates of both bilbies and brushtail possums meant spooling did not successfully identify multiple burrows for individuals, and hence burrow data collected at that site was inadequate to calculate burrow range for any individuals. Regardless, the advantages of using spooling in this study were significant: 1) spools were low cost, which enabled data collection from a larger number of individuals, and; 2) there

was low impact on individual bilbies due to the light weight of spools and the fact they readily detached once spool thread ran out.

The methods used to investigate paternity and genetic diversity in this study had advantages and disadvantages, and were limited by some external factors. I used 10 microsatellites for the analysis of paternity, and also carried out a basic assessment of genetic diversity using these same loci. Microsatellite loci are powerful for parentage analysis, however, they can be limited in species where there is low polymorphism (Flanagan and Jones 2019). I was unable to resolve the paternity of all pouch young sampled during the study, and the low number of alleles at some loci may have contributed. In the Venus Bay population, where genetic diversity measures were generally low, four of the loci used had four or less alleles (Chapter 5). When multiple individuals share similar alleles, it may be difficult to resolve paternity with high confidence. Another reason that likely impacted my ability to assign paternity to some pouch young was that not all males that lived in the populations could be trapped. It is likely that some unassigned pouch young were sired by males we were unable to trap, particularly at Thistle Island where the population density of bilbies was extremely high and we also recorded high numbers of by-catch species (Chapter 3). Another limitation was that we did not have access to genetic samples from founder individuals of the populations. Therefore, I was unable to assess whether genetic diversity measures had remained similar over time or had declined following release. I also compared the genetic diversity in the two temperate populations I studied to samples from wild populations that had been collected more than a decade prior to our study. Given that bilbies have continued to decline in the wild in recent decades (Bradley e*t al.* 2015), it is possible the genetic diversity of wild populations has changed over time.

Microsatellites require a significant initial investment to identify polymorphic loci, develop primers and optimise PCR conditions (Flanagan and Jones 2019). This study was fortunate to utilise primers developed for 10 loci for bilbies by Moritz *et al.* (1997), and two additional loci were developed during this study. Therefore, the initial investment in loci identification and primer development was lower due to previous genetic work on this species. It was also of interest to compare diversity measures across the same loci as previous studies on the species, which allowed direct comparison of differences in the number of alleles per

locus and observed heterozygosity between our sites and those sampled in other studies, particularly between translocated, captive and wild populations.

Next-generation sequencing technologies have become popular in recent years for genetic diversity estimates and population-level diversity studies (Zimmerman *et al.* 2020). These new sequencing technologies have enabled the use of single nucleotide polymorphisms (SNPs) for sampling large numbers of loci for relatively less cost than traditional microsatellite techniques (Schlötterer 2004). Although SNPs can also be used for parentage analysis, their power to resolve parentage is usually similar to that of microsatellites (Flanagan and Jones 2019). In my study the decision was made to use microsatellites for two reasons: First, the use and analysis of microsatellite data is well established and powerful for parentage analysis, which was the main analysis we were interested in, and secondly because 10 polymorphic loci had already been identified and successfully used in previous studies. However, if this study focused more on population-level diversity and differentiation between populations across Australia, then SNPs may have been a more suitable option, although the analytical techniques to efficiently utilise SNPs were not available at the time my genetic analyses were conducted.

6.6 Future research

6.6.1 Improving the persistence of the bilby in the future

My research showed that adult bilbies exhibited a similar survival rate in a population with feral cats present compared to a feral-cat free population (Chapter 3). However, the results highlighted that young and sub-adult bilbies were still highly susceptible to cat predation (Chapter 3). The predation of young bilbies appeared to be so severe that it resulted in a lack of recruitment into the adult population despite high levels of reproductive output by adult females. This resulted in a declining population trend at the site with feral cats (Venus Bay). There have been several recent studies on the effectiveness of predator avoidance training or low level predator exposure on the survival and behaviour of adult bilbies. These studies have indicated that when bilbies are exposed to predator scent and are trained to avoid it prior to their release into an area with live predators, this can elicit behavioural changes in predator-exposed bilbies compared to control individuals (Moseby e*t al.* 2012; Ross *et al.* 2019). These predator-exposed bilbies are more likely to survive when released into areas with feral cats (Blumstein e*t al.* 2019, Ross *et al.* 2019). In areas where a remnant

population of bilbies has co-existed in the wild with feral cats for 150 years, the bilbies showed an anti-predator behavioural response to cat scent, and hence even this short period of co-existence seems to have been long enough for the development of predator recognition (Steindler and Letnic 2021). However, all of these studies focus on the survival of adult bilbies in the presence of feral cats, and the issue of low sub-adult mortality has had little attention up until now.

Given wild remnant populations of bilbies still appear be on a trajectory of decline, severe sub-adult mortality due to predation is likely occurring in other bilby populations that coexist with feral cats. Therefore, the next logical step in terms of research priorities in this area is to determine if this is true, and understand possible mechanisms to improve the survival of small, younger bilbies following pouch emergence in the presence of feral cats. It could be that young bilbies are easier prey for feral cats, given their smaller size, but it could also be that they are more predator naïve than adult bilbies. Given there is evidence that bilbies are now able to recognise feral cats as a predator after only 150 years of coexistence, predator-avoidance behaviour may continue to develop and improve in this species in the presence of exotic predators. However, given the continued decline of the bilby, any potential evolutionary response to these new predators may develop too slowly for the species to ever truly be able to persist in the presence of exotic predators without considerable assistance from humans. Therefore, at this point in time, research into more effective large-scale predator control methods should remain a priority.

A further research question of interest could be to determine whether recruitment is the demographic parameter most affected by predation in other threatened and declining medium sized-marsupials, and whether this differs between feral cat and fox predation. Most in-depth research on predator-prey interactions and demographic parameters to date has occurred in ungulates. In carnivore-ungulate systems, larger prey body mass was associated with juveniles making up a larger proportion of prey individuals killed (Gervasi e*t al.* 2015). There is also evidence from the large members of the Felidae family that smaller predators may expand their range of available prey through increased killing of juveniles in some prey species compared to larger predators (Annear e*t al.* 2023). However, this is complicated by research from other continents that has shown that different types of predators prey on different age cohorts of the same species quite differently due to their specific predation patterns (Gervasi e*t al.* 2012). The impact on prey populations likely

varies, however in black-tailed deer it was demonstrated that the predation and subsequent survival of younger age cohorts plays a larger role in population growth estimates than adult survival (Marescot *et al.* 2015). Very little research has been carried out how predation impacts demographic parameters of different age-cohorts in Australian marsupials. However, in the northern quoll, population persistence has been found to be most sensitive to changes in juvenile survival (Moro *et al.* 2019). My research does suggest that feral cat predation rates vary between different age-cohorts in the bilby, therefore study of age-specific demographic parameters in threatened medium-sized marsupials warrants further study, as does the impact of different predator species on threatened prey demographics.

6.6.2 Managing translocated and captive populations

This study indicated that the two temperate zone translocated populations I studied may have lower genetic diversity measures than other managed captive populations and samples from wild populations, however further research is needed to verify this (Chapter 5). The Venus Bay population was lower density and occurred over a smaller area than the Thistle Island population, due to predation by feral cats (Chapter 3). It is likely that the population processes acting at Venus Bay that were limiting its growth were also contributing to a loss of genetic diversity over time, and small isolated populations such as Venus Bay are more likely to have lower genetic diversity (Frankham 1996). In bilbies and other Australian marsupials, supplementing translocated populations with individuals from other populations has been shown to effectively maintain genetic diversity over time (Miller *et al.* 2015; White *et al.* 2018). Ideally a future study would compile contemporary and historical samples collected from different parts of the bilbies extant range, and compare them to all of the captive and translocated populations from around Australia to develop a management plan to maintain and improve genetic diversity across managed populations. Work has commenced on this, and as I was completing the final revisions of this thesis, a paper by Hogg *et al.* (2024) was released that included genetic diversity parameters from a number of populations. However, not all wild populations had been able to be included, and for some the number of individuals sampled was very low (< 6; Hogg *et al.* 2024). Given that wild bilbies in arid areas are often extremely difficult to capture, this is not surprising. However, it should continue to be a priority to collect genetic samples from any wild bilbies that are able to be captured to improve our understanding of

connectivity between wild populations and whether translocated populations need further supplementation.

6.6.3 Bilby ecology and behaviour

Several questions remain unanswered about bilby ecology and behaviour. My study tracked individual bilbies to their burrows, and on occasion we recorded bilbies using the same burrows as another bilby on the same night (Chapter 4). However, my ability to determine how frequently burrow sharing occurred was limited, as I could only spool a small number of bilbies successfully per night. Therefore, burrow sharing could be occurring far more frequently than we currently realise. Investment in other more costly monitoring techniques would be required to resolve this, such as infra-red motion activated cameras and remote data loggers at the entrance to burrows that record the microchip of bilbies passing in to burrows are both possible options. If technology such as this was used to further investigate burrow use, the frequency of burrow sharing, as well as other questions of interest such as how long young-at-foot stay with their mothers, could potentially be answered.

The present study focused on overall population processes such as survival and recruitment (Chapter 3), which is how the impact of feral cats was identified. However, recent research has also found that the presence of novel predators such as feral cats, where there is only a very short period of evolutionary history between the two species, can elicit behavioural changes in individual bilbies. Bilbies exposed to feral cats have been found to spend more time under the cover of vegetation, move burrows more frequently and also reduce the time spent on burrow maintenance activities after a feral cat visit (Moseby *et al.* 2012; Ross *et al.* 2019; Chen *et al.* 2024). However, it has also been ascertained that the magnitude of behavioural response is smaller when bilbies are faced with a feral cat compared to a predator such as the dingo which they have coexisted with longer (Steindler *et al.* 2018). Although our research showed adults are able to survive in the presence of feral cats, our study did not shed light on the effect of feral cats on bilby behaviour. What would be of interest is the effect on behaviour that has an impact on individual's ability to seek food, shelter and reproduce. For example, given that bilbies showed reduced burrow maintenance activities and increased vigilance following a feral cat visit (Moseby *et al.* 2012; Chen *et al.* 2024), could this mean that cats also influence the foraging and mate seeking behaviours of individuals? Although feral cats affect population processes such as

recruitment (Chapter 3), it could also be that the presence of feral cats also has significant impacts on the behaviours of survivors within those populations.

6.6.4 Bilbies in temperate ecosystems

There is still much to learn about the bilby, and its possible impacts on temperate ecosystems. Although the thermal properties of relic bilby burrows has been investigated (Chapman 2013), the thermal properties of occupied bilby burrows has not. Of particular interest would be to determine whether burrow construction and orientation are used to maximise the thermal properties of burrows. For example, our research found burrow orientation in a temperate zone site was non-random, which differs from studies in the arid zone (Chapter 4; Moseby and O'Donnell 2003). It would be of particular interest to determine whether high use burrows that had a northerly aspect had improved thermal properties compared to burrows orientated in other directions. Future research could also investigate whether bilby burrows have benefits for other burrow commensal species in the temperate zone, as has been observed in other parts of Australia (Read *et al*. 2008; Hofstede and Dziminski 2017; Dawson *et al*. 2019). It is possible that burrows could provide important refuges during bushfires for non-burrowing species in temperate and forest areas (Newsome *et al*. 1975; Long 2009; Linley *et al.* 2024). An interesting area of future research would be to determine if survival in non-burrowing ground dwelling species is improved in the presence of bilby burrows.

Ecosystem engineers, such as the bilby, dig extensively for food and to construct burrows, and move large quantities of soil during digging activities. This movement of soil is important for soil function, fungi dispersal, native seedling germination, soil carbon sequestration and leaf litter breakdown (Fleming *et al.* 2014; Hayward e*t al.* 2016; Dundas *et al.* 2018; Davies *et al.* 2019; Decker 2020). Soil and ecosystem function across the continent has likely been impaired by the loss of native digging mammals across large areas of Australia (Fleming *et al.* 2014; Decker 2020). Introduced rabbits in arid areas, which are widespread and also dig, create fewer digging pits overall and create structurally different digging pits that support far fewer native seedlings than the pits of native digging mammals so they likely do not fill the ecological role of native mammals (James *et al.* 2011). However, we are yet to determine the impact of the loss of native digging mammals in temperate ecosystems. Much research to date on the impact of bilby diggings on soil has occurred in populations in the arid zone, and there is scope for further research on comparative studies

in the temperate zone between areas with reintroduced bilbies and adjacent areas without. It could be that the lack of bilbies throughout large parts of their historic range has had detrimental impacts on nutrient cycling, native seed germination and fungi dispersal since their extinction. Comparisons of these ecosystem processes pre- and post-release could also be considered for future bilby reintroduction sites.

Finally, there are still broad questions surrounding the behaviour of translocated animals, and whether the behaviours observed in translocated populations are an adaptation to new environments or adaptations they developed in their source environment. This likely varies significantly depending on the behavioural plasticity of species. Within a species, translocation itself can lead to changes in behaviour of individuals, with increased vigilance and moving associated with translocated individuals compared to resident individuals (Eline *et al.* 2023). Some of this is likely due to the short-term acute or sometimes long-term chronic stress caused by translocating individuals to unfamiliar environments (Dickens e*t al.* 2010). There are calls for more research into individual personality plasticity to assist in more appropriate choices of individuals for release to unfamiliar environments (Wu *et al.* 2024). However, there is a lack of research on the long-term population level behavioural changes that might be induced by translocating captive individuals to new sites. My study showed remarkably similar burrow use behaviour in bilbies in the temperate zone populations studied to wild bilbies in the arid zone, despite the founding individuals of the populations I studied being from a captive colony. This indicates there are some behaviours that may be retained in all environments due to their importance to survival, such as burrow construction and use. However, it could be that there are some long-term behavioural adaptations when species are moved to very different environments that have not yet been identified, and more in-depth observational behaviour studies over long time periods would be required to identify these.

6.7 Conclusion

My research has demonstrated that greater bilby populations translocated to the temperate zone of South Australia can provide opportunities to learn more about the ecology of this species in different climatic zones and habitats. This research has also highlighted that translocated populations require ongoing management to remain viable and contribute to the long-term security of this species. The bilby can adapt its reproductive strategy to suit local conditions and can exist at high population densities in the higher

rainfall parts of Australia when exotic predators are absent. However, other aspects of its ecology, such as burrow use behaviour, are remarkably similar between populations in the arid zone and the temperate zone. The work detailed in this thesis has added to the growing body of evidence that feral cats can be catastrophic for the long-term persistence of medium-sized marsupials. This thesis also provides several suggestions to improve the management of translocated populations: 1) that founder groups are female-biased and large females are selected for translocation to improve the initial rate of increase; 2) that feral cats and red foxes are excluded completely from translocation sites, and; 3) that translocated bilby populations have ongoing supplementation with unrelated individuals from other populations to maintain genetic diversity. It is hoped that the work contained within this thesis makes a contribution to the long-term conservation of bilbies both within South Australian and nationally.

Appendices

Appendix 1

Primers used to amplify 11 microsatellite loci and the. ML1 and ML2 were developed for this study, all loci with the 'Bil' prefix were developed by Moritz et al. 1997.

Locus name	Repeat motif	Fluoresc. label	Forward Primer	Reverse Primer
ML1	(GTT)9	VIC	AATTGAAGAGATAGGAGATGGAATG	TGGGATCATGAAGAGGTGGAC
ML2	(GGA)19	FAM	GCTGGGCTAACTTTCAAGGG	TCCTGATGTGACAGCCTTAC
Bil02	(CA)17	FAM	GCATGTACTTAACCCCCTTTGCC	CCCGACAATCCAGCCTGTTATTC
Bil16	(CA)12	NED	GTTAGAAGACAGGATGGGGATTG	TGAGTACACAGTGCAGCAAGAGC
Bil17	(CA)16	FAM	GAGAACTGGAACTTCGCGTAGGC	CTCCAATTCACTTTTCCTGAGAC
Bil22	(CA)16	VIC	GGTATGAGGAATTAGAATTACAGG	CGGTATTAAATGGGCTATGGAGT
Bil41	(CA)14	PET	CATTGTCATAGAAGCATCATCAAC	GGAAAAGTTTTTAGCCTAATAGTGG
Bil55	(CA)24	FAM	GCACCAACCTATCCTCTTCATTC	CTACAAGTCTGATAATTCCAGGC
Bil56	(CA)23	VIC	GCATGAATGAATCTCCTGGCTCC	CACTAACAAATATGCTTGGGAAAGG
Bil63	(CA)14	NED	CTTAGGCAAATAGGGTGAAGTGG	CAGAACCATTAGGAAGGAGTTTC
Bil66	(CA)45	PET	GCCTTGGGTATGCTGTCATCTGA	CTCCAAGCCAAGAATCCCATTCC

Appendix 2

Loci characteristics based on 69 adult greater bilbies from Venus Bay Conservation Park. The number of alleles, allele size range, allelic richness (N_{AR}), the observed (H_O) and expected (H_E) heterozygosity, the significance of tests for Hardy-Weinberg expectation departures (HWE) with the Bonferroni correction (*** is significant at the 0.1% level; NS is not significant) and the frequency of null alleles at eleven loci are given.

Locus	No. alleles	Allele size range	N_{AR}	H_O	H_E	HWE	F(Null)
Bil16	3	207-217	5.997	0.435	0.452	NS	0.019
Bil17	5	249-259	6.942	0.754	0.697	NS	-0.046
Bil22	3	183-193	2.758	0.667	0.574	NS	-0.082
Bil41	6	247-257	4.997	0.841	0.783	NS	-0.040
Bil55	4	186-192	5.997	0.797	0.712	NS	-0.062
Bil56	5	228-240	7.459	0.594	0.501	NS	-0.108
Bil63	4	237-243	5.000	0.609	0.641	NS	0.030
Bil66	10	261-289	-	0.435	0.801	**	0.296
Bil02	5	167-187	6.997	0.783	0.754	NS	-0.022
ML1	7	138-219	6.701	0.623	0.557	NS	-0.064
ML2	7	195-297	6.987	0.797	0.775	NS	-0.016

Appendix 3

Loci characteristics based on 91 adult greater bilbies from Thistle Island. The number of alleles, allele size range, allelic richness (N_{AR}), the observed (H_O) and expected (H_E) heterozygosity, the significance of tests for Hardy-Weinberg expectation departures (HWE) with Bonferroni correction (*** is significant at the 0.1% level; NS is not significant) and the frequency of null alleles at eleven loci are given.

Locus	No. alleles	Allele size range	N_{AR}	H_O	H_E	HWE	F(Null)
Bil16	6	207-231	5.897	0.747	0.750	NS	-0.002
Bil17	7	249-263	7.000	0.879	0.812	NS	-0.043
Bil22	3	191-197	3.429	0.385	0.393	NS	0.013
Bil41	5	249-263	6.839	0.593	0.590	NS	0.000
Bil55	6	156-192	5.897	0.780	0.786	NS	0.000
Bil56	8	228-242	6.786	0.813	0.736	NS	-0.060
Bil63	5	233-241	5.994	0.758	0.751	NS	-0.010
Bil66	5	273-281	-	0.231	0.668	***	0.493
Bil02	7	161-187	7.897	0.890	0.807	NS	-0.055
ML1	7	141-219	6.786	0.780	0.803	NS	0.012
ML2	7	195-297	8.000	0.868	0.818	NS	-0.034

Appendix 4

Genetic diversity measures for greater bilby populations at Venus Bay Conservation Park and Thistle Island derived from only the "Bil" loci previously described by Moritz *et al*. (1997) and comparison with two captive colonies in Western Australia, a wild Northern Territory (NT) population and a wild Queensland (QLD) population. H_O = observed heterozygosity, H_E = expected heterozygosity.

Population	No. of individuals	N_A	H_O	H_E
Venus Bay	69	4.4 (± 0.4)	0.685	0.639
Thistle Island	91	5.9 (± 0.5)	0.731	0.703
WA captive colony A [A]	216	6.4 (± 0.6)	0.681	0.709
WA captive colony B [A]	266	7.6 (± 0.6)	0.664	0.758
Wild NT [B]	19	8.9	-	0.813
Wild QLD [B]	27	7.7	-	0.654

[A] Data from Miller e*t al.* 2015

[B] Data from Moritz e*t al.* 1997

Appendix 5

Models to test for an effect of male bilby body mass and relative testes size on reproductive success (Sire, 1 = fathered young, 0 = did not father young) for the Venus Bay (GLMM; family = binomial) and Thistle Island (GLM; family = binomial; random effect = year). Models are ranked by AICc and model weights.

Site	Model structure	d.f.	AICc	ΔAICc	ω
Venus Bay	Sire ~ body mass	3	57.28	0.00	0.41
	Sire ~ 1 (null model)	2	57.59	0.31	0.35
	Sire ~ body mass + relative testes	4	59.70	2.42	0.12
	Sire ~ relative testes	3	59.73	2.45	0.12
Thistle Island	Sire ~ body mass	2	46.10	0.00	0.42
	Sire ~ 1 (null model)	1	47.38	1.28	0.22
	Sire ~ body mass + relative testes	3	47.38	1.29	0.22
	Sire ~ relative testes	2	48.32	2.22	0.14

Appendix 6

Model averaged estimates of the effect size of factors featured in the top candidate models for Venus Bay (GLMM; family = binomial; random effect = year) and Thistle Island (GLM; family – binomial). The adjusted standard error is given, and estimates are based on the full (not conditional) average.

Site	Independent variable	Estimate	S.E.	z-value	P
Venus Bay	Intercept	-0.93	0.47	1.97	0.05
	Body mass	0.66	0.86	0.76	0.44
Thistle Island	Intercept	-4.42	2.97	1.49	0.14
	Body mass	1.72	1.51	1.14	0.25
	Relative testes	0.61	1.64	0.37	0.71

Appendix 7

Paternity assignments and likely candidate fathers for pouch young at Venus Bay Conservation Park and Thistle Island using six loci for the analysis.

Site	Pouch Young ID	Date sampled	Litter number	Mother ID	Assigned paternity?	Assigned father	Assignment method*	For pouch young with unassigned paternity	
								Most likely candidate (> 80 %)	Other potential fathers (positive LOD score > 0.8 in CERVUS and < 2 loci mismatches at trio level)
Venus Bay	VBPY01	2/07/2008	1	VB04F	No	-	-	VB19M	-
Venus Bay	VBPY02	2/07/2008	1	VB04F	Yes	VB19M	CERVUS	-	-
Venus Bay	VBPY03	4/07/2008	2	VB10F	Yes	VB35M	Combination	-	-
Venus Bay	VBPY04	5/07/2008	3	VB11F	No	-	-	VB12M	VB02M, VB60M
Venus Bay	VBPY05	5/07/2008	4	VB13F	No	-	-	VB12M	-
Venus Bay	VBPY06	5/07/2008	4	VB13F	No	-	-	VB12M	VB02M
Venus Bay	VBPY07	6/07/2008	5	VB23F	Yes	VB28M	COLONY + Combination	-	-
Venus Bay	VBPY08	6/07/2008	6	VB25F	No	-	-	VB24M	VB35M, VB38M, VB03M, VB26M, VB19M
Venus Bay	VBPY09	6/07/2008	6	VB25F	No	-	-	-	VB24M
Venus Bay	VBPY10	22/09/2008	7	VB09F	No	-	-	-	VB12M
Venus Bay	VBPY13	26/11/2008	8	VB13F	No	-	-	-	VB29M, VB58M
Venus Bay	VBPY14	26/11/2008	8	VB13F	Yes	VB58M	Combination	-	-
Venus Bay	VBPY15	26/11/2008	9	VB23F	No	-	-	-	VB31M
Venus Bay	VBPY16	26/11/2008	9	VB23F	No	-	-	-	VB31M, VB33M, VB27M
Venus Bay	VBPY17	26/11/2008	10	VB32F	Yes	VB35M	Combination	-	-
Venus Bay	VBPY18	26/11/2008	10	VB32F	Yes	VB35M	Combination	-	-
Venus Bay	VBPY19	27/11/2008	11	VB37F	No	-	-	-	VB05M, VB58M, VB29M
Venus Bay	VBPY20	27/11/2008	11	VB37F	Yes	VB58M	CERVUS + combination	-	-

Site	Pouch Young ID	Date sampled	Litter number	Mother ID	Assigned paternity?	Assigned father	Assignment method*	For pouch young with unassigned paternity	
								Most likely candidate (> 80 %)	Other potential fathers (positive LOD score > 0.8 in CERVUS and < 2 loci mismatches at trio level)
Venus Bay	VBPY21	27/11/2008	12	VB10F	No	-	-	-	VB27M, VB06M
Venus Bay	VBPY22	27/11/2008	12	VB10F	No	-	-	-	VB27M, VB44M
Venus Bay	VBPY23	28/11/2008	13	VB04F	No	-	-	-	VB08M , VB35M, VB03M, VB26M
Venus Bay	VBPY24	28/11/2008	13	VB04F	No	-	-	VB08M	
Venus Bay	VBPY25	29/11/2008	14	VB11F	No	-	-	-	VB05M, VB58M, VB29M
Venus Bay	VBPY26	29/11/2008	14	VB11F	Yes	VB58M	CERVUS + combination	-	-
Venus Bay	VBPY27	29/11/2008	15	VB41F	No	-	-	-	VB24M, VB30M, VB58M
Venus Bay	VBPY28	25/02/2009	16	VB11F	Yes	VB58M	Combination	-	-
Venus Bay	VBPY29	25/02/2009	17	VB23F	Yes	VB28M	COLONY + Combination	-	-
Venus Bay	VBPY30	25/02/2009	17	VB23F	Yes	VB28M	COLONY + Combination	-	-
Venus Bay	VBPY31	25/02/2009	18	VB10F	Yes	VB35M	Combination	-	-
Venus Bay	VBPY32	25/02/2009	18	VB10F	No	-	-	-	-
Venus Bay	VBPY33	26/02/2009	19	VB25F	No	-	-	-	VB42M, VB31M
Venus Bay	VBPY34	22/06/2009	20	VB10F	No	-	-	VB39M	VB31M
Venus Bay	VBPY35	1/09/2009	21	VB47F	Yes	VB18M	COLONY + Combination	-	-
Venus Bay	VBPY36	1/09/2009	22	VB50F	Yes	VB18M	COLONY + Combination	-	-
Venus Bay	VBPY37	2/09/2009	23	VB13F	No	-	-	-	VB28M
Venus Bay	VBPY38	2/09/2009	24	VB25F	No	-	-	-	VB58M, VB19M
Venus Bay	VBPY39	2/09/2009	24	VB25F	No	-	-		VB29M, VB58M
Venus Bay	VBPY40	5/09/2009	25	VB09F	No	-	-	VB28M	-
Venus Bay	VBPY41	6/09/2009	26	VB57F	Yes	VB55M	CERVUS	-	-

Site	Pouch Young ID	Date sampled	Litter number	Mother ID	Assigned paternity?	Assigned father	Assignment method*	For pouch young with unassigned paternity	
								Most likely candidate (> 80 %)	Other potential fathers (positive LOD score > 0.8 in CERVUS and < 2 loci mismatches at trio level)
Venus Bay	VBPY43	17/11/2009	27	VB57F	Yes	VB18M	COLONY + Combination	-	-
Venus Bay	VBPY44	17/11/2009	27	VB57F	Yes	VB18M	COLONY + Combination	-	-
Venus Bay	VBPY45	17/11/2009	28	VB09F	Yes	VB18M	COLONY + Combination	-	-
Venus Bay	VBPY46	17/11/2009	29	VB13F	Yes	VB18M	COLONY + Combination	-	-
Venus Bay	VBPY47	17/11/2009	29	VB13F	Yes	VB18M	COLONY + Combination	-	-
Venus Bay	VBPY48	22/11/2009	30	VB50F	Yes	VB44M	CERVUS	-	-
Venus Bay	VBPY49	27/01/2010	31	VB50F	No	-	-	VB26M	VB01M
Venus Bay	VBPY50	27/01/2010	31	VB50F	No	-	-	-	VB26M, VB58M
Venus Bay	VBPY51	29/01/2010	32	VB25F	Yes	VB44M	CERVUS	-	-
Venus Bay	VBPY52	29/01/2010	32	VB25F	No	-	-	-	VB52M
Venus Bay	VBPY53	30/01/2010	33	VB10F	No	-	-	-	VB44M
Venus Bay	VBPY54	30/01/2010	33	VB10F	No	-	-	-	VB44M
Venus Bay	VBPY55	26/01/2010	34	VB47F	Yes	VB18M	COLONY + Combination	-	-
Venus Bay	VBPY56	26/01/2010	34	VB47F	Yes	VB18M	COLONY + Combination	-	-

* Assignment methods are 1) CERVUS – father assigned paternity at ≥95 % likelihood by CERVUS; 2) COLONY – father assigned at ≥95 % likelihood by COLONY or; 3) Combination – assigned most likely father by both CERVUS and COLONY and likelihood is > 80 % for at least one program.

Site	Pouch Young ID	Date sampled	Litter number	Mother ID	Assigned paternity?	Assigned father	Assignment method*	For pouch young with unassigned paternity	
								Most likely candidate (> 80 %)	Other potential fathers (positive LOD score > 1.4 in CERVUS and < 2 loci mismatches at trio level)
Thistle Is.	THPY28	24/09/2009	1	TH027F	No	-	-	TH155M	TH193M
Thistle Is	THPY29	24/09/2009	2	TH146F	No	-	-	-	-
Thistle Is	THPY30	24/09/2009	3	TH134F	No	-	-	-	-
Thistle Is	THPY31	25/09/2009	4	TH096F	No	-	-	-	-
Thistle Is	THPY32	26/09/2009	5	TH137F	Yes	TH097M	COLONY + Combination	-	-
Thistle Is	THPY33	26/09/2009	5	TH137F	Yes	TH097M	CERVUS + COLONY + combination	-	-
Thistle Is	THPY34	25/09/2009	6	TH143F	No	-	-	-	TH150M
Thistle Is	THPY35	25/09/2009	6	TH143F	No	-	-	-	TH150M, TH158M
Thistle Is	THPY36	26/09/2009	7	TH040F	No	-	-	-	
Thistle Is	THPY37	27/09/2009	8	TH095F	No	-	-	-	
Thistle Is	THPY38	27/09/2009	9	TH120F	No	-	-	-	TH142M, TH135M
Thistle Is	THPY39	27/09/2009	10	TH131F	No	-	-	TH150M	
Thistle Is	THPY40	28/09/2009	11	TH156F	No	-	-	-	
Thistle Is	THPY41	28/09/2009	12	TH111F	Yes	TH160M	Combination	-	-
Thistle Is	THPY42	28/09/2009	13	TH122F	Yes	TH097M	COLONY + Combination	-	-
Thistle Is	THPY44	20/09/2009	14	TH114F	No	-	-	TH168M	
Thistle Is	THPY46	28/11/2009	15	TH181F	Yes	TH097M	COLONY + combination	-	-
Thistle Is	THPY47	27/11/2009	16	TH111F	No	-	-	-	TH170M, TH160M
Thistle Is	THPY48	26/11/2009	17	TH166F	Yes	TH160M	Combination	-	-
Thistle Is	THPY49	26/11/2009	18	TH167F	Yes	TH097M	COLONY + combination	-	-

Site	Pouch Young ID	Date sampled	Litter number	Mother ID	Assigned paternity?	Assigned father	Assignment method*	For pouch young with unassigned paternity	
								Most likely candidate (> 80 %)	Other potential fathers (positive LOD score > 1.4 in CERVUS and < 2 loci mismatches at trio level)
Thistle Is	THPY50	26/11/2009	18	TH167F	Yes	TH097M	CERVUS + COLONY + combination	-	-
Thistle Is	THPY52		19	TH122F	No	-	-	TH147M	-
Thistle Is	THPY53		19	TH122F	No	-	-	TH147M	-
Thistle Is	THPY54		20	TH164F	No	-	-	-	TH189M, TH191M
Thistle Is	THPY55	30/11/2009	21	TH095F	Yes	TH168M	CERVUS + combination	-	-
Thistle Is	THPY56	30/11/2009	21	TH095F	No	-	-	-	TH168M, TH189M, TH176M
Thistle Is	THPY57	29/11/2009	22	TH156F	No	-	-	-	
Thistle Is	THPY58	29/11/2009	22	TH156F	No	-	-	-	TH150M
Thistle Is	THPY59	29/11/2009	23	TH114F	No	-	-	-	TH168M
Thistle Is	THPY60	29/11/2009	23	TH114F	Yes	TH168M	Combination	-	-
Thistle Is	THPY61	9/02/2010	24	TH164F	No	-	-	-	TH168M

* Assignment methods are 1) CERVUS – father assigned paternity at ≥95 % likelihood by CERVUS; 2) COLONY – father assigned at ≥95 % likelihood by COLONY or; 3) Combination – assigned most likely father by both CERVUS and COLONY and likelihood is > 80 % for at least one program.

Appendix 8

Paternity assignments and likely candidate fathers for pouch young at Venus Bay Conservation Park and Thistle Island using 10 loci for the analysis.

Site	Pouch Young ID	Date sampled	Litter number	Mother ID	Assigned paternity?	Assigned father	Assignment method*	For pouch young with unassigned paternity	
								Most likely candidate (> 80 %)	Other potential fathers (positive LOD score > 1.2 in CERVUS and < 2 loci mismatches at trio level)
Venus Bay	VBPY01	2/07/2008	1	VB04F	Yes	VB19M	CERVUS	-	-
Venus Bay	VBPY02	2/07/2008	1	VB04F	Yes	VB19M	CERVUS	-	-
Venus Bay	VBPY03	4/07/2008	2	VB10F	Yes	VB35M	Combination	-	-
Venus Bay	VBPY04	5/07/2008	3	VB11F	No	-	-	VB12M	VB02M, VB39M
Venus Bay	VBPY05	5/07/2008	4	VB13F	Yes	VB12M	CERVUS	-	-
Venus Bay	VBPY06	5/07/2008	4	VB13F	Yes	VB12M	CERVUS	-	-
Venus Bay	VBPY07	6/07/2008	5	VB23F	Yes	VB28M	COLONY + combination	-	-
Venus Bay	VBPY08	6/07/2008	6	VB25F	No	-	-	VB24M	VB35M, VB38M, VB03M, VB26M
Venus Bay	VBPY09	6/07/2008	6	VB25F	No	-	-	VB19M	-
Venus Bay	VBPY10	22/09/2008	7	VB09F	No	-	-	VB12M	-
Venus Bay	VBPY13	26/11/2008	8	VB13F	No	-	-	VB29M	VB58M
Venus Bay	VBPY14	26/11/2008	8	VB13F	Yes	VB58M	Combination	-	-
Venus Bay	VBPY15	26/11/2008	9	VB23F	Yes	VB31M	CERVUS	-	-
Venus Bay	VBPY16	26/11/2008	9	VB23F	Yes	VB31M	CERVUS	-	-
Venus Bay	VBPY17	26/11/2008	10	VB32F	Yes	VB35M	CERVUS + COLONY	-	-
Venus Bay	VBPY18	26/11/2008	10	VB32F	Yes	VB35M	COLONY + Combination	-	-
Venus Bay	VBPY19	27/11/2008	11	VB37F	Yes	VB58M	Combination	-	-
Venus Bay	VBPY20	27/11/2008	11	VB37F	Yes	VB58M	Combination	-	-
Venus Bay	VBPY21	27/11/2008	12	VB10F	Yes	VB27M	CERVUS + COLONY	-	-
Venus Bay	VBPY22	27/11/2008	12	VB10F	Yes	VB27M	CERVUS + COLONY	-	-

Site	Pouch Young ID	Date sampled	Litter number	Mother ID	Assigned paternity?	Assigned father	Assignment method*	For pouch young with unassigned paternity	
								Most likely candidate (> 80 %)	Other potential fathers (positive LOD score > 1.2 in CERVUS and < 2 loci mismatches at trio level)
Venus Bay	VBPY23	28/11/2008	13	VB04F	Yes	VB08M	CERVUS + COLONY	-	-
Venus Bay	VBPY24	28/11/2008	13	VB04F	Yes	VB08M	CERVUS + COLONY	-	-
Venus Bay	VBPY25	29/11/2008	14	VB11F	Yes	VB58M	CERVUS + combination	-	-
Venus Bay	VBPY26	29/11/2008	14	VB11F	Yes	VB58M	CERVUS + combination	-	-
Venus Bay	VBPY27	29/11/2008	15	VB41F	No	-	-	VB30M	VB24M, VB58M
Venus Bay	VBPY28	25/02/2009	16	VB11F	Yes	VB58M	CERVUS + Combination	-	-
Venus Bay	VBPY29	25/02/2009	17	VB23F	Yes	VB28M	CERVUS + COLONY	-	-
Venus Bay	VBPY30	25/02/2009	17	VB23F	Yes	VB28M	CERVUS + COLONY	-	-
Venus Bay	VBPY31	25/02/2009	18	VB10F	No	-	-	VB33M	VB06M, VB35M
Venus Bay	VBPY32	25/02/2009	18	VB10F	No	-	-	VB29M	VB58M
Venus Bay	VBPY33	26/02/2009	19	VB25F	No	-	-	VB42M	VB31M
Venus Bay	VBPY34	22/06/2009	20	VB10F	Yes	VB39M	CERVUS	-	-
Venus Bay	VBPY35	1/09/2009	21	VB47F	Yes	VB18M	CERVUS + Combination	-	-
Venus Bay	VBPY36	1/09/2009	22	VB50F	Yes	VB18M	CERVUS + Combination	-	-
Venus Bay	VBPY37	2/09/2009	23	VB13F	Yes	VB28M	CERVUS + COLONY	-	-
Venus Bay	VBPY38	2/09/2009	24	VB25F	No	-	-	VB19M	VB46M, VB07M, VB58M
Venus Bay	VBPY39	2/09/2009	24	VB25F	No	-	-	VB29M	VB58M
Venus Bay	VBPY40	5/09/2009	25	VB09F	Yes	VB28M	CERVUS	-	-
Venus Bay	VBPY41	6/09/2009	26	VB57F	Yes	VB55M	CERVUS	-	-
Venus Bay	VBPY43	17/11/2009	27	VB57F	Yes	VB18M	CERVUS + Combination	-	-

Site	Pouch Young ID	Date sampled	Litter number	Mother ID	Assigned paternity?	Assigned father	Assignment method*	For pouch young with unassigned paternity	
								Most likely candidate (> 80 %)	Other potential fathers (positive LOD score > 1.2 in CERVUS and < 2 loci mismatches at trio level)
Venus Bay	VBPY44	17/11/2009	27	VB57F	Yes	VB18M	Combination	-	-
Venus Bay	VBPY45	17/11/2009	28	VB09F	Yes	VB18M	CERVUS + Combination	-	-
Venus Bay	VBPY46	17/11/2009	29	VB13F	Yes	VB18M	CERVUS + Combination	-	-
Venus Bay	VBPY47	17/11/2009	29	VB13F	Yes	VB18M	CERVUS + Combination	-	-
Venus Bay	VBPY48	22/11/2009	30	VB50F	Yes	VB44M	CERVUS + combination	-	-
Venus Bay	VBPY49	27/01/2010	31	VB50F	No	-	-	VB26M	VB34M, VB15M
Venus Bay	VBPY50	27/01/2010	31	VB50F	Yes	VB26M	CERVUS	-	-
Venus Bay	VBPY51	29/01/2010	32	VB25F	Yes	VB44M	CERVUS + combination	-	-
Venus Bay	VBPY52	29/01/2010	32	VB25F	No	-	-	VB44M	VB52M
Venus Bay	VBPY53	30/01/2010	33	VB10F	Yes	VB44M	CERVUS + combination	-	-
Venus Bay	VBPY54	30/01/2010	33	VB10F	Yes	VB44M	CERVUS + combination	-	-
Venus Bay	VBPY55	26/01/2010	34	VB47F	Yes	VB18M	CERVUS + Combination	-	-
Venus Bay	VBPY56	26/01/2010	34	VB47F	Yes	VB18M	CERVUS + Combination	-	-

* Assignment methods are 1) CERVUS – father assigned paternity at ≥95 % likelihood by CERVUS; 2) COLONY – father assigned at ≥95 % likelihood by COLONY or; 3) Combination – assigned most likely father by both CERVUS and COLONY and likelihood is > 80 % for at least one program.

Site	Pouch Young ID	Date sampled	Litter number	Mother ID	Assigned paternity?	Assigned father	Assignment method*	For pouch young with unassigned paternity	
								Most likely candidate (> 80 %)	Other potential fathers (positive LOD score > 1.4 in CERVUS and < 2 loci mismatches at trio level)
Thistle Is.	THPY28	24/09/2009	1	TH027F	Yes	TH193M	CERVUS	-	-
Thistle Is.	THPY29	24/09/2009	2	TH146F	No	-	-	152M	-
Thistle Is.	THPY30	24/09/2009	3	TH134F	No	-	-	168M	-
Thistle Is.	THPY31	25/09/2009	4	TH096F	No	-	-	-	-
Thistle Is.	THPY32	26/09/2009	5	TH137F	Yes	TH097M	CERVUS + combination	-	-
Thistle Is.	THPY33	26/09/2009	5	TH137F	Yes	TH097M	CERVUS + combination	-	-
Thistle Is.	THPY34	25/09/2009	6	TH143F	No	-	-	TH150M	-
Thistle Is.	THPY35	25/09/2009	6	TH143F	Yes	TH150M	CERVUS	-	-
Thistle Is.	THPY36	26/09/2009	7	TH040F	No	-	-	TH153M	-
Thistle Is.	THPY37	27/09/2009	8	TH095F	No	-	-	TH060M	
Thistle Is.	THPY38	27/09/2009	9	TH120F	Yes	TH142M	CERVUS	-	-
Thistle Is.	THPY39	27/09/2009	10	TH131F	Yes	TH150M	CERVUS	-	
Thistle Is.	THPY40	28/09/2009	11	TH156F	No	-	-	-	-
Thistle Is.	THPY41	28/09/2009	12	TH111F	Yes	TH160M	CERVUS + combination	-	-
Thistle Is.	THPY42	28/09/2009	13	TH122F	Yes	TH147M	CERVUS	-	-
Thistle Is.	THPY44	20/09/2009	14	TH114F	Yes	TH168M	CERVUS + combination	-	-
Thistle Is.	THPY46	28/11/2009	15	TH181F	Yes	TH097M	CERVUS + combination	-	-
Thistle Is.	THPY47	27/11/2009	16	TH111F	Yes	TH160M	CERVUS + COLONY	-	-

Site	Pouch Young ID	Date sampled	Litter number	Mother ID	Assigned paternity?	Assigned father	Assignment method*	For pouch young with unassigned paternity	
								Most likely candidate (> 80 %)	Other potential fathers (positive LOD score > 1.4 in CERVUS and < 2 loci mismatches at trio level)
Thistle Is.	THPY48	26/11/2009	17	TH166F	Yes	TH160M	CERVUS + COLONY	-	-
Thistle Is.	THPY49	26/11/2009	18	TH167F	Yes	TH097M	CERVUS + combination	-	-
Thistle Is.	THPY50	26/11/2009	18	TH167F	Yes	TH097M	CERVUS + combination	-	-
Thistle Is.	THPY52		19	TH122F	Yes	TH147M	CERVUS + COLONY	-	-
Thistle Is.	THPY53		19	TH122F	Yes	TH147M	CERVUS + COLONY	-	-
Thistle Is.	THPY54		20	TH164F	Yes	TH189M	CERVUS	-	-
Thistle Is.	THPY55	30/11/2009	21	TH095F	Yes	TH168M	CERVUS + combination	-	-
Thistle Is.	THPY56	30/11/2009	21	TH095F	Yes	TH168M	CERVUS + combination	-	-
Thistle Is.	THPY57	29/11/2009	22	TH156F	No	-	-	TH150M	
Thistle Is.	THPY58	29/11/2009	22	TH156F	No	-	-	-	-
Thistle Is.	THPY59	29/11/2009	23	TH114F	Yes	TH168M	CERVUS + combination	-	-
Thistle Is.	THPY60	29/11/2009	23	TH114F	Yes	TH168M	CERVUS + combination	-	-
Thistle Is.	THPY61	9/02/2010	24	TH164F	Yes	TH168M	CERVUS + combination	-	-

* Assignment methods are 1) CERVUS – father assigned paternity at ≥95 % likelihood by CERVUS; 2) COLONY – father assigned at ≥95 % likelihood by COLONY or; 3) Combination – assigned most likely father by both CERVUS and COLONY and likelihood is > 80 % for at least one program.

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