



THE EFFECTS OF EXCLUSION FENCING ON THE  
YELLOW-FOOTED ROCK-WALLABY  
(*Petrogale xanthopus celeris*)

A Thesis submitted by  
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For the award of  
Doctor of Philosophy  
2020

This thesis is dedicated to the memory of

***Margaret Mary Hayden (née Arnold)***

(1931-2019)

who was my grandmother, a courageous and gentle soul, an avid reader, a passionate  
birdo/twitcher, and a third parent to me.

&

***Harold Graham Smith***

(1928-2015)

who was my grandfather, a lover of reading, the natural world and science, and an  
inspiring and interesting mind.

## Abstract

Many land managers have recently constructed exclusion fences around their properties in an effort to increase the productivity of sheep (*Ovis aries*) farming in central-western Queensland, Australia. These fences (known as cluster fences when they enclose more than one property) are used to exclude dingoes (*Canis familiaris*) from grazing properties and thereby eliminate livestock predation. The fences are also used by land managers as a tool to better manage total grazing pressure on their properties, usually through the removal of pest herbivores such as feral goats (*Capra hircus*) and red kangaroos (*Osphranter rufus*). While these fences are considered highly effective in this goal, what is less known is whether they have substantial impacts on other extant wildlife. This thesis aimed to elucidate this issue through an examination of positive and negative effects of pest cluster fences on a non-target wildlife species. First, potential positive effects were explored by investigating where threatened species distributions overlap with the cluster fencing in central western Queensland. Where species distributions did overlap with cluster fencing, threats to those species were checked against the proposed management of pest species within fences. From this examination, the yellow-footed rock-wallaby (YFRW; *Petrogale xanthopus celeris*) was identified as one species whose range and threats suggested it may benefit from pest species management within cluster fencing. As such, several potential effects of cluster fences (identified in a literature review) were also investigated for the species. From habitat scoring and camera trapping data, we found that YFRW habitat use and behaviour were similar both inside and outside the fences, although diel activity was considerably different. These data also revealed extensive spatial and temporal overlap between goats and YFRW, suggesting potential benefits to YFRW might accrue should goats be removed within fences. Finally, a genetic assessment of YFRW tissue samples collected at several colonies revealed that, whilst infrequent, YFRW do move between colonies over large distances. This aspect of the study indicated that fence placement may genetically isolate some colonies, which has the potential to create negative genetic consequences for the affected colonies in the long term. The project as a whole was limited by a lack of pre-fence data, and reliable data on predators. Despite these limitations, my research concluded that both positive and negative effects on non-target wildlife are likely to arise following the erection of cluster fences, but net effects remain unclear. I therefore propose that (1) non-target species be taken into account before and after exclusion fences are constructed, (2) at-risk species within the system be monitored to ensure negative effects do not exceed acceptable thresholds, and (3) mitigation strategies be employed if necessary to ensure negative outcomes do not outweigh the potential benefits of exclusion fencing to non-target species.

## Certification of Thesis

This Thesis is the work of Deane A. Smith except where otherwise acknowledged, with the majority of the authorship of the papers presented as a Thesis by Publication undertaken by the Student. The work is original and has not previously been submitted for any other award, except where acknowledged.

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## Statement of Contribution

#	Article	Contributions
1.	<p><b>Chapter 2:</b> SMITH, D., KING, R., &amp; ALLEN, B.L. (2020)</p> <p>Impacts of exclusion fencing on target and non-target fauna: a global review.</p> <p>Biological Reviews. doi:10.1111/brv.12631</p>	<p>DS – 75% RK – 10% BLA – 15%</p> <p>BLA and DS both contributed to the conception and design of the research that led to this manuscript. DS acquired the data. DS and RK analysed and interpreted the data. DS drafted the initial manuscript and all authors contributed critically to the drafts and gave final approval for publication.</p>
2.	<p><b>Chapter 3:</b> SMITH, D., WADDELL, K., &amp; ALLEN, B.L. (2020)</p> <p>Expansion of vertebrate pest exclusion fencing and its potential benefits for threatened fauna recovery in Australia.</p> <p>Animals, 10(9). doi:10.3390/ani10091550</p>	<p>DS – 70% KW – 10% BLA – 20%</p> <p>BLA, DS and KW contributed to the conception and design of the research that led to this manuscript. DS and KW acquired the data. DS analysed and interpreted the data. DS drafted the initial manuscript and all authors contributed critically to the drafts and gave final approval for publication.</p>
3.	<p><b>Chapter 6:</b> SMITH, D., &amp; ALLEN, B.L. (2021)</p> <p>Effects of predator exclusion fencing on habitat use by yellow-footed rock-wallabies in Australia.</p> <p>Journal of Arid Environments, 184. Doi: 10.1016/j.jaridenv.2020.104329</p>	<p>DS – 80% BLA – 20%</p> <p>The concept of this study was developed by BLA and DS. DS collected all data (with the help of volunteers) and conducted all data analysis. DS created the first draft of the manuscript. Both DS and BLA contributed to the editing of the manuscript for submission.</p>
4.	<p><b>Chapter 7:</b> SMITH, D., TAYLOR, M., &amp; ALLEN, B.L. (in review)</p> <p>Pest management effects and diel activity of yellow-footed rock-wallabies and sympatric pest animals.</p>	<p>DS – 70% MT – 15% BLA – 15%</p> <p>The concept of this study was developed by BLA. DS and MT collected and entered all data (with the help of volunteers) and DS conducted all data analysis. DS created the first draft of the manuscript. All authors contributed to the editing of the manuscript for submission.</p>
5.	<p><b>Chapter 8:</b> SMITH, D., LETHBRIDGE, M.R., ALLEN, B.L., &amp; ANDREW, R.L. (in review)</p> <p>Movement between yellow-footed rock-wallaby (<i>Petrogale xanthopus</i>) colonies, inferred from estimates of kinship</p>	<p>DS – 65% MRL – 20% BLA – 5% RLA – 10%</p> <p>DS and MRL collected tissues samples from Queensland and South Australia, respectively. The methods and study design was decided on by DS and RLA as part of a broader project conceptualised by DS and BLA. DS performed all analysis. The main body of the writing was completed by DS. All authors contributed to the editing and review of the manuscript pre-submission.</p>

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A handwritten signature in black ink, appearing to read 'Deane A. Smith', written in a cursive style.

- Deane A. Smith

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## **List of acronyms (alphabetically listed)**

- i. AEC – **A**nimal **E**thics **C**ommittee
- ii. AGRF – **A**ustralian **G**enome **R**esearch **F**acility
- iii. CE – **C**ritically **E**ndangered
- iv. DArT – **D**iversity **A**rrays **T**echnology
- v. DBF – **D**ingo **B**arrier **F**ence
- vi. DNA – **D**eoxyribonucleic **A**cid
- vii. EGK – **E**astern **G**rey **K**angaroo (*Macropus giganteus*)
- viii. EPBC – **E**nvironmental **P**rotection and **B**iodiversity **C**onservation Act (1999)
- ix. HS – **H**abitat **S**core
- x. IUCN – **I**nternational **U**nion for the **C**onservation of **N**ature and **N**atural **R**esources
- xi. LWDEFS – **L**ongreach **W**ild **D**og **E**xclusion **F**encing **S**cheme
- xii. PMST – **P**rotected **M**atters **S**earch **T**ool
- xiii. RAPAD – **R**emote **A**rea **P**lanning and **D**evelopment (Board)
- xiv. RK – **R**ed **K**angaroo
- xv. SILO – **S**cientific **I**nformation for **L**and **O**wners (resource)
- xvi. SNP – **S**ingle **N**ucleotide **P**olymorphism
- xvii. SPRAT – **S**pecies **P**rofile and **T**hreats **D**atabase
- xviii. TGP – **T**otal **G**razing **P**ressure
- xix. TSP – **T**hreatened **S**pecies
- xx. YFRW – **Y**ellow-footed **r**ock-**w**allaby

## Glossary (alphabetically listed)

- i. At-risk species – Species listed as threatened, endangered or critically endangered by any level of government or the IUCN.
- ii. Camera-day – A 24 hour period that any given camera operates over. If Camera-1 was active 10 camera-days, and Camera-2 for 8 camera-days concurrently, then Site-1<sup>(Camera-1 + Camera-2)</sup> was active for 18 camera-days.
- iii. Cluster fence – An exclusion fence built around multiple properties for the purpose of pest exclusion.
- iv. DArTseq – A form of genetic (complexity reduction) sequencing performed by Diversity Arrays Technology (DArT).
- v. Diel – occurring in a twenty-four hour period.
- vi. Dingo Barrier Fence (DBF) – An historical exclusion fence, running ~5000km from South Australia through Queensland. Erected in the late 19<sup>th</sup> century and maintained since.
- vii. Euro – Common name used for *Osphranter robustus*, also known as a Wallaroo (previously *Macropus robustus*).
- viii. Exclusion fence – A fence built for the purpose of excluding a single or multiple species for a variety of ecological or economic purposes.
- ix. Macropod/id/ae – Macropods or macropodids or macropodidae is a family of marsupials consisting of kangaroos, rock-wallabies and many other hopping marsupials. Macro = large, Pod = foot.
- x. Metapopulation – A group of spatially separated populations of the same species that are connected through interactions or inter-population movement.
- xi. Pest – Species (both native and exotic) that can be controlled by farmers and land holders due to a stated negative impact.
- xii. Risk-effect – A change in species behaviour as a result of fear of predation, often described in the context of a trade-off between resources and predation risk.
- xiii. SNP – (see common acronyms) Pronounced ‘snip’, a change is a single base nucleotide of DNA that can be used to inform genetic diversity.
- xiv. Wild dog – Dingoes, feral dogs and their hybrids, *Canis familiaris*.



## Provisions

- 1) This thesis is formatted with the intention of publishing each primary chapter as discrete journal articles. As a result, some content may be repeated in multiple chapters.
- 2) Due to the editorial process and formatting requirements of various journals, there may be some minor stylistic differences between the contents of the chapter in this thesis and its corresponding published manuscript, though the underlying data and analyses remain the same. This has also resulted in some inconsistent formatting within this thesis.
- 3) The contributions of each co-author to each chapter have been listed in the *Author Contributions* section of each chapter, and in the Statement of Contribution section. Where no *Author Contributions* section is present, the entirety of that chapter's contents are attributed to D. Smith.
- 4) All scientific species names within this document follow binomial nomenclature guidelines and are sourced from the Australian Faunal Directory (AFD) website ([biodiversity.org.au/afd/home](http://biodiversity.org.au/afd/home)) or from the Mammal Diversity Database ([mammaldiversity.org](http://mammaldiversity.org)). If both these databases did not contain a record of the species, the Integrated Taxonomic Information System (ITIS) website ([www.itis.gov](http://www.itis.gov)) was queried.
- 5) The study and use of animals for this thesis was approved by the University of Southern Queensland's Animal Ethics Committee: AEC-USQ-17REA011
- 6) This thesis may be cited as:  
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## **Chapter 1 : Introduction**

Biodiversity is in decline globally, with species currently going extinct at much higher rates than historical averages (Butchart et al., 2010; De Vos et al., 2015) as the human population grows and habitats are destroyed or fragmented (Hoffmann et al., 2010). Australia has a particularly poor record, with more than 10% of its terrestrial mammal species having gone extinct in the last 200 years (Woinarski, Burbidge, & Harrison, 2015). The causes of these extinctions have been attributed to a number of threatening processes and the mitigation of these processes continue to be a focus of conservation efforts (Australian Government, 2015; Burbidge & McKenzie, 1989). Extinctions and declines of native Australia fauna have occurred broadly across the Australian continent, including on agricultural lands. While unintentional, management practices on agricultural lands can often lead to negative outcomes for wildlife species through habitat degradation and fragmentation, and landscape modifications that result in heightened predation risk (Woinarski, Burbidge, & Harrison, 2015). Given that livestock grazing occurs across more than half of the Australian land mass (Allen, 2011) and is known to cause considerable harm to biodiversity (see Williams & Price, 2010), an in-depth understanding of threats and conservation of wildlife species on agricultural lands may be critical to their persistence.

Exclusion fencing is a style of fencing defined by its goal of completely restraining all movement of a target species into an enclosure. These fences have been employed globally for a wide variety of purposes (Chapter 2). While they are often highly effective at achieving their stated goal, such as the protection of threatened species on conservation lands (e.g. Hayward et al., 2015; Short & Hide, 2014, 2015) or stemming the flow of pest species onto agricultural lands (RAPAD & QFPI, 2018a), such fences may have unintended impacts on non-target species that are present around and within the fenced area. This is evidenced by literature from an array of fence types that details fences causing adverse effects such as death through entanglement (e.g. Ferronato, Roe, & Georges, 2014), changes in behaviours (e.g. Davies-Mostert, Mills, & Macdonald, 2013), and genetic consequences for species (e.g. Hepenstrick et al., 2012). While fences may have these and other negative outcomes, there is also the potential for the fences to unintentionally benefit

species when the target species excluded is also a threat (via predation or competition) to the non-target species.

In parts of Australia, wild dogs (*Canis familiaris*) are extensively controlled through lethal baits and trapping to prevent stock losses, primarily of sheep (*Ovis aries*), as a result of wild dog predation (Allen & West, 2013, 2015). Exclusion fencing has also been a commonly employed tool for the management of wild dogs and other pests in post-European settlement Australia. The most famous instances of exclusion fencing in Australia are the Dingo Barrier Fence (DBF), a ~5,600 km fence that stretches from the Great Australian Bight to south-east Queensland, and the ~3,200 km of rabbit-proof fences of Western Australia (Holder et al., 1893; Rolls, 1969; Yelland, 2001). These barrier fences aim to exclude wild dogs and European rabbits (*Oryctolagus cuniculus*) from large, agriculturally productive portions of Australia and formed just a part of extensive exclusion fencing networks of the time. Up until the 1930s, there was also an estimated 46,000 km of rabbit-proof fencing and 32,000 km of dog-proof fences in Queensland alone, though there was some overlap of the two (Department of Primary Industries and Fisheries, 2007). This network of wild dog cell fences (areas of exclusion fenced land for the purpose of wild dog exclusion) largely fell into disrepair after the DBF Scheme was introduced in 1948, effectively excluding wild dogs from the most productive sheep grazing areas of south-east of Australia (Department of Primary Industries and Fisheries, 2007). Despite the DBF being maintained to this day, wild dogs have again become a problem in sheep grazing areas and graziers on both sides of the DBF have begun re-constructing the former network of cell fences to protect their livestock, using modern fencing materials. In the last ~10 years, thousands of kilometres of exclusion fences have been erected around groups or clusters of adjacent livestock properties and the wild dogs removed from within. Such ‘cluster fences’, as they have come to be known, prevent immigration of wild dogs and other pests (such as feral goats and red kangaroos) from neighbouring properties, and also prevent livestock escaping. Cattle, sheep, goats, kangaroos and wild dogs are the primary targets of cluster fencing. Though the fences do occasionally have breaches (Giumelli & White, 2016), they are reportedly highly effective at reducing livestock losses and therefore economic losses caused by wild dogs (an average increase in lamb survival of over 40%; see RAPAD & QFPI, 2018a). However, cluster fences are likely to have unintended impacts on non-target species just as other analogous fencing has had (Chapter 2). Within Australia there are already several examples of fencing negatively impacting native species

(e.g. Bradby et al., 2014; Ley & Tynan, 2008). Thus it is imperative that we understand what species are present in the system and assess whether they may experience unintended impacts to avoid further destruction of biodiversity. The effects of fencing on wildlife may be broad and different types of fencing may also affect species in different ways, both positive and negative.

To synthesise an understanding of how cluster fencing affects wildlife, a review of exclusion fencing related literature and an examination of the impacts on wildlife species globally is required. Although similar reviews have been performed in the past, they have focused on only one type of fencing or one geographic location (e.g. Ferguson & Hanks, 2010; Hayward & Kerley, 2009; Mbaiwa & Mbaiwa, 2006). These reviews also stress the need for more empirical research on fencing impacts. As such, this thesis begins with a comprehensive review of all fencing literature, assessing what positive and negative impacts have been documented, and strategies that have been employed in the past to mitigate these effects (Chapter 2). From this review it was clear that cluster fencing could have positive, negative, and/or neutral effects on non-target species in central-western Queensland, the study region of relevance to this thesis research.

Given the above assessment, I aimed to identify which threatened species occupy the same region as the cluster fences and may have costs and benefits from the fence's construction. As previously stated, the primary goal of cluster fencing in central-western Queensland is to exclude and remove or reduce the predators and competitors of livestock within fences, such as dingoes and kangaroos. Australia has the highest rate of mammal extinction in the world (Woinarski, Burbidge, & Harrison, 2015) and this is largely attributed to predation pressure and competition from introduced species (Burbidge & McKenzie, 1989; Department of the Environment, 2019c; Dickman, 1996; Legge et al., 2017), including many of the same species controlled within exclusion fences (Giumelli & White, 2016; Hacker, Sinclair, & Pahl, 2019). Hence, while the goal of the cluster fencing is not conservation of threatened species *per se*, such species may benefit from fencing and pest removal activities. To test this potential, we compared threatened species distributions with fence placement, and further compared threats to those non-target species with the target species controlled/managed within cluster fencing (Chapter 3).

From these two preliminary evaluations (Chapter 2 and Chapter 3), the yellow-footed rock wallaby (YFRW, *Petrogale xanthopus*) was identified as one native species that may experience both positive and negative outcomes from exclusion fencing. There are two subspecies of YFRW: *P. x. xanthopus* in South Australia and New South Wales and *P. x. celeris* which inhabits the rocky remnant cliffs of the Grey Range in central-western Queensland (Copley, 1983; Gordon, McGreevy, & Lawrie, 1978; Wilson, Gerritsen, & Milthorpe, 1976). Like all rock wallabies, YFRW are a medium sized macropod that have a strong preference for complex rocky habitats (Gordon et al., 1993; Lim & Giles, 1987). Though adults are outside the critical weight range (CWR; 35 to 5500 g) most at risk from predation by smaller invasive predators particularly foxes (Burbidge & McKenzie, 1989), their young (joeys) evacuate the pouch while small, and hence fall within the CWR, which contributes to high juvenile mortality (Lethbridge et al., 2010; Sharp, 2002). While evidence of wild dog predation on YFRW is largely anecdotal/opportunistic, wild dogs are known predators of other rock-wallaby species (Allen et al., 2012; Brook & Kutt, 2011; Whitehouse, 1977). Furthermore, YFRW have a large dietary overlap with feral goats (Allen, 2001; Dawson & Ellis, 1979) and competition with goats is a key threatening process for the species (Threatened Species Scientific Committee, 2016a). Feral goats are often partly managed within clusters. Control or management of these pest species within cluster fences may therefore benefit YFRW. However, YFRW may also experience negative impacts as a result of fences, such as behavioural adaptation to the reduced density of predators (Chapter 6 and Chapter 7), or genetic consequences (Chapter 8). As a result of their habitat specificity, the YFRW and other *Petrogale* species' habitat is already naturally fragmented, and colonies of rock-wallabies are often isolated from other populations. This can result in inbreeding, and other genetic consequences, such as those observed by Eldridge et al. (1999) who found strong evidence of inbreeding depression in an isolated population of the closely related black-footed rock-wallaby (*Petrogale lateralis*). Cluster fencing may further exacerbate this issue, due to reduced dispersal opportunities across fenced areas. Consequently, this thesis aimed to examine the potential positive and negative effects of exclusion fences on non-target species using YFRW in Queensland as a case study.

To resolve the stated aims of this thesis, YFRW were assessed based on their overlap in distribution with the exclusion fenced areas (Chapter 3), whether pest species removal was likely to be of benefit to them (Chapter 3 and Chapter 7), their habitat use across fence lines

(Chapter 6), diel behaviour across fence lines (Chapter 7), and the genetic diversity and relatedness of separated colonies (Chapter 8). Additional information on the study site and methods are given in Chapter 4 and Chapter 5. The results of these research components were then discussed in light of similar global studies to form an overall evaluation of the potential impacts of cluster fencing on non-target species on this topic (Chapter 9). Additionally, while several peripheral and expanded research directions could have been undertaken alongside this project, the scope of this project was restricted by project funding and to those achievable within a three year timeframe. These research opportunities, as well as future research priorities, are also outlined Chapter 9.

## **Chapter 2 : Impacts of exclusion fencing on target and non-target fauna: a global review**

**Reference:** Smith, D., King, R., & Allen, B.L. (2020). Impacts of exclusion fencing on target and non-target fauna: a global review. *Biological Reviews*. doi:10.1111/brv.12631  
(Published: 28<sup>th</sup> of July, 2020)

### **2.1 Abstract**

Exclusion fencing is a common tool used to mitigate a variety of unwanted economic losses caused by problematic wildlife. While the potential for agricultural, ecological and economic benefits of pest animal exclusion are often apparent, what is less clear are the costs and benefits to sympatric non-target wildlife. This review examines the use of exclusion fencing in a variety of situations around the world to elucidate the potential outcomes of such fencing for wildlife and apply this knowledge to the recent uptake of exclusion fencing on livestock properties in the Australian rangelands. In Australia, exclusion fences are used to eliminate dingo (*Canis familiaris dingo*) predation on livestock, prevent crop-raiding by emus (*Dromaius novaehollandiae*), and enable greater control over total grazing pressure through the reduction of macropods (Macropodidae) and feral goats (*Capra hircus*). A total of 208 journal articles were examined for location, a broad grouping of fence type, and the reported effects the fence was having on the study species. We found 51% of the literature solely discusses intended fencing effects, 42% discusses unintended effects, and only 7% considers both. Africa has the highest proportion of unintended effects literature (52.0%) and Australia has the largest proportion of literature on intended effects (34.2%). We highlight the potential for exclusion fencing to have positive effects on some species and negative effects on others (such as predator exclusion fencing posing a barrier to migration of other species), which remain largely unaddressed in current exclusion fencing systems. From this review we were able to identify where and how mitigation strategies have been successfully used in the past. Harnessing the potential benefits of exclusion fencing while avoiding the otherwise likely costs to both target and non-target species will require more careful consideration than this issue has previously been afforded.



## 2.2 Introduction

As humans' land usage spreads further into the natural environment in pursuit of resources there has been an increased effort to separate natural and anthropogenic environments. This task has fallen almost completely to fences. Exclusion fences separate wildlife from humans (Kassily, 2002), agriculture from pest species (McKnight, 1969), at-risk wildlife from threatening processes (Hayward & Kerley, 2009), and vectors of disease from livestock (Jori & Etter, 2016). While these fences often fulfil their intention, both target and non-target species within the system may experience unintended side effects.

Ecological cost/benefit reviews of exclusion fences used for conservation have been conducted both worldwide (Hayward & Kerley, 2009) and also as a focus within Australia (Dickman, 2012). Similarly, the costs and benefits of reserve and veterinary cordon fences (VCFs) in Africa has been explored (Hoare, 1992; Taylor & Martin, 1987). These reviews emphasise the need for empirical studies on the effects of fences and broadly highlight similar benefits and costs. The ecological benefits in the case of conservation fencing is the separation of an at-risk species and the factors that may threaten it (Hayward & Kerley, 2009), usually with the aim of decreasing mortality in the threatened species. This is typically in the form of restricting the movement of poachers or excluding invasive predators and competitors. On the other hand, the aggregation of costs (external to economic costs) listed by Hayward and Kerley (2009), Taylor and Martin (1987), and Dickman (2012) are entanglement, restriction of access to resources, unnatural or uneven resource use, the blocking of migration routes, overabundance, prey naivety, restriction of evolutionary potential, inbreeding and isolation.

Though each of these reviews are concerned with the costs and benefits of one type of exclusion fencing, it could reasonably be assumed that similar ecological costs and benefits may be found in all other forms of exclusion fencing. Other forms of exclusion fence have been used stop the spread of species (McKnight, 1969) and disease (Taylor & Martin, 1987), hold livestock species that cannot be held by standard (simple or less restrictive) livestock fences (Webb et al., 2009), stop the movement of wildlife onto roadways (Bissonette & Rosa, 2012), and control human movement rather than wildlife (Linnell et al., 2016; Peters et al., 2018; Pokorny et al., 2017).

Most exclusion fences have been designed with target species in mind, but these designs can also affect other species (non-target species) which may experience net positive or negative effects. For example, conservation fence construction and the removal of predators may also have a positive outcome on non-target prey species, as small species and proficient climbers, may use fenced enclosures as refuge zones (Arthur, Pech, & Dickman, 2005). These species pass through the fence and leave their predators behind (Dickman, 2012). Conversely, the same conservation fence may preclude other species and have undesirable effects. One such example from Australia found restricted movement and death caused by entanglement in several reptilian species on a conservation fence designed to exclude invasive predators from native mammalian species (Ferronato, Roe, & Georges, 2014). This study also acknowledged that the fence may be of benefit to the same entangled reptile species within the enclosure due to the decrease in nest raiding and direct predation from red foxes (*Vulpes vulpes*), feral cats (*Felis catus*) and dingoes (*Canis familiaris dingo*).

Pest exclusion fencing has been used to exclude dingoes from agricultural properties in Australia since the 1870s (Long & Robley, 2004) and its use had become widespread as a method of pest exclusion in Australia by the middle of the 20<sup>th</sup> century (McKnight, 1969). Fences currently being built in the eastern states of Australia have the intention of restricting the movement of dingoes onto sheep (*Ovis aries*) grazing properties (Clark, Clark, & Allen, 2018). They also should allow graziers to manage their total grazing pressure (TGP) better by enabling longer-lasting control of introduced species and abundant native species, such as the goat (*Capra hircus*) and red kangaroo (*Osphranter rufus*; Waters et al., 2019). These exclusion fences (also referred to as ‘cluster fences’ due to the fence enclosing a group or ‘cluster’ of properties) have been successful in significantly decreasing predation rates on livestock when combined with other forms of dingo control (RAPAD & QFPI, 2018a). This is unsurprising given the success of similarly designed fences and control programs used against the same predator species in conservation (Dickman, 2012), and also the historical success of using such fences to eradicate dingoes from large tracts of land in the early 20<sup>th</sup> century (McKnight, 1969; Yelland, 2001).

Understanding the effects of exclusion fencing on the environment remains an important research priority in Australia and globally. As more fences are constructed, recognising

what species are present and determining whether mitigation strategies can be employed may be incredibly important to the preservation of natural processes and ecosystem services. Novel solutions to some of these impacts have been utilised in other forms of fencing, in other parts of the world (e.g. Dupuis-Desormeaux et al., 2016). A review of the global literature on all fence types would allow land managers to assess how exclusion fences have impacted wildlife species in the past and consider the best mitigation strategies for the species present in their system, with their fence.

Given the knowledge gaps and the recent uptake of cluster fencing use in agriculture in Australia, we assess the possible effects of exclusion fencing on both target and non-target fauna through a review of exclusion fencing literature from across the world. We observe the spatial and temporal distribution of exclusion fencing literature and identify the environmental benefits and costs of fencing discussed in this literature. We also highlight strategies that have been employed elsewhere to mitigate exclusion fencing costs and discuss whether they should be explored before and after new exclusion fences are constructed.

## 2.3 Methods

We searched the Web of Science database on the 26<sup>th</sup> of July, 2018, using the Advanced Search tab, for the following terms:

**TS = ("barrier fence\*" OR "exclusion fence\*" OR "conservation fence\*" OR "veterinary cordon fence\*" OR "pest fence\*" OR "predator fence\*" OR "fenced reserve\*" OR "proof\* fence\*" OR "veterinary fence\*" OR "exclosure fence\*" OR "cluster fence\*")**

The field tag 'TS' indicates a Topic Search, which inspects the title, abstract and keywords of the literature in the database for the search terms. Single-word search terms such as "fence\*" and "reserve\*" were deliberately avoided because they yielded excessive noise (>4 million results). Our search included all years and document types. After removing duplicates, the abstract from each document was then read to confirm its applicability to the research topic being explored. The ultimate purpose of these search criteria was to identify original articles reporting effects of exclusion fences primarily on terrestrial vertebrate mammals. As such, papers were excluded if they (1) solely described effects on soil, plants, insects, fish and invertebrates, (2) only discussed economic costs, (3) were a

reply or comment paper, (4) only mentioned fences as a potential future solution to a problem, (5) had no obvious relevance to the research area, such as political or border security literature concerning fences or walls, or (6) did not measure or acknowledge any effect of the fence (intended or unintended). The remaining articles were all read in full and searched for further relevant literature cited in their reference lists. Additional papers known to the authors but not identified during the search were also added. Notes were taken on all papers during reading, including information on the (1) study location, (2) target and non-target species involved, and the (3) type of fence and its intended function, so that the effects of the fences could be classified as intended or unintended.

We used the following definitions to distinguish different types of fences:

- **Barrier fence** – A linear barrier erected for the purpose of pest species control (e.g. the Dingo Barrier Fence in Australia).
- **Border fence** – A linear fence along the boundary of two countries, erected for the purpose of controlling human migration (e.g. the USA-Mexico border fence).
- **Conservation fence** – A typically enclosed or circular fence, or one that dissects a coastal peninsula, erected and cleared of undesirable species for the purpose of introducing another (usually threatened or endangered) species of conservation concern (e.g. Arid Recovery Reserve in South Australia).
- **Agricultural exclusion fence** – Similar to a conservation fence but erected and cleared of undesirable species for the purpose of protecting livestock or crops (e.g. fences excluding feral pigs from crops in the USA, or cluster fences excluding dingoes from sheep properties in Australia).
- **Experimental fence** – A small circular fence erected for scientific or experimental purposes, often for later use in pest exclusion projects.
- **General fence** – A fence built for a general fencing purpose, such as containing domestic livestock species, but still inadvertently impacting some wildlife species.
- **Reserve fence** – A typically enclosed fence built around an area to conserve biodiversity. Species may also be released into these fenced areas, but differs from the conservation fence definition (above) in that the species protected are not necessarily of high conservation concern (e.g. Addo Elephant Park in South Africa).
- **Road fence** – Erected along edges of major roads or railways to reduce wildlife/transport collisions, often by redirecting wildlife to under- or overpasses.

- **Veterinary cordon fence (VCF)** – Barrier fences (above) erected to separate managed livestock from wild-living disease vector species that pose a risk to livestock. Such fences often consist of two parallel fences with space in between that is not populated by either the vector species or the livestock species (e.g. foot-and-mouth fence in northern Kruger National Park).

All fence effects were recorded and classified as entanglement, pest exclusion, predator exclusion, separation (of disease vectors from other species), barrier to movement, behavioural change, overpopulation, barrier to migration, redirection (of species towards over- or under-passes, or fence gaps and gates), barrier to gene flow, or evolutionary restriction (see Discussion – Genetic implications). These were included because previous work had identified these effects to be the most common and important environmental effects of fences. Multiple fence effects could be recorded for each paper. Pest exclusion, predator exclusion, separation and redirection were typically deemed to be the intended purpose of fence construction, and all others were considered unintended effects.

Chi-squared tests were used to assess whether each continent was represented equally in the literature-search results. Chi-squared tests were also used to assess whether the fence class categories, the fence effects, and the proportions of intended and unintended effects were equally represented in the literature search results. This analysis required some pooling of categories where expected frequencies were too low (less than 5). For the analysis of intended and unintended consequences the fence types were pooled into barriers (barrier fence, border fence), conservation-style fences (conservation and reserve), and agricultural/pest enclosures (agricultural exclusion, general fences and experimental fences). VCFs are mostly used for agricultural purposes by creating a linear barrier of two parallel fences, and road fencing often has an end point or wildlife crossing, so these classes were not pooled. For comparison, fences were also pooled into linear (barrier, border, VCF and road fences) and enclosure (conservation, agricultural exclusion, experimental, general and reserve fences) groups. The proportion of literature for each fence class was calculated with 95% confidence intervals (1000 bootstraps). We also observed an increase in fencing literature over time and fitted with a generalized linear model; however, there are several limitations to the meaningfulness of this analysis (see Discussion). Statistical analyses were performed in R v3.6.2 (R Core Team, 2019). All graphs were produced in Microsoft Excel

or R v3.6.2 using the package `ggplot2` v3.2.1 (Wickham, 2016). A map showing the percentage of papers per country was produced in ArcMap v10.5.1 (Environmental Systems Research Institute, 2019).

## 2.4 Results

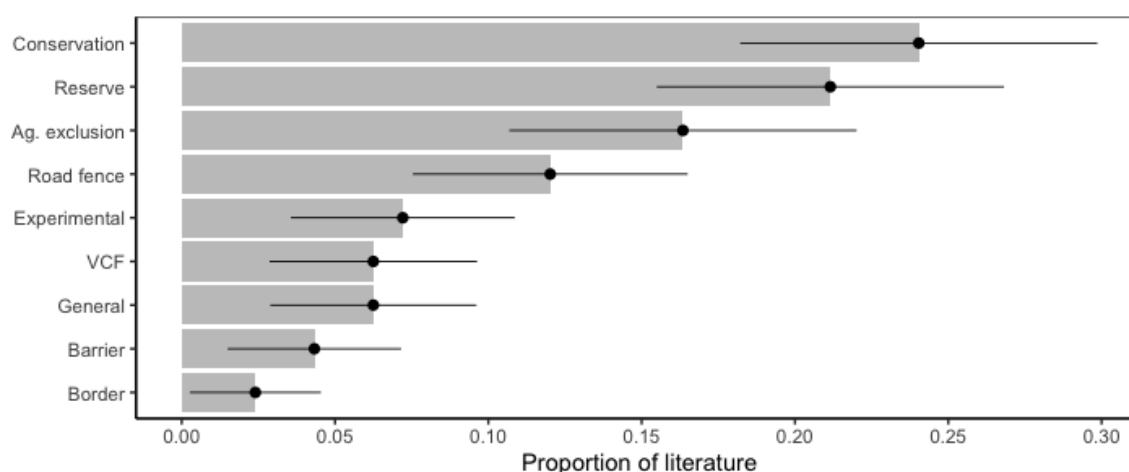


**Figure 2.1: Proportion of fencing literature from each continent**

Tree map displaying the proportion of identified literature from each continent that discusses unintended effects ( $\Delta$ ), discusses intended effects ( $\times$ ), or discusses both intended and unintended effects ( $\bullet$ ). N/A, no specific location identified.

Our search initially found 254 articles, but after applying our inclusion-exclusion criteria, only 93 articles were read in full. We added another 71 articles from their reference lists, and a further 44 from their reference lists too, making 208 articles in total (Table S1). Papers describe fences on six continents, including Africa (28.8%), Australasia (24.5%), North America (24.0%), Europe (13.9%), Asia (4.8%) and South America (1.4%; Figure 2.1). A small number of papers (2.4%) did not address a specific location. Papers per continent ( $n = 203$ , excluding Antarctica and papers with no specific location) were not equally distributed, ( $\chi^2(5) = 82.25, p < 0.001$ ), with a higher proportion related to fences in Africa, Australasia and North America and a lower proportion associated with Asia and South America. Papers originated from 29 countries (Figure S1) and 86 states or regions. The most represented countries were the USA (19.7%), Australia (16.3%) and South Africa (15.4%), which collectively represent 51.4% of all papers.

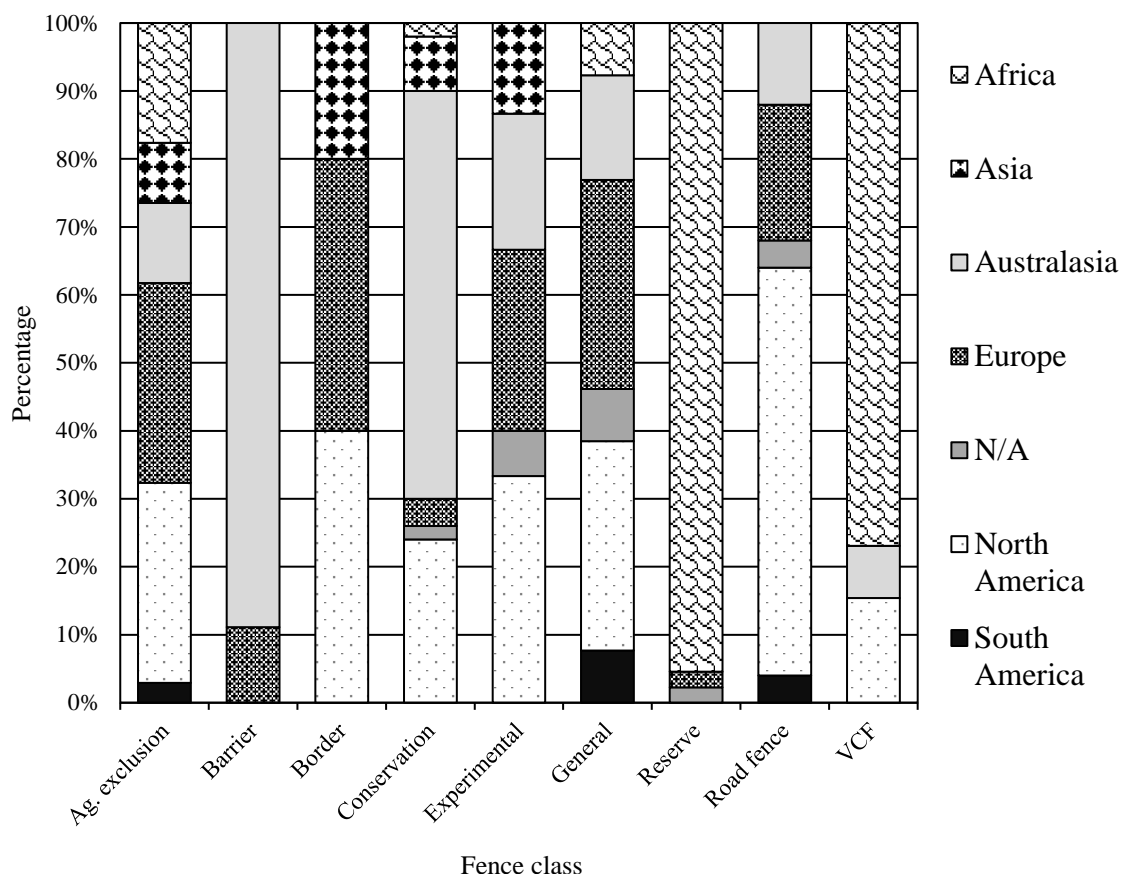
The division of fences by class can be seen in Figure 2.2. Chi-squared tests showed that the proportions of papers (n = 208) were not equally distributed among fence types ( $\chi^2(8) = 89.95, p < 0.001$ ), with a higher proportion related to conservation and reserve fences and a lower proportion associated with border barriers and barrier fences. Australasia had the largest percentage of papers on barrier fences (80.0%) and conservation fences (57.7%), and Africa on reserves (95.2%) and VCFs (77.0%; Figure 2.3).



**Figure 2.2: Proportion of literature regarding each fence class**

Bar chart displaying the proportion of identified literature for each exclusion fence class [Conservation, Reserve, Agricultural exclusion, Road fence, Experimental, Veterinary cordon fencing (VCF), General, Barrier and Border] with 95% confidence intervals. Total number of studies = 208.

Effects on at least 164 vertebrate species were discussed (Table S2), with African elephant (*Loxodonta africana*; 9.1%), lion (*Panthera leo*; 8.7%), red fox (7.6%) and domestic cattle (*Bos Taurus*; 7.2%) most often mentioned. A total of 304 fence effects were discussed in all 208 papers (Table 2.1). The breakdown of how often each effect was reported in the literature can be viewed in Figure S2. A chi-squared test indicated that the literature (n = 304) was not evenly distributed among the fencing effect categories ( $\chi^2(10) = 171.24, p < 0.001$ ).



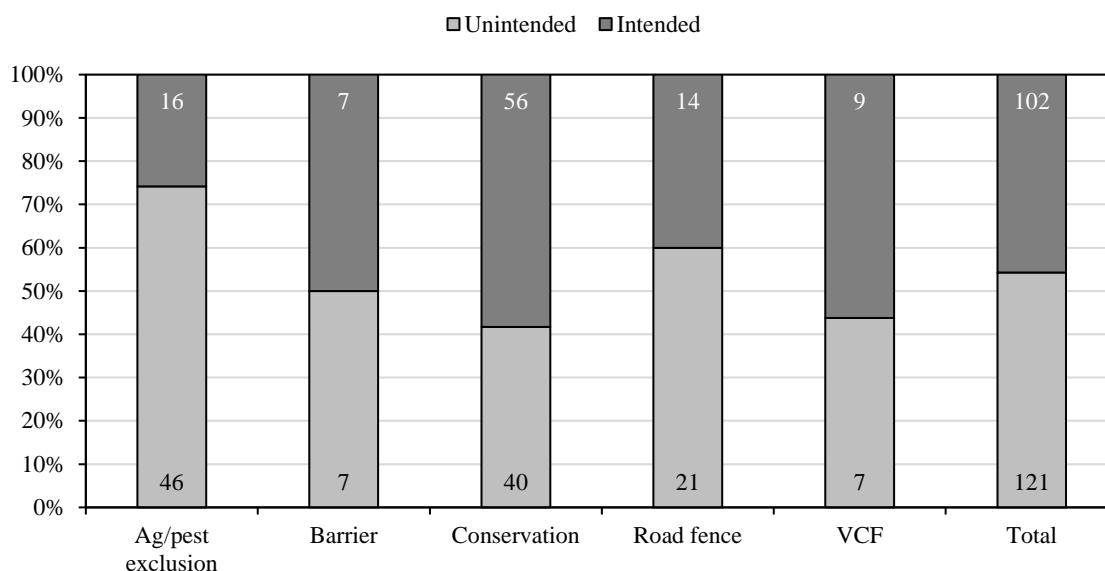
**Figure 2.3: Proportion of all fencing classes' literature in each continent**

Proportions of fencing class literature in each continent (total number of studies = 208). N/A, no specific location identified. Ag. Exclusion, agricultural exclusion fence; VCF, veterinary cordon fencing.

Approximately 51.0% of the literature discussed only intended fence effects, 41.8% discussed only unintended effects, and 7.2% discussed both intended and unintended effects (Figure 2.1). A chi-squared test assessing whether intended and unintended effects were evenly distributed among continents was not performed due to low samples in some categories. However, a total of 52.0% of papers that discuss possible negative fence effects have occurred in Africa, and 13.7% in North America. The largest group of papers discussing intended fence effects papers occurred in Australasia (34.2%), followed by North America with 33.3%. A total of 11.1% of papers reported fences directly causing death, 15.3% discussed an unintended effect on a non-target species, and 36.1% showed an unintended effect on the target species. A total of 30 papers (14.4%) discussed possible genetic consequences, nine of which present primary evidence (4.3%). A chi-squared test indicated that the literature ( $n = 223$ ) was not evenly distributed among fence classes ( $\chi^2(4) = 17.34, p < 0.005$ ), when fences classes were pooled into five categories as previously described (Figure 2.4). However, when fence classes were pooled into linear barrier and



enclosure groups, the chi-squared test was not significant ( $\chi^2(1) = 6.8^{-31}$ ,  $p = 1$ ). Publication year ranged from 1938 to 2018, with 2011 as the median year and 2016 the modal year (20 publications). An average of 2.6 papers have been published per year, which appears to be increasing, from 1938–2018 (Figure S3).



**Figure 2.4: Proportion of recorded intended and unintended fence effects by fence group**

Percentage of papers discussing intended and unintended effects by fence classes (grouped by style): Agricultural/pest enclosures, Barrier fences, Conservation fences, Road fence and veterinary cordon fencing (VCF). Literature that discussed both intended and unintended effects were included in both groups. Values in bars show counts of papers

**Table 2.1: Table of fence effects by fence class**

Table of fence effects by fence class. Numbers indicate counts of effects recorded across each fence class from the literature. Ag. Exclusion, agricultural exclusion fence; VCF, veterinary cordon fencing.

Fence Class	Ag. exclusion	Barrier	Border	Conservation	Experimental	General	Reserve	Road fence	VCF	Total
Barrier to movement	2	1	5	8	1	4	35	14	9	79
Predator exclusion	1	4	0	33	7	0	0	0	0	45
Pest exclusion	30	3	0	4	6	0	0	0	0	43
Behavioural change	0	0	0	4	0	1	23	1	3	32
Barrier to gene flow	0	1	3	3	1	0	11	8	1	28
Redirection	0	0	0	0	0	0	1	21	0	22
Entanglement	0	1	2	2	0	10	1	1	2	19
Overpopulation	0	0	0	5	1	0	5	0	2	13
Separation	1	0	0	0	1	0	1	0	7	10
Barrier to migration	0	0	0	1	0	1	1	1	5	9
Evolutionary restriction	0	0	0	3	0	0	1	0	0	4
<b>Total</b>	<b>34</b>	<b>10</b>	<b>10</b>	<b>63</b>	<b>17</b>	<b>16</b>	<b>79</b>	<b>46</b>	<b>29</b>	<b>304</b>

## 2.5 Discussion

Our review showed that fencing has a myriad of intended and unintended effects on many fauna species all over the world. Thus, whether or not a fence is considered effective, successful, good, or bad largely depends on what the stated purpose of the fence is, and which effects are investigated. We found the identified literature on exclusion fencing to have a patchy global distribution (Figure S1). Fence effects, fence classes (Figure 2.3) and whether fence effects were unintended or unintended (Figure 2.1) also have geographic biases. Fence effects and fence classes were also not reported equally in the literature. Finally, intended and unintended effects per fence group (based on five categories) were also unevenly represented in the literature, indicating research on different fence classes may report effects disproportionately. These results make it difficult to assess the net benefits of fences and identify ways to mitigate unintended effects at a general level.

### (i) *Spatial Distribution*

The distribution of literature by continent was not uniform across the world. Hayward and Kerley (2009) noted potential geographic biases, and this bias is observable in the distribution of conservation and reserve fence papers (77% in the Southern Hemisphere) and agricultural exclusion fencing papers in the Northern Hemisphere (65.7%). This may be a reflection of the need for these fence types. For example, conservation fencing papers largely originate in Australasia, and reserve fence papers in Africa (Figure 2.3). The abundance of literature on these fence classes may be a reflection of the many at-risk species endemic to both continents (Grenyer et al., 2006; Woinarski, Burbidge, & Harrison, 2015). Additionally, road fencing papers came mostly from North America and Europe, potentially due to greater human population density leading more vehicle-wildlife collisions (Conover, 2019). Like the fence class biases, fence effects also appear to have location biases. Over half (52.0%) of literature reporting unintended effects derives from Africa (Figure 2.1). An emphasis on fencing for wildlife preservation rather than agriculture may draw attention to the unintended fencing effects; a majority of the unintended effects literature from Africa and Australia were reserve and conservation fencing papers (73.6% and 61.5%).

The spatial distribution of the literature was also likely affected by our search methods. Though the search was not limited to English literature, the search terms were in English

language, and this may have influenced our results. The greatest numbers of papers did come from English speaking countries (69.0%; Figure S1). Spatial distribution biases of fence classes and effects by continent would require more detailed examination by language to fully explore this issue.

(ii) *Temporal Distribution*

Since the early 1900s there has been a gradual increase in papers on all forms of exclusion fencing (Figure S3). Pressures to biodiversity and rapid species extinction from human encroachment, while not ‘new’, are developing phenomena (De Vos et al., 2015), spurring the use of conservation and reserve fencing to save biodiversity before it disappears (Hayward & Kerley, 2009). Similar to fencing for conservation, the uptake in fencing in agriculture (pest exclusion fences and barrier fences) and roads may be a reflection of the pressures being placed on wildlife species from human encroachment into wildlife regions. Human population growth also has an indirect effect on the use of VCFs. VCFs ensure an international market for beef from Africa (Darkoh & Mbaiwa, 2002). These increasing pressures and therefore exclusion fences may be the cause of the increasing literature; however, using literature as a proxy for increased use of exclusion fencing is problematic. The temporal distribution of recorded literature might be influenced by increases in available journals for publication and the search methods. For the methods applied here, the perceived increase in exclusion fencing literature may be a function of the search methods. Web of Knowledge restricts searches from the year 1985-present, and older literature may not be digitally recorded or have tagged keywords that would allow them to be located through electronic searches. We attempted to address this as best we could by searching the reference lists of each paper, but as there is no way to fully extricate the influence of the search methods on the resulting temporal distribution of the literature, these results were included only to demonstrate that effects of fencing are well represented in the literature over time, and particularly within the last 20 years (Figure S3). Similarly to the spatial distribution of literature, to achieve reliable results, global fencing data on year of construction and effects would be required; again, this was not within the scope of this review.

(iii) *Identified benefits of fencing*

The principal goal and benefit of fencing is the restriction of movement of a target species, though this intention varies across the different fencing classes. This restriction is

envisioned as either complete cessation of target species movement, such as conservation fences or VCFs, or a restriction of where the target species is ‘allowed’ to move, such as the redirection of target species to over- or under-passes.

The most common beneficial effects of fencing described in the literature are pest and predator exclusion (Figure 2.3). The application of fencing for pest exclusion has been broad, from restricting pig (*Sus scrofa*) damage in North American crops (Geisser & Reyer, 2004) to mitigating Asiatic black bear (*Ursus thibetanus*) damage to apiaries in Japan (Huygens & Hayashi, 1999). Papers that discussed pest exclusion as a beneficial outcome focused most frequently on barrier fencing, experimental fencing, and agricultural exclusion fencing (Table 2.1). Agricultural exclusion fences allow the farmer to better manage their resources, restricting the movement of unwanted herbivores and predators. Barrier fences inhibit the spread of unwanted species into large areas of high value, such as the western barrier fence, the rabbit fence, and the Dingo Barrier Fence in Australia (McKnight, 1969), which is intended to control dingo movement to- and rabbit movement from- south-east Australia. Non-target species may also benefit from these fences given that removal of a predator or competitor would likely benefit other species in the system (Dickman, 2012; Pedler et al., 2016). Predator exclusion was the prevailing beneficial outcome of conservation fencing in the literature, but there is potential for most forms of fencing described here to impede a predator and benefit non-target prey species if the predator’s movements are restricted. Exclusion fences around species of conservation concern enables improved management of vulnerable species as it puts a hard boundary on the geographical area of concern, focusing conservation efforts. Along with control of the predator, even agricultural exclusion fences might be utilized for conservation purposes (Allen, 2017).

VCFs ensure financial stability for farmers, particularly in regions that rely on international export. VCFs prevent the spread of disease into these agricultural areas. A primary example a VCF is the Northern and Southern Buffalo Fences that control the spread of foot and mouth disease in the Okavango Delta. These fences restrict the movement of Cape buffalo (*Syncerus caffer* - which transmit disease) to the central parts of the delta, away from the cattle grazing areas (Mbaiwa & Mbaiwa, 2006). The Buffalo Fences were then further expanded with Contagious Bovine Pleuropneumonia (CBPP) Fences after the outbreak of this disease in the delta (Mbaiwa & Mbaiwa, 2006). Though most frequently employed in

African nations, there are examples of successful implementations of VCF fences in other parts of the world (see Brook, 2010; Cowan & Rhodes, 1992; Gooding & Brook, 2014). Other classes of exclusion fencing discussed would also potentially limit the spread of disease through the separation of domestic and wildlife species, though only one example was found in this review (Poole, Western, & McKillop, 2004). Pest exclusion fences built to limit crop raiding by badgers (*Meles meles*) in the United Kingdom also help prevent the spread of bovine tuberculosis (*Mycobacterium bovis*).

Road fences redirect wildlife to prevent vehicle/animal collisions. The benefits of road fences are largely self-explanatory, preventing wildlife mortality and ensuring human safety in vehicles (Conover, 2019). Further to this however is the use of road fences to redirect wildlife to under or overpasses and promote connectivity across the barrier (see Bissonette & Rosa, 2012). A summary of identified benefits can be seen in Table 2.2.

**Table 2.2: Projected benefits of exclusion fencing**

Benefits of each fence class identified in the literature, and the potential outcome of that benefit

Fence Class	Type of Benefit	Outcomes
Reserve Fence	Pest Exclusion	-Prevents crop raiding -Prevents predation of stock
	Separation	-May prevent spread of disease
	Conservation	-Limits poaching -Reduction in human-wildlife conflict/persecution -Preserves landscapes of conservation value
Conservation Fence	Predator Exclusion	-Prevents predation on vulnerable species
	Conservation	-Manage populations
	Pest exclusion	-Prevents competition
Exclusion Fence	Pest Exclusion	-Prevents predation of stock -Manage resources
	Separation	-Inhibits spread of disease
	Predator Exclusion	-May prevent predation on non-target species
Barrier Fence	Pest Exclusion	-Prevents the spread of stock predators into high value areas -Prevents the spread of other pests into high value areas
	Predator Exclusion	-May prevent predation on non-target species
VCF	Separation	-Prevents spread of disease -Prevents economic losses
Road Fence	Redirection	-Avoid vehicle/animal collisions -Redirect wildlife to reconnect previously separated populations

Though obvious, it is important to reiterate that the primary goal of all exclusion fences is to restrict target species' movement. Secondary benefits then also become apparent over time. For example, reserve fencing in Africa prevents economic loss to farmers by restricting the movement of wildlife into populated areas, where they may cause damage to crops, infrastructure and livestock. This in turn has the added benefit of reducing retaliatory killing of native species, and allows for easier conservation management of the species (Hayward & Kerley, 2009). The reserve fences also inhibit the movement of poachers into protected areas, which alleviates pressures on targeted species of conservation concern such as the black rhinoceros (*Diceros bicornis*). These secondary benefits are a key part of the extensive use of exclusion fencing and are important part of understanding the use of individual fence classes. However, these secondary benefits are many and varied, and often are reported in literature that solely discusses economic benefits without discussing effects on target or non-target species, and so were not explicitly recorded.

*(iv) Identified negative impacts of fencing and potential mitigation strategies*

The potential for negative impacts in conservation fencing has been thoroughly explored. Past reviews have identified the need for more empirical research into the effects of conservation and reserve fencing specifically (Hayward & Kerley, 2009) and this recommendation appears to hold true for all forms of exclusion fencing. Much of the literature documented in this review reports that there 'may be' substantial negative effects of exclusion fencing (Figure 2.4) but then made no attempt to measure those effects or suggest mitigation strategies or solutions. This is particularly evident for papers recognizing a barrier to gene flow as potential impact of fencing (see '*Genetic Impacts*' below). This section classifies each of the impacts and presents any mitigation strategies that have been employed in the past.

*(a) Barrier to movement*

The most common negative impact identified in the literature was the potential for the fence to be a barrier to movement and its flow-on effects (Figure S2), after all, this is really the sole purpose of any fence – to stop movement of a target species from one side to the other. The definition of barrier to movement used here is the inability for a species to freely move between areas of suitable habitat for the maintenance of natural processes, where this restriction was not the intended goal of the fence. To this definition, the literature often

described uneven or unnatural use of resources, fragmentation of populations, and the inability for species to freely move into areas of suitable habitat that may have otherwise been utilised in the absence of the fence (Ben-Shahar, 1993; Boone & Hobbs, 2004). The potential for an exclusion fence to present a barrier to species is both obvious and widely discussed in literature found in this review, and discussions of fences as a barrier to movement were found in all fence categories (Table 2.1).

When wildlife cannot move freely, both as an individual within its home range or as a species within its distribution, it appears adverse effects are to be expected, and are often intended. Mitigation of this impact is dependent on the exclusion fences' purpose and the species affected. In road fencing, wildlife over- and under-passes are often utilised to varying degrees of success against a wide variety of species (e.g. Bager & Fontoura, 2013; Bissonette & Rosa, 2012; Chachelle et al., 2016). Of the 13 publications testing wildlife passes identified in this review, 12 reported general success, though five of these also reported caveats to success such as a target or small species failing to use the pass (McCollister & Van Manen, 2010; Taylor & Goldingay, 2003), or pass use declining with increased vehicular traffic (Olsson, Widen, & Larkin, 2008). For conservation and reserve fencing, species-specific gates and gaps have been used (e.g. Coates, 2013; Dupuis-Desormeaux et al., 2016; Weise et al., 2014). Six publications testing effectiveness of gates and gaps were included in this review and all reported general success. Similarly, bump gates in VCFs have proven successful in allowing free movement of stock whilst restricting wildlife movement (see Barasona et al., 2013; VerCauteren et al., 2009). One-way gates such as those used for burrowing bettongs (*Bettongia lesueur*; Butler, Paton, & Moseby, 2019), while worth mentioning, are not a true mitigation of this impact. One-way gates don't allow the bidirectional movement of species and therefore does not fulfil the requirements outlined in our definition of barrier to movement. Though one-way gates would allow more natural utilisation of resources, and species to disperse into areas of suitable habitat outside the fence, resource utilisation may still be affected, particularly in species with large home-ranges.

#### (b) Overpopulation

Overpopulation has been defined here as the increase in density of species within a fenced-off area until resource availability declines to a level that negatively impacts a species. In this review, overpopulation was always associated with the barrier to movement effect

(above) and several papers reported overpopulation as a consequence of fencing (see Boonstra & Krebs, 1977; Harding et al., 2007; Moseby, Lollback, & Lynch, 2018; Treydte et al., 2001). Overpopulation was always discussed as an outcome of enclosures, rather than linear barriers, suggesting it is most associated with reduced dispersal ability in all directions. Early Australian pastoralists remarked that the advent of fences is what ultimately led to overgrazing and land degradation because it prevented natural dispersal of herbivores in response to fluctuating environmental conditions (Idriess, 2001).

Though a potential positive result for a vulnerable target species, the increase of one species in the system beyond carrying capacity has downstream effects to other species (see Moseby, Lollback, & Lynch, 2018), including starvation of both target and non-target species. As a flow-on effect to barrier to movement, similar mitigation strategies to those mentioned above can be utilised. Species-specific gates and gaps can be used to mitigate overpopulation of some species within fences, but notably for this effect, one-way gates (Butler, Paton, & Moseby, 2019) could be utilised as well. Overpopulation may also be mitigated through active population management, for example the relocation of individuals to other suitable locations (Treydte et al., 2001), contraception (e.g. Doughty et al., 2014), or if the species is not of conservation concern, culling (Clark, Clark, & Allen, 2018).

#### *(c) Barrier to migration*

Similarly to overpopulation (above), fences as a barrier to migration may cause starvation as the fences restrict the seasonal movement of species to resources (Allen & Hampton, 2020). The literature highlighted VCFs as the most common fence class associated with barriers to migration (Table 2.1). VCF papers almost exclusively originated in Africa, so this association likely arises from the prevalence of large-bodied, migrating species in that region. Barrier to migration was also associated with other negative outcomes, including entanglement of species that persist with migration routes after the fence has been constructed (Mbaiwa & Mbaiwa, 2006; Taylor & Martin, 1987) and behavioural changes (see below) in species that do not (Bartlam-Brooks, Bonyongo, & Harris, 2011; Bennitt, Bonyongo, & Harris, 2016). While most associated with African literature, other continents had examples of this also, particularly around road fences blocking migratory routes of ungulates (see Harrington & Conover, 2006; Sawyer, Lebeau, & Hart, 2012).



Fence gaps that allow bidirectional movement may again be used to mitigate this effect, though in this case it may be the only feasible solution for existing fences. There is evidence that wildlife species will re-establish migration routes if given a plausible path (Bartlam-Brooks, Bonyongo, & Harris, 2011). Prior to fence construction, investigations into existing migration routes could negate the need to mitigate for this effect at all. Constructing fences parallel to species' migration, or wildlife corridors that emulate their paths would allow seasonally migrating species to move freely between locations and resources. In the case of road fences, under- or over-passes has been shown to help allay barrier to migration effects (see Olsson, Widen, & Larkin, 2008; Sawyer, Lebeau, & Hart, 2012).

#### *(d) Entanglement*

The most direct impact, entanglement, was reported in nearly all fence classes (Table 2.1). Entanglement was categorized as the snaring (and often death; Allen & Hampton, 2020) of animals in the exclusion fence. Reports of entanglement of non-target species was far more common than target species and affected a wide variety of species across the world. Entanglement appears to be most often associated with migrating and wide-ranging species (Harrington & Conover, 2006; Mbaiwa & Mbaiwa, 2006; Taylor & Martin, 1987), reptiles (Burger & Branch, 1994; Ferronato, Roe, & Georges, 2014) and low-flying birds. Bird species appear to be of particular risk when barbed-wire fencing is used (see Allen & Ramirez, 1990; van der Ree, 1999).

Mitigation of death due to entanglement could be achieved through regular fence checks, however this would likely be prohibitively time consuming or costly. Prior to fence construction surveys of species in the area would be valuable as fence placement and design evidently play a role in the rate of entanglement. After fence construction, surveys of the species found entangled should be a primary focus to aid the development of species specific mitigation strategies. The latter method has been successful in reducing cases of entanglement in several species (Baines & Andrew, 2003; Burger & Branch, 1994; Van Lanen et al., 2017). While fences may kill some non-target or target individuals (Allen & Hampton, 2020), populations of the same species may experience a net benefit after their threats have been removed by the fence (see Ferronato, Roe, & Georges, 2014).

(e) Behavioural change

Behavioural changes caused by exclusion fencing appear to be challenging to both evaluate and alleviate. The literature discussing potential behavioural changes was heavily biased towards reserve fencing and African countries. The most commonly assessed behavioural changes were changes in predator-prey dynamics (e.g. Bissett, Bernard, & Parker, 2012; Tambling et al., 2013), altered species demographics (e.g. Bissett & Bernard, 2011), use of fences for prey-trapping (e.g. Bojarska et al., 2017; Davies-Mostert, Mills, & Macdonald, 2013), prey naïveté in the absence of predators (e.g. Ikuta & Blumstein, 2003), changes in ratios of migrating and resident individuals (e.g. Bartlam-Brooks, Bonyongo, & Harris, 2011), and variances in a species space utilization within fences when compared to free ranging equivalents (e.g. Welch et al., 2015). The high percentage of mentioned behavioural shifts of African origin appears to be mostly explained due to the large number of species with altered demographics, densities and interspecies interactions that is a by-product of reserve-style fencing and active population management and monitoring within reserves.

Prey naïveté is of particular note when discussing behavioural change in species of conservation concern. The usual purpose of a conservation fence, isolating a species from other predatory species, may act against the long-term benefit for the threatened species. In a single lifespan individuals within the species can become more accustomed to altered conditions, and an example of this type of behavioural change was included in this review (see Ikuta & Blumstein, 2003). This concept may then be extended to the long term where generations of species isolated from a predator may unlearn instinctual antipredator responses (Moseby, Blumstein, & Letnic, 2016). Over time it is theoretically possible for species to lose their evolutionary solutions to predation risk (see also Gittleman & Gompper, 2001), and therefore in a conservation fence scenario the fence would be preserving a behaviourally unequipped species that would never be capable of surviving outside an exclusion fence (this concept is discussed further below). The solution to this issue may be the purposeful use of leaky barriers to permit a low density of predators to periodically reinforce instinctive response to predators (Moseby, Blumstein, & Letnic, 2016).

Solutions for alleviating processes that alter behaviour would clearly be diverse. Several of the identified behavioural changes appear to be negatively correlated with increasing

enclosure size, which may be addressed by connecting reserves or removing internal fences (e.g. Druce, Pretorius, & Slotow, 2008). This can also be addressed by prioritising fund allocation to the construction of new fences that allow for existing enclosures to be expanded before funding new and separate enclosures; though, this would be dependent on the fences' purpose, budgetary requirements and land availability.

*(f) Genetic implications*

Of the 32 papers that discussed the potential for adverse genetic effects of fences, only nine presented primary data. Of these nine, four discussed linear barriers with a genetic discontinuity between two now split, but still viable populations (Burkart et al., 2016; Hepenstrick et al., 2012; Kuehn et al., 2006; Wilson et al., 2015). A further three papers describe the genetic effects of reserve and conservation fences on a target species (Miller et al., 2015; Ottewell et al., 2014; White et al., 2018). Only two studies focused on a non-target species (Flesch et al., 2010; Linnell et al., 2016) and both of these papers did not test the post-fence genetic consequences, only showing that a proposed or recently constructed fence would pose a threat to known paths of gene flow. From this review it is clear that more focus should be placed on non-target species and fenced enclosures, where adverse genetic effects would likely be a larger issue than for linear barriers.

This review divided genetic consequences into two separate but connected categories. The first, a barrier to gene flow, was characterised as the restriction of genetic transfer between populations separated by an exclusion fence that may result in negative genetic outcomes, such as inbreeding. The second potentially negative genetic outcome, evolutionary restriction, was characterised as the continuing genetic divergence from other populations as a result of (1) the lack of gene flow to the natural population and (2) altered environmental factors. This scenario may eventually lead to localised adaptations and a population less likely to persist outside an enclosure (Hayward & Kerley, 2009).

Mitigation of genetic impacts relies heavily on the identification of species within the system that have a life history, ecology and conservation status which put them at particular risk. Species with high conservation concern and low population densities, solitary behaviours, already fragmented distributions, or a combination of these factors would be at greater threat of genetic isolation due to the addition of a fence (Lacy, 1997). Once identified, species-specific fence gaps or bidirectional gates would be the most useful and

cost effective mitigation method for facilitating gene flow. When neither of these methods are viable, meta-population management would need to be used to ensure genetic diversity is maintained within the isolated populations. This method has been used effectively in conservation and reserve fencing (see Frankham, 2015; Miller et al., 2015). After the implementation of any or all of these mitigation strategies, continuing genetic monitoring would ensure the continuing diversity of the enclosed population and should be considered. Further to this, regular checks of other species in the system might also be necessary in order to ensure detrimental genetic implications are not starting to compromise populations of other species that were not identified by the initial assessments.

(v) *Exclusion fencing in Australian agriculture*

Exclusion fencing in Australia has clear benefits and studies on fencing worldwide have a strong record for success, particularly in conservation. For Australian agriculture the use of fences to relieve effects of predation on livestock species and allow land managers to better monitor grazing pressure is undeniable (Clark, Clark, & Allen, 2018; Yelland, 2001). However, as demonstrated, agricultural exclusion fencing has the potential to create a number of negative impacts, particularly to non-target species (Allen & Hampton, 2020). The most damage to non-target species occurs when poor fencing alignment occurs because environmental assessments were ignored or not completed at all (Hayward & Kerley, 2009). This exact scenario holds true in Australia, as no formal impact assessments of agricultural exclusion fencing on wildlife have been required or completed prior to their construction (RAPAD & QFPI, 2019). This leaves a knowledge gap that may have adverse outcomes, particularly for non-target wildlife (e.g. Wilson & Edwards, 2019).

Entanglement of a diverse range of Australian species as a result of fencing has already been documented on several occasions (Ferronato, Roe, & Georges, 2014; Ley & Tynan, 2008; van der Ree, 1999). Similar to this, mass mortality events may occur when fences block wildlife movement. Mass mortality events have been recorded on African VCFs when the fences have been misaligned with seasonal migratory routes (Ferguson & Hanks, 2010). Although not seasonal migration, kangaroos and emus have been known to move large distances in pursuit of resources (Bailey, 1971; Bradby et al., 2014; Hill, 1982), and reports of large scale deaths in both species as a result of fencing have occurred (Bradby et al., 2014; Wilson & Edwards, 2019). This highlights the need for assessments of species in the area and fence designs and placement to be adjusted accordingly.

One-way gates have been successfully used in Australia (Butler, Paton, & Moseby, 2019; Crisp & Moseby, 2010) and worldwide (Schumann et al., 2006) in the past to help mitigate over-abundance and the dispersal of individuals to outside populations whilst still excluding the target species, though this would not alleviate genetic pressures on animals within fences (Dickman, 2012). As previously stated, the optimal scenario would be fence gaps or gates designed to allow non-targets free bidirectional movement, while effectively restricting the target species. This goal maybe difficult to achieve in Australia given the physiological traits of the most commonly excluded target species (fox, cat, dingo, kangaroo, emu), but innovative solutions have been successfully implemented for other species worldwide (see Barasona et al., 2013; Dupuis-Desormeaux et al., 2016; Schumann et al., 2006; Weise et al., 2014). At a minimum, effects of isolation on vulnerable non-target species should be offset by metapopulation management, just as has been advised for target species in conservation fencing (Miller et al., 2015; White et al., 2018).

(vi) *Publication biases*

The results of this review show that fence types and fence effects are not reported in the literature in equal proportions, and that intended and unintended impacts are not evenly reported across fence classes (Figure 2.4). This may be an artefact of publication bias in fencing literature. Publication biases skewed towards positive results may have led to research indicating ‘no effects’ to be left unpublished. Additionally, the type of fence may also skew whether the intended or unintended effects are the most often recorded, and then reported in publications. These publication biases would affect any attempt to accurately quantify increases or changes in fence types or fence effects using the methods we have employed here. To correct for these effects the researcher would need to collect and collate unpublished data and results, a near impossible task. However, this weakness does not discount the outcomes of this review. The papers identified and analysed here represent the papers most easily found and read by managers and decision makers when it comes to the use of exclusion fencing and the effects that they may have on the species of interest. The biases and shortcomings of this literature are the same biases directing management decisions, for example, the lack of literature concerning potential genetic effects may lead decision makers to miss or discount the potential for this impact to non-target species.

(vii) *Future research focuses*

From this assessment it is clear that there are a variety of intended and unintended effects facing species in exclusion fenced systems. Several of these effects (such as long-term genetic effects, separation, overpopulation and barriers to migration) are underrepresented in the literature (Figure S2), and future research should prioritise these areas. Additionally, a more holistic approach to fencing effects is needed, along with more empirical studies. The vast majority of literature focuses on a single effect of fencing. Future research should aim to take into account both the costs and benefits for species so that the net outcomes of exclusion fencing can be better explored.

## **2.6 Conclusions**

1. Articles related to exclusion fencing effects are well represented in the literature, particularly in the last 20 years. This increase may be a reflection of the recent, rapid uptake of fencing as a tool to alleviate economic and ecological pressures caused by pest and predatory species.
2. This literature comes from all continents, excluding Antarctica. However, a large proportion of the literature addresses Australia, South Africa and North America. Spatial biases of both fence types and reported impacts may give insight into the pressures faced in these areas.
3. Based on the possible positive and negative fencing effects explored in this paper, harnessing benefits and avoiding costs is best achieved through a proper assessment of these impacts prior to fence construction and continuing assessment of outcomes following construction.
4. Prior to construction, assessments of the species in the area should be conducted to ensure a sound understanding of the potential impacts that may need to be mitigated. This is particularly important where the direction or style of the fence might affect migration routes, or species prone to entanglement.
5. Post construction, regular fence checks offer a good opportunity to record negative outcomes. This is particularly essential in cases where low population densities, habitat fragmentation, or species of high conservation concern were identified within or around the fenced area.

6. If negative outcomes are recorded, species-specific mitigation strategies can be utilised that alleviate the impact. In particular, one-way and bi-directional gates, fence gaps and translocations have been utilised with success in the past.
7. Much of what is understood about potential genetic impacts of fencing (both short and long term) is inferred from comparable barriers to gene flow. This knowledge gap requires further investigation. There is a distinct lack of primary research into the genetic impacts of fencing.

Some unintended aspects of exclusion fencing may ultimately prove beneficial to both target and non-target species, but robust assessments need to be performed to ensure fences are not doing more harm than good. For this reason it is imperative that potential adversely affected species in the system are documented, ecological costs and benefits of exclusion fencing are assessed prior to their use, and mitigation strategies be employed during and after their construction, for both target and non-target species.

## **2.7 Author Contributions**

BLA and DS both contributed to the conception and design of the research that led to this manuscript. DS acquired the data. DS and RK analysed and interpreted the data. DS drafted the initial manuscript and all authors contributed critically to the drafts and gave final approval for publication.

## **Chapter 3 : Expansion of vertebrate pest exclusion fencing and its potential benefits for threatened fauna recovery in Australia**

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### **3.1 Abstract**

The global effort to conserve threatened species relies heavily on our ability to separate these species from the processes that threaten them, and a common tool used for this purpose is exclusion fencing. In Australia, pest animal exclusion fencing has been repeatedly used on conservation land on a small scale to successfully exclude introduced predators and competitors from threatened native fauna populations. But in recent years, ‘cluster fencing’ on agricultural land has re-emerged on a large scale, and is used by livestock producers seeking to reduce predation losses by dingoes (*Canis familiaris*) and manage total grazing pressure from native and introduced herbivores, including red kangaroos (*Osphranter rufus*). Given that the primary threats to at-risk native fauna are also predation and overgrazing, there may be potential for cluster fencing on livestock land to achieve additional fauna conservation benefits. Understanding the number, location and potential conservation value of cluster fenced livestock lands is critical for determining how these areas might contribute to broader threatened fauna recovery goals. Drawing from publicly available databases maintained by the Australian Government, we assessed the spatial overlap of threatened species’ distributions with 105 cluster fences erected in Queensland since 2013, which cover 65,901 km<sup>2</sup> of land. These cluster fenced areas represent 18 biogeographic subregions and may contain 28 extant threatened mammals, birds and reptiles including 18 vulnerable species, 7 endangered species, and 3 critically endangered species. An average of 9 threatened species or their habitat were identified per cluster, and over three quarters (78.6%) of these species face at least one threat that is being mitigated within clusters. The true status of threatened and pest species within clusters is largely unknown or unrecorded in most cases, but some examples of pest eradication and threatened species recovery are already emerging. Given the vast size of the cluster fenced estate, the many different biomes and species it represents, and the nature of the threats



being removed within these fenced areas, we contend that agricultural cluster fencing may offer an unprecedented opportunity to advance threatened fauna conservation goals for some species at scales previously thought impossible, and should be a research priority for threatened species managers.

### **3.2 Introduction**

Globally there is a need to manage wildlife species, both to alleviate economic pressures caused by invasive or pest species, and also to mitigate anthropogenic pressures on threatened wildlife. Australia has the highest number of mammal extinctions worldwide and many other species have experienced rapid declines in abundance or distribution (Woinarski, Burbidge, & Harrison, 2015). This decline is largely attributed to competition and predation from introduced species such as feral goats (*Capra hircus*), European red foxes (*Vulpes vulpes*), and feral cats (*Felis catus*; Allen, 2011; McKenzie et al., 2007; Woinarski, Burbidge, & Harrison, 2014). Many of Australia's most at-risk species are on the brink of extinction and exist only within fenced conservation reserves or on offshore islands (Ringma et al., 2018). Exclusion fencing has become a common tool to alleviate the pressures that introduced or invasive pest animals have on native species (Hayward & Kerley, 2009). Fences create a hard boundary around the control/management area and prevent the immigration of undesirable pest species following their removal. Fences of this style have been deployed worldwide to conserve a wide variety of species from Australian bilbies (*Macrotis lagotis*) to African elephants (*Loxodonta africana*; e.g. Druce, Pretorius, & Slotow, 2008; Moseby & O'Donnell, 2003). Exclusion fencing has also been utilised globally for the benefit of agricultural industries, excluding pest species that economically affect productivity. The applications of these fences have been broad, and include fencing multiple species out of large portions of countries and continents (McKnight, 1969; Smith, King, & Allen, 2020; Chapter 2) through to fencing single species out of small enclosures (Allen & West, 2013; Cockfield, Botterill, & Kelly, 2018; Darkoh & Mbaiwa, 2002; Otto, 2013).

Within Australia, exclusion fencing has been successfully employed for the conservation of small mammals, birds and reptiles (Dickman, 2012; Legge et al., 2018; Ringma et al., 2019). The feral cat and red fox were introduced into Australia shortly after European settlement and spread quickly to occupy the majority of the country (Abbott, 2002; Rolls, 1969). These two mesopredators have been repeatedly linked to extinctions and other

negative effects on populations of native mammal species (Doherty et al., 2015; Doherty et al., 2017; Woinarski, Burbidge, & Harrison, 2014) with weights between 35–5,500 g (Burbidge & McKenzie, 1989). For this reason the Australian Government has identified predation by cats and foxes as Key Threatening Processes (Department of the Environment, 2019c). Dingo (*Canis familiaris*) predation also represents a Key Threatening Process to many threatened fauna (Allen & Fleming, 2012; Allen & Leung, 2012; Major, 2009), as does competition with vertebrate herbivores including feral goats, feral pigs (*Sus scrofa*) and European rabbits (*Oryctolagus cuniculus*; Pedler et al., 2016). Consequently, these predators and competitors of threatened fauna are actively removed and managed within almost all fenced conservation reserves in Australia (Australian Wildlife Conservancy, 2019a, 2019b), which totalled ~360 km<sup>2</sup> of protected land in 2017 (Legge et al., 2017), and has since increased to 594 km<sup>2</sup>. Many threatened species rapidly recover when these threats are removed (Moseby, Lollback, & Lynch, 2018; Ringma et al., 2018; Short, 2009).

Exclusion fences have also been used effectively in Australia for over 100 years to exclude species that cause negative economic impacts to agricultural enterprises (McKnight, 1969). Pest animal barrier fences were erected throughout much of Australia in the late 1800s and early 1900s (Department of Primary Industries and Fisheries, 2007) to slow the expansion of rabbits and exclude dingoes and emus (*Dromaius novaehollandiae*) from areas of high agricultural productivity. The fences assisted with eradicating or reducing these animals to manageable levels inside fenced areas within a few decades, but in the case of dingoes, after they had been removed the fences eventually fell into disrepair and were replaced with standard livestock fencing of a kind that provides no impediment to dingoes and other pest animals (Allen & West, 2013). Pest animal numbers then predictably increased over the following decades, and small groups of properties have again begun to surround themselves with pest animal exclusion fencing, colloquially known as ‘cluster fencing’ as they encompass a cluster of properties (RAPAD & QFPI, 2018b). Such fences are netted (i.e. ‘hinge-joint’ or ‘ring-lock’ fencing), ~1.8 m high, and typically feature a strained 30–50 cm apron extending away from the base of the fence along the ground, though sometimes this apron is buried (Figure 3.1). As it was historically, the goal of cluster fencing is to facilitate local eradication or suppression of agricultural pest animals inside the fences while inhibiting reinvasion from animals on the outside. It is worth noting here that feral cats are not pests to agriculture and are not controlled by cluster fencing. In central and central-western Queensland, the primary target species for exclusion are dingoes and

kangaroos (*Macropod* spp., most commonly red kangaroos *Osphranter rufus*). Dingoes and kangaroos, as well as secondary target species including feral pigs, feral goats, and foxes, are considered pests to agriculture. The dingo is considered a pest species on agricultural land because of its proclivity to kill livestock, particularly sheep (Allen & West, 2013, 2015). Kangaroos and feral goats are considered pest species because they contribute substantially to total grazing pressure (TGP) and land degradation (Hacker, Sinclair, & Pahl, 2019; Pahl, 2019; Waters et al., 2019). The lethal control of these species is widespread within cluster fences (Allen, 2017) which, by 2019, now encompass ~66,000 km<sup>2</sup> of protected livestock grazing land in central-western Queensland alone (see below).



**Figure 3.1: Examples of exclusion fence design**

Examples of netted cluster fence types in western Queensland, showing those that have strained aprons with one or two barbed top-wires (A, B) and those without top wires and buried aprons (C). A typical creek crossing is also shown (D), with an excluded emu.

Though their motivations and objectives are ostensibly different, conservation fencing and agricultural cluster fencing share a common enemy – vertebrate predators and competitors, and the management of TGP and land degradation. Both efforts also have a long track record of achieving their objectives, having removed pest animals and conserved livestock or threatened fauna. But while conservation fencing has demonstrated its value on small

scales, there are several limitations hindering its larger-scale use, including the cost of construction and maintenance (Dickman, 2012) and the unavailability of suitable land not already used for other purposes (see Bode et al., 2012). Utilising agricultural land already being pest-fenced could alleviate both of these limitations (as discussed in Allen, 2017). However, no information presently exists about the potential utility of cluster fenced areas to threatened fauna conservation objectives in Australia. Understanding the location of existing cluster fences, the biomes they represent, and the status of and threats to extant threatened fauna within these fences, could advance the implementation of threatened fauna recovery actions on a scale previously thought impossible.

Here, we explore the potential utility of cluster fenced areas for fauna conservation by assessing the threatened species thought to be present within the recently established cluster fences in central-western Queensland. Our aim is to demonstrate the variety of ecosystems and threatened species these cluster fences represent, and identify the threatened species that may potentially benefit from the pest animal and land management activities occurring within these clusters.

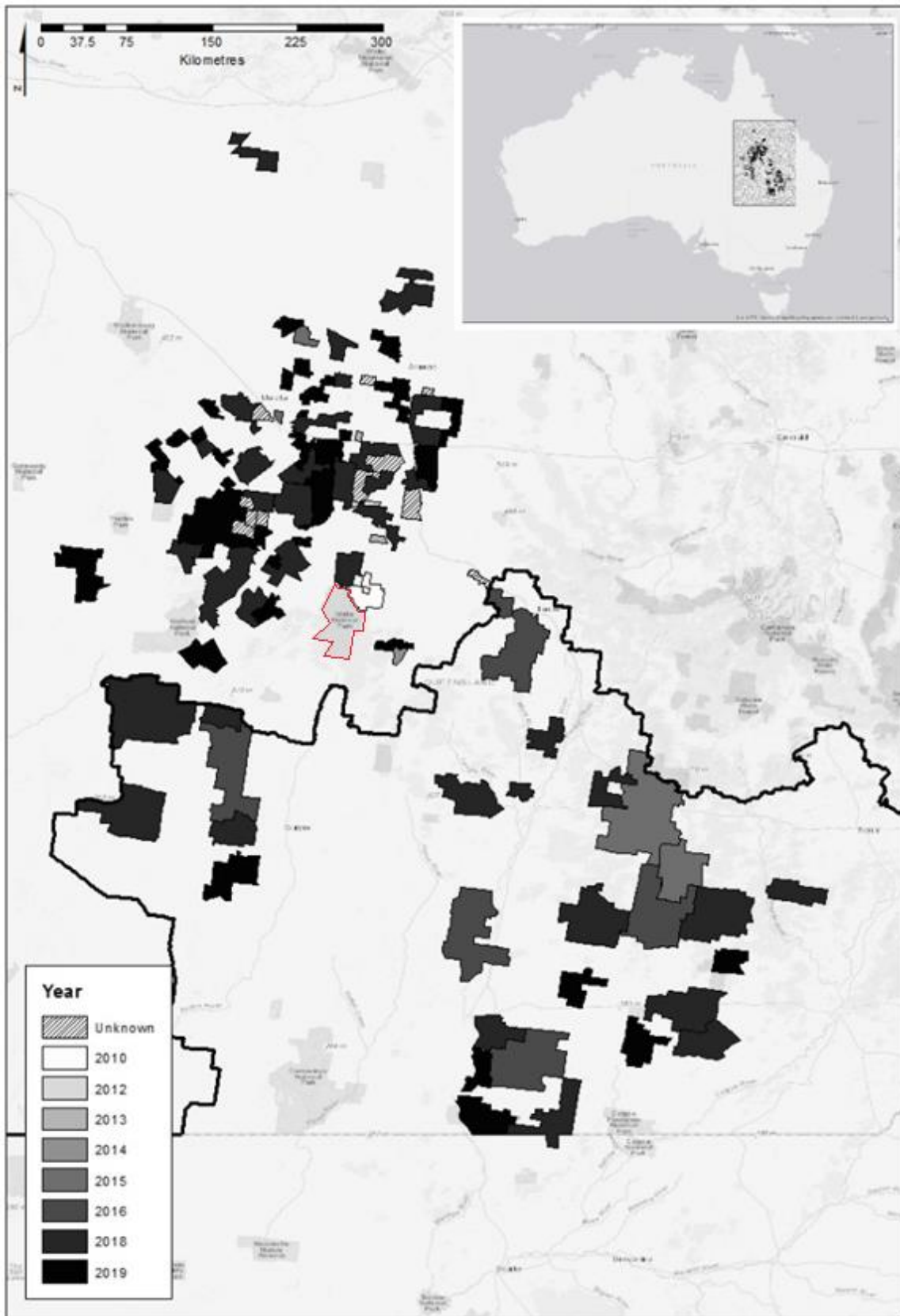
### **3.3 Methods**

The names, locations, size (km<sup>2</sup>), and estimated completion dates of cluster fences in western Queensland were sourced from publicly available maps provided by the government agencies that funded the construction of the cluster fences. The individually fenced clusters of properties were then geolocated using ArcMap v10.5.1 and the ‘Rural properties – Queensland’ dataset (Department of Natural Resources, Mines and Energy, 2014; Figure 3.2). If the size of a cluster was unreported, the size was projected using the ‘calculate geometry’ tool within ArcMap based on the co-ordinate system used (GDA2020 MGA – zone 55) and specified polygons (in this case cluster fence boundaries). Next, cluster fence boundaries data were used to determine which biogeographic regions and sub-regions were represented within the clusters using the ‘Biogeographic sub regions – Queensland’ dataset (Department of Natural Resources, Mines and Energy, 2019). The GPS coordinates for each clusters’ centroid were then used in the Australian Government’s Protected Matters Search Tool (PMST) interactive map (available at [www.environment.gov.au](http://www.environment.gov.au)) to generate a Protected Matters Report which listed extant threatened and pest fauna, and their habitat, known or thought to be present within each cluster. The search radius of the PMST was limited to 10 kilometres, meaning that

threatened fauna or their habitat within an area of 314.2 km<sup>2</sup> around the cluster centroid were identified. From enclosure sizes, the percentage of cluster fenced land each species might occupy was also calculated, with the assumption that the species may be present in the entirety of any cluster it was recorded in, as shown in Table 3.1. Given that this assumption is likely unsupported for many species, particularly for habitat specialists, more-accurate distributions of some (but not all) species were able to be generated through the ‘Modelled potential habitat for selected threatened species – Queensland’ dataset (Department of Natural Resources, Mines and Energy, 2019; see also Table 3.1).

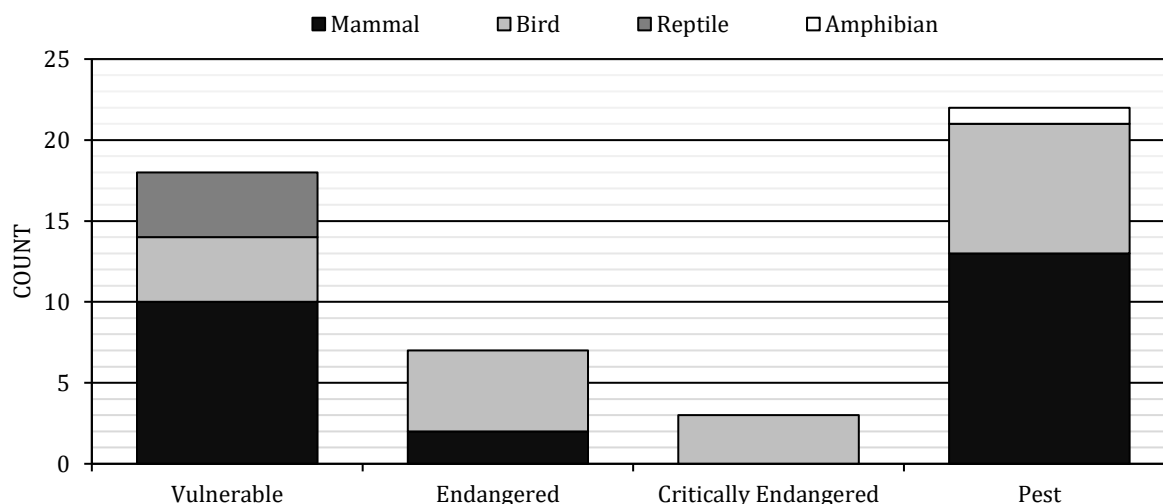
Once identified, the listed threats to each species were extracted from the SPRAT (Species profile and threats) database (also available at [www.environment.gov.au](http://www.environment.gov.au)). We categorised and considered the relevance of whether each threat may be alleviated with clusters. These threats were Dietary Competition (Relevant), Dietary Competition (Irrelevant), Habitat Degradation (Relevant), Habitat Degradation (Irrelevant), Predation by a Controlled Species, Predation by an Uncontrolled Species, Altered Fire Regimes, Habitat Loss, Exotic Weeds, and Human Disturbance. ‘Irrelevant’ threats were those that would not likely be mitigated within a cluster, such as degradation or competition from livestock species. ‘Relevant dietary competition’ (i.e. competition with a controlled pest), ‘Relevant habitat degradation’ (i.e. degradation caused by a controlled pest), and ‘Predation by a controlled species’ are the threats most likely to be alleviated within cluster fences, so, we assessed which extant threatened species within cluster fences are most likely to benefit from the reduction or removal of these threats. We focused our attention on the threats being directly managed within cluster fences (e.g. predation and competition by a controlled species) even though most other threats (e.g. fire, habitat loss, weeds, and human disturbance) are also being managed or mitigated indirectly.





**Figure 3.2: Map of exclusion fenced areas in central-west and south-west Queensland as at December 2019.** Shaded by final funding year or known year of completion. Unknown completion years of privately funded exclusion fences are indicated by hatching. Black line shows the location of the national Dingo Barrier Fence (designed to help manage dingoes in the southern part of this area). Map generated in ArcMap v10.5.1. Red polygon shows Idalia National Park (see Discussion for details).

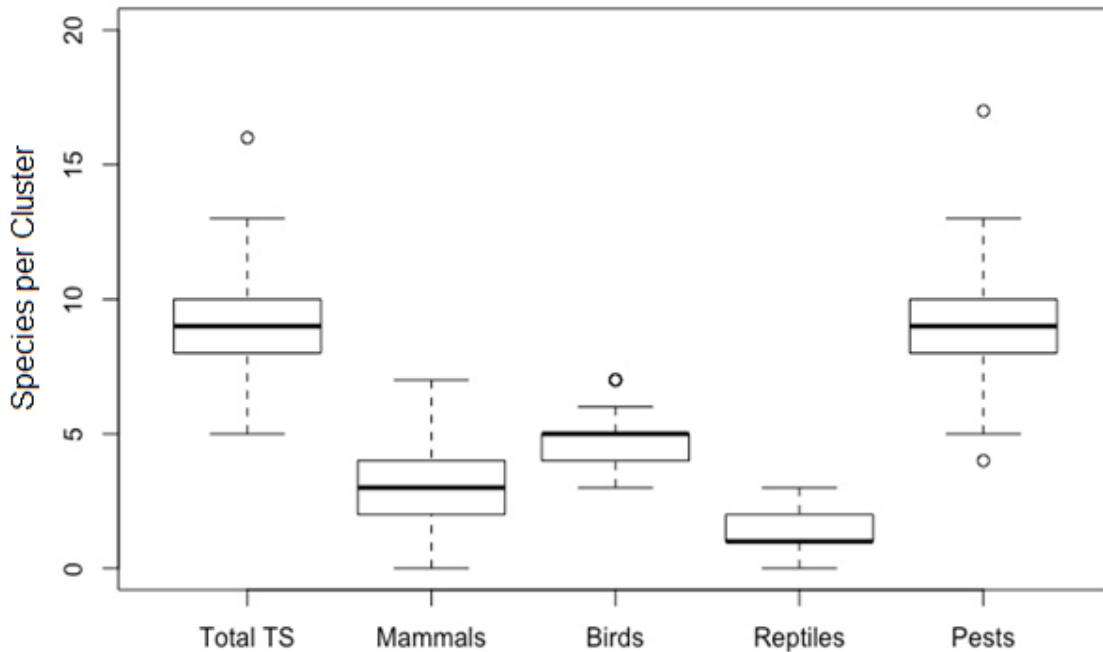
### 3.4 Results



**Figure 3.3: Count of fauna species identified in each class across conservation status**

Bar chart presenting the count of identified species at each level categorised by SPRAT database: Critically Endangered, Endangered, Vulnerable or Pest Species (x-axis). Columns divided by species class. Black – mammal, Light grey – birds, Dark grey – reptiles, and White – amphibian.

The former South-West Natural Resource Management Board (SWNRM; now known as Southern Queensland Landscapes) administered three fence funding rounds beginning after 2010. SWNRM reported that 28 clusters were fenced over the three rounds of funding, totalling 39,773 km<sup>2</sup> of land located south of the Dingo Barrier Fence (Figure 3.2). Most of the larger, original clusters within the SWNRM area have since been subdivided into dozens of smaller fenced areas or cells, but these have not been separately assessed here. The Remote Area Planning and Development Board (RAPAD) reported an additional 32 clusters over three funding rounds, totalling 18,461 km<sup>2</sup> of land located north of the Dingo Barrier Fence. The additional 27 clusters that sourced funding from the Longreach Wild Dog Exclusion Fence Scheme (LWDEFS) and a further 18 known, privately-fenced clusters were also included the dataset, adding another 3,782 km<sup>2</sup> and 3,885 km<sup>2</sup> to the total cluster fenced area, respectively. Thus, we assessed 105 individual clusters representing approximately 65,901 km<sup>2</sup> of pest-fenced agricultural land as at December 2019 (Figure 3.2).

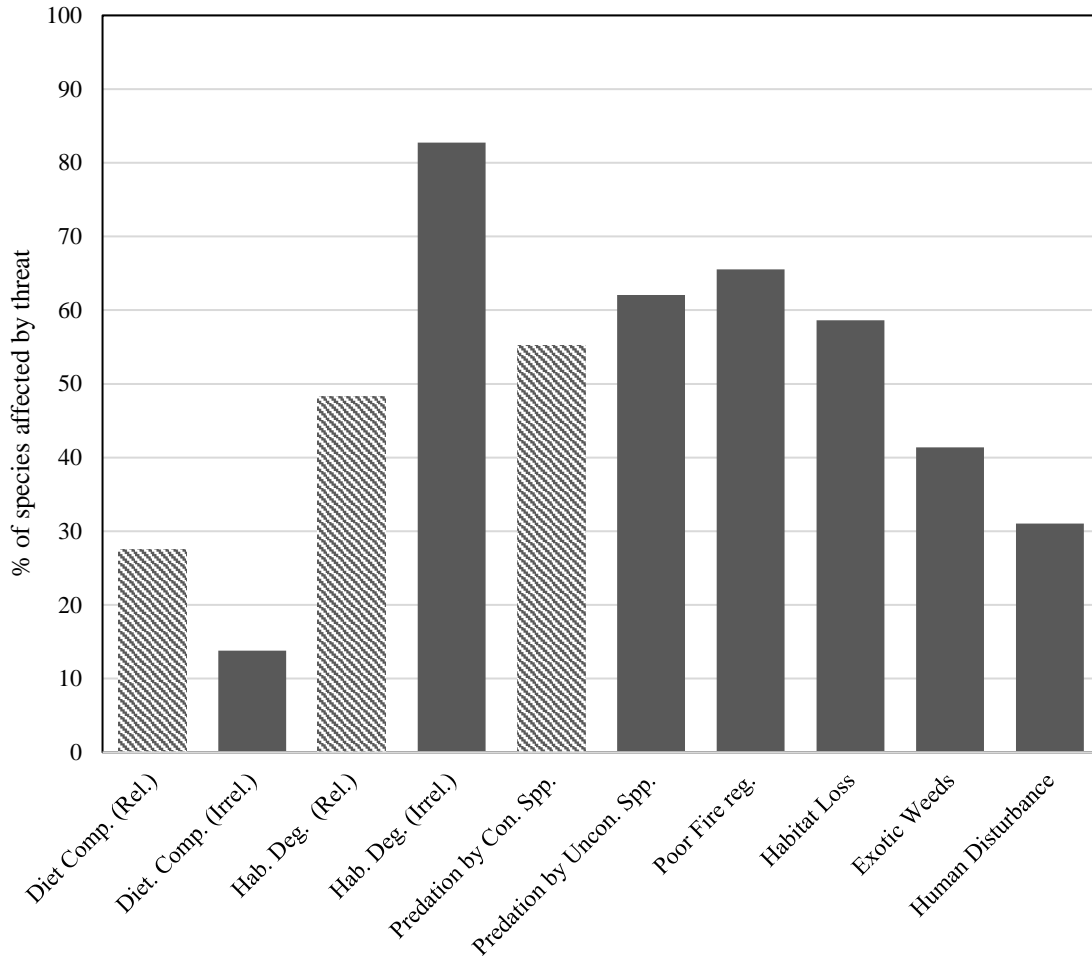


**Figure 3.4: Boxplot showing count of threatened species per cluster by phylum**

Included is the total threatened species (TS) per cluster and total pest species per cluster. Bolded line shows median value, boxes show interquartile range. Outlier values shown as open circles. Average TS per cluster is 9.09 (SE=0.19); mammals (mean=2.8, SE=0.09); birds (mean=4.9, SE=0.08); reptiles (mean= 1.3, SE=0.07); Pests (mean=9.3, SE=0.20).

The 105 clusters included parts of 18 biogeographic subregions (Figure S4), and 28 threatened species or their habitat were identified using the PMST. These included 18 vulnerable species, 7 endangered species, and 3 critically endangered (CE) species (Figure 3.3). These three CE species – the Curlew Sandpiper (*Calidris ferruginea*), Plains Wanderer (*Pedionomus torquatus*), and Eastern Curlew (*Numenius madagascariensis*) – feature in 100%, 18% and 1% of PMST reports, respectively; the proportion of the cluster fenced estate that these species could occupy were 100%, 35% and 4% of cluster fenced land, respectively (Table 3.1). Detailed models of habitat distribution were available for 21 of the 28 threatened species (Table 3.1). Of note, 4,031.9 km<sup>2</sup> of yellow-footed rock-wallaby (*Petrogale xanthopus*) habitat and 686.7 km<sup>2</sup> of Julia Creek Dunnart (*Smithinthopsis douglasi*) habitat is available within cluster fenced areas (Table 3.1, Figure S5). Additionally, 1 amphibian, 8 avian and 13 mammalian pest animals are listed as being present within clusters (Figure 3.3, Table S4). Pest predators (or their habitat) occurring in high proportions of fenced land (based on PMST reports) were the dingo (50%), feral cat (94%), fox (96%), and the invasive herbivores feral goat (95%), feral pig (96%), and rabbit (97%).





**Figure 3.5: Threats to identified species**

Threats recorded from individual species SPRAT file (Department of Environment and Energy). Patterned entries indicate the primary threats being actively mitigated within clusters; threats like fire, habitat loss, weeds, and human disturbance are also mitigated within clusters, but are not the focus of clusters.

An average of 9 (SE = 0.19) threatened species and 9 (SE = 0.20) invasive species were identified per cluster (Figure 3.4). All documented threatened species have a variety of threatening process listed in the SPRAT database, most commonly ‘irrelevant habitat degradation’ (72.4%), ‘altered fire regimes’ (65.5%), and ‘habitat loss’ (58.6%). We found that ‘predation by a controlled species’, ‘relevant habitat degradation’, and ‘relevant dietary competition’ were recorded as threats for 55.2%, 48.2%, and 27.6% of threatened species, respectively. Over three quarters (78.6%) of identified threatened species experience at least one threat that may be alleviated within clusters. Four identified species (14.3%; night parrot *Pezoporus occidentalis*, Julia Creek dunnart, yellow-footed rock-wallaby, and Bridled Nail-tail Wallaby *Onychogalea fraenata*), face all three relevant threats, a further 28.6% face two relevant threats, and 35.7% face one relevant threat (Figure 3.5).

**Table 3.1: List of threatened species recorded in PMST reports and their key threats**

The list of threatened species identified by PMST reports to be present within Clusters. Table lists their threat level status (S), critically endangered (CE), endangered (E), and vulnerable (V). (%) show the percentage of clustered land the species may appear in based on assumed 100% occupancy per cluster. (km<sup>2</sup>) details the area of potential species distribution in km<sup>2</sup> within clusters (where data exists – QSpatial). The ‘Potentially alleviated’ threats only identify those threats being directly mitigated within clusters, and do not identify the additional threats being indirectly mitigated within clusters.

	Species	S	%	km <sup>2</sup>	Threats	Alleviated Threats
1	<b>Curlew Sandpiper</b> , <i>Calidris ferruginea</i>	CE	100.0	-	3,4,5,6,9	-
2	<b>Plains-wanderer</b> , <i>Pedionomus torquatus</i>	CE	35.1	481.2	3,4,5,6,7,8,9	5
3	<b>Eastern Curlew</b> , <i>Numenius madagascariensis</i>	CE	3.66	-	3,4,5,6,9	-
4	<b>Star Finch</b> , <i>Neochmia ruficauda</i>	E	44.1	-	1,2,3,4,5,6,7	3,5
5	<b>Black-throated finch</b> , <i>Poephila cincta</i>	E	10.1	4.2	3,4,5,6,8,9	3,5
6	<b>Australian Painted Snipe</b> , <i>Rostratula australis</i>	E	99.5	208.3	1,4,5,6,7,8,9	3,5
7	<b>Night Parrot</b> , <i>Pezoporus occidentalis</i>	E	26.0	-	1,3,4,5,6,7,9	1,3,5
8	<b>Bulloo Grey Grass-wren</b> , <i>Amytornis barbatus barbatus</i>	E	5.16	0	3,4,5,6,8,9,10	3,5
9	<b>Northern Quoll</b> , <i>Dasyurus hallucatus</i>	E	4.77	0	2,3,4,6,8,10	1,5
10	<b>Bridled Nailtail Wallaby</b> , <i>Onychogalea fraenata</i>	E	1.84	0	1,2,4,7,8	1,3,5
11	<b>Squatter Pigeon</b> , <i>Geophaps scripta scripta</i>	V	22.9	124.5	2,3,7,8	3,5
12	<b>Painted Honeyeater</b> , <i>Grantiella picta</i>	V	96.3	14,710.0	1,4,5,6,7	3
13	<b>Red Goshawk</b> , <i>Erythrotriorchis radiatus</i>	V	37.8	0	5,7,8,10	1
14	<b>Masked Owl</b> , <i>Tyto novaehollandiae kimberli</i>	V	3.66	-	1,3,5,6,8,9	3
15	<b>Greater Bilby</b> , <i>Macrotis lagotis</i>	V	40.9	0	4,5,7,8,10	1,5
16	<b>Koala</b> , <i>Phascolarctos cinereus</i>	V	69.8	193.6	1,3,5,7,8	5
17	<b>Julia Creek Dunnart</b> , <i>Sminthopsis douglasi</i>	V	34.0	686.7	4,5,6,7,8,10	1,3,5
18	<b>Corben's Long-eared Bat</b> , <i>Nyctophilus corbeni</i>	V	41.7	193.4	1,4,6,7,8,10	5
19	<b>Yellow-footed rock-wallaby</b> , <i>Petrogale xanthopus</i>	V	46.3	4,031.9	4,6,7,8	1,3,5
20	<b>Semon's Leaf-nosed Bat</b> , <i>Hipposideros semoni</i>	V	3.66	0	4,7,10	5
21	<b>Ghost Bat</b> , <i>Macroderma gigas</i>	V	4.47	0	4,7	1
22	<b>Greater Glider</b> , <i>Petauroides volans</i>	V	3.66	-	3,4,6,7	-
23	<b>Spectacled Flying Fox</b> , <i>Pteropus conspicillatus</i>	V	3.66	0	4,5,6,7	-
24	<b>Bare-rumped Sheath-tail-bat</b> , <i>Saccolaimus nudicluniatius</i>	V	3.66	0	3,4,6	-
25	<b>Plains Death Adder</b> , <i>Acanthophis hawkei</i>	V	45.5	-	4,7,9	3
26	<b>Yakka Skink</b> , <i>Egernia rugosa</i>	V	49.1	2,202.6	3,4,5,6,9	5
27	<b>Ornamental Snake</b> , <i>Denisonia maculata</i>	V	12.9	0	3,4,5,6,7,8,9	3
28	<b>Adorned Delma</b> , <i>Delma torquata</i>	V	10.9	1.3	3,4,5,6,9	-

Threats identified were (1) Relevant dietary competition, (2) Irrelevant dietary competition, (3) Relevant habitat degradation, (4) Irrelevant habitat degradation, (5) Predation by a controlled species, (6) Predation by an uncontrolled species, (7) Poor fire regimes, (8) Habitat loss, (9) Exotic weeds, and (10) Human disturbances.

### 3.5 Discussion

There has been a rapid resurgence of pest-proof netting fencing on agricultural land across central-west Queensland in recent years. Since the first two cluster fences were completed in 2013 near the towns of Tambo and Morven, there are now over 105 clusters of livestock properties totalling ~66,000 km<sup>2</sup> that are enclosed by fences intended to exclude dingoes, kangaroos, feral pigs, feral goats, foxes and some other animals (Figure 3.2). The true status of each of these species within clusters is largely unknown or unrecorded in most cases. However, anecdotal case studies (e.g. Clark, Clark, & Allen, 2018) and limited in-progress fauna monitoring data collected since 2013 (B. Allen, unpublished data) indicate that wild dogs, foxes, and feral cats are either absent or at near undetectably-low densities in many clusters, as are feral pigs, feral goats and rabbits. Kangaroos have also been reduced by 90–95% of their former densities in some clusters (Allen, 2019). These pest removals and declines have the potential to benefit some of the 28 threatened fauna thought to be present within these cluster fences, including the 8 endangered or critically endangered species (Figure 3.3). The predation and competition threats that are being actively alleviated within cluster fencing could potentially benefit at least 22 of the 28 (78.6%) threatened fauna known or expected to be present inside these cluster fences. Although not all land or habitats within cluster fences will be suitable for each threatened species, our results indicate that in many cases, hundreds or even thousands of square kilometres of land within cluster fences is suitable for some species provided their key threats have indeed been eliminated or neutralised (Table 3.1). The vast size of the cluster fenced estate, the many different biomes and species it represents, and the nature of the threats being removed within these fenced areas offers a remarkable opportunity to potentially advance threatened fauna conservation goals. Recovery of extant threatened fauna and/or reestablishment of locally-extinct threatened fauna within cluster fences could dramatically increase global populations of these species (Allen, 2017).

The high cost of conservation fences remains a limiting factor in their application and extensive research has gone into developing low-cost and effective fencing designs (Dickman, 2012; Helmstedt et al., 2014; Long & Robley, 2004; Moseby & Read, 2006). Depending on the materials used and the topography of the land, conservation fences typically cost about \$15,000 to \$18,000 per kilometre whereas cluster fences cost about \$5,000 to \$8,000 per kilometre, with the primary difference being the extra effort required

to exclude feral cats (e.g. a taller fence with smaller gauge mesh and a floppy top). Moreover, seldom are conservation fences erected on land that generates an income capable of paying for ongoing fence maintenance, which is typically funded by governments, philanthropy and public donations. In contrast, cluster fenced agricultural land produces incomes (i.e. sales of livestock products such as wool or red meat) that can sustainably fund ongoing fence maintenance without external support. Indeed, all government-subsidised cluster fences already feature perpetual and legally-binding fence maintenance funding arrangements, and new and additional funding arrangements are also being proposed (Cockfield, Botterill, & Kelly, 2018). Research on choosing cost-effective locations for conservation fences also raises valid concerns about constraints on finding suitable locations that minimise outlaid economic costs and maximise species conserved (Bode et al., 2012). A major reason for the unavailability of suitable conservation land is that it is being used for extensive livestock grazing; approximately 50% of Australia's land mass is used for this purpose (Allen, 2011). But what if this livestock land was made suitable for conservation? Using cluster fences as pseudo-conservation reserves would be a 'land sharing' initiative that has the potential to help relieve each of these issues. If current cluster fences are suitable for a given threatened species, then no additional fencing need be constructed. But if current cluster fences require 'upgrading' to be suitable for a given threatened species (e.g. to make them 'cat proof'), then conservation agencies might consider partnering with livestock producers to establish 'conservation fences' for a fraction of the cost of a new conservation fence, and at scales much larger than is typically possible for conservation agencies. Fence construction savings might then be used towards eradication of cats within fences, which is not a priority activity undertaken by livestock producers. The ~66,000 km<sup>2</sup> of cluster fenced land identified here dwarfs the ~360 km<sup>2</sup> of land contained within all the high-security fenced conservation reserves dotted across Australia (Legge et al., 2017); and these are just those cluster fences that exist in central and central-western Queensland, which does not include the rapidly growing number of cluster fences in other states including New South Wales, South Australia and Western Australia. The 105 cluster-fenced areas we assessed also cover a variety of different biogeographic subregions (Figure S4), not all of which are fully represented in the national reserve estate (Ringma et al., 2018). In the effort to improve agricultural productivity, livestock producers have potentially handed conservationists a powerful tool in the fight against threatened species decline.

Given that some predators (i.e. feral cats) and competitors (i.e. livestock) may persist in some cluster fenced areas, cluster fences are probably most suitable for larger-sized threatened species and/or those that are able to tolerate low levels of predation and competition; they are unlikely to be suitable for highly threatened species that cannot tolerate any predation at all. For example, the yellow-footed rock-wallaby (YFRW) is a ‘vulnerable’ species present in 55% of PMST reports, 4,031 km<sup>2</sup> of cluster fenced land represents suitable habitat for them (Table 3.1), and they have been recently confirmed as present in several clusters (D. Smith, unpublished data). Three key threats to this species are directly eliminated or reduced within cluster fences, and four other threats are indirectly alleviated (Table 3.1). The conservation advice for YFRW lists predation by foxes and cats, and dietary competition and land degradation by feral herbivores and livestock as the key threats to the species (Threatened Species Scientific Committee, 2016b). Dingoes also threaten YFRW (Allen & Fleming, 2012) but do not appear as threats in most previous conservation advices because dingoes had been effectively eradicated and were absent from YFRW habitat for many decades at the time these advices were generated. Dingoes are known to predate other rock-wallaby species (Moseby et al., 1998; Whitehouse, 1977. Control of canid predators has yielded positive results for analogous rock-wallaby species Kinnear et al., 2010) and fox and goat control across populations of the South Australian subspecies of YFRW (*P. x. xanthopus*), resulted in increases in abundance and distribution (Lethbridge & Alexander, 2008; Lethbridge, Harper, & Strauss, 2010). For this species at least, recent creation of cluster fences within the present distribution of YFRW and the subsequent removal of their key threats within these fences are likely to dramatically benefit them. Using the estimates of species density calculated across 40 km<sup>2</sup> of similarly suitable habitat (Gordon et al., 1993), the total cluster fenced area in Queensland could potentially hold approximately 23,000 YFRW, which is more than double the current YFRW population estimate of around 10,000 mature individuals (Copley, Ellis, & van Weenen, 2016). Examples like this highlight the substantial potential gains for extant threatened species if their local threats can be effectively managed within cluster fences.

Cluster fences may also be valuable locations for reintroduction of locally extinct species if historical ranges or analogous habitat for the species is identified within cluster fenced areas. Bridled Nail-tail Wallabies (*Onychogalea fraenata*) occur in only 2% of cluster fenced land based on PMST reports and its modelled distribution did not fall within any cluster erected by the end of 2019 (Table 3.1). However, the species faces all three threats

being alleviated within clusters and existing clusters cover some suitable habitat for the species within its former range (Pople et al., 2001). The species had been successfully reintroduced in the past to the unfenced Idalia National Park which borders some of the assessed clusters (see red polygon, Figure 3.2; Pople et al., 2001), but the effort ultimately failed years later due to drought, predation, and competition – the very threats being alleviated in adjacent cluster fenced areas. Given reintroductions of this species and others have been shown to benefit from exclusion fencing in the past, and fail in its absence (see Hayward et al., 2012; Moseby et al., 2011; Short, 2016; West et al., 2017), we contend that cluster fenced areas should be seriously considered for this and other species' reintroductions, particularly when they enclose suitable habitat for the species. The density of wallabies within the National Park was reported to be 0.3/km<sup>2</sup> in 1999 and increasing (Pople et al., 2001), and up to 12/km<sup>2</sup> in suitable habitat (Fisher, Hoyle, & Blomberg, 2000). Applying these densities to just the two cluster fenced properties that share a boundary with the National Park (Figure 3.2) show that utilisation of these two clusters for reintroductions could increase the population by 326 to 13,052 individuals. Bridled nail-tail wallabies and yellow-footed rock-wallabies are just two of the many extant threatened species we identified that could potentially benefit from cluster fences on agricultural land (Table 3.1).

Our study was limited by available spatial data on potential habitat and accurate estimates of species densities from healthy populations. Habitat data represents only potential habitat within a 200 km convex hull of species records that are 50 years and younger (see metadata, 'Modelled potential habitat for selected threatened species; Department of Natural Resources, Mines and Energy, 2019), and does not cover all threatened species identified within PMST reports (Table 3.1). Given species may have experienced rapid and vast declines in their range previous to the last 50 years, such as the Bridled Nail-tail wallaby (Pople et al., 2001), this limited our ability to accurately determine all locations where cluster fencing could be used in specific species' conservation. In other words, our results likely underestimate the potential value of cluster fences to threatened species. Our assessment was also confined to central and central-western Queensland, a unique area of broadly similar biogeography (Mulga Lands and Mitchell Grass Downs, see Environmental Resources Information Network, 2016). Despite this, the fenced areas still enclose parts of 18 different biogeographic sub-regions (Figure S4) and we identified 22 threatened species that may benefit from cluster fencing within these regions. Cluster fencing on agricultural land is rapidly increasing across Australia and additional cluster fences also now occur in

south-east Queensland; the north-eastern, Monaro, and western regions of New South Wales; and also in Western Australia. Cluster fencing in these areas might also benefit additional threatened species not discussed here. These limitations mean that our results merely show the *potential* for extant threatened species recovery on agricultural lands based on theoretical responses to cluster fencing and its accompanying animal management activities. On-ground research is greatly needed to determine whether or not these theoretical predictions are realistic.

Conservationists might understandably have some reservations about the utility of agricultural land for threatened species recovery. The type of fences used for cluster fencing are not exactly the same as those used for conservation, nor are the animal management activities that occur within fenced areas the same (see Bode & Wintle, 2010; Giumelli & White, 2016; Norbury et al., 2014). Livestock producers have little incentive to control some pest species, such as feral cats, which are of great concern to threatened fauna but are of negligible concern to livestock. Cluster fences may also be considered more permeable than conservation fences for some pest species, though this may not be as great a concern as might be supposed. Dingoes, foxes and cats are each known to breach even the best conservation fences at times (Moseby et al., 2012); intruding predators have decimated at-risk species in conservation zones (Moseby et al., 2011) and prey naivety has been identified as a potential driver of their vulnerability. Some have proposed that exposing species to low densities of predators may assist the development of antipredator behaviours and overcome prey naivety (Berger, Swenson, & Persson, 2001; Moseby, Blumstein, & Letnic, 2016; Ross et al., 2019). Pest exclusion fences could therefore be utilised for this purpose as an intermediary step between high-security reserves and completely unfenced populations (see Allen, 2017). Cluster fencing may also have adverse effects on native species, such as barriers to movement and gene flow. However, many these adverse effects also occur in conservation fencing (Smith, King, & Allen, 2020; Chapter 2). With appropriate monitoring and use of mitigation strategies (such as metapopulation management) these adverse effects can be minimised to ensure net positive outcomes.

Some conservationists might also assume that cluster fenced agricultural land does not have equivalent conservation value to conservation fenced reserve land. Though this will undoubtedly be true in some cases, there are at least three important reasons not to glibly dismiss cluster fenced land as unsuitable for conservation purposes in many or perhaps

most cases. First, many current National Parks, reserves and conservation-fenced lands were formerly degraded livestock properties sold cheap to conservation organisations in the last few decades because their ecosystems were no longer productive enough to produce livestock. Had these lands been productive and profitable they would not likely have been converted to non-agricultural land uses. Hence, we should not automatically assume that land currently zoned for conservation has greater ecological value than land currently zoned for agriculture when the difference between the two might only be an administrative label. Second, there are often large tracts of land (e.g. 25–100 km<sup>2</sup>) on agricultural properties that have never been grazed by livestock or have been grazed by livestock only temporarily in the distant past. Examples include the >4,000 km<sup>2</sup> of rugged YFRW habitat that is rarely, if ever, grazed by sheep or cattle because such rugged areas are too difficult to muster or supply water to in quantities sufficient for ruminant livestock. So even if much or most of a cluster is currently utilised by livestock, there are often many areas within clusters that have always been and are still essentially managed as ‘reserves’. Third, even if cluster fenced land really was not as productive or not as beneficial for threatened species as a reserve, and threatened species might never reach ‘reserve densities’ on livestock lands, such lands nonetheless represent areas where at least some threatened species may be able to re-establish populations. Achieving lower ‘cluster densities’ in areas where they are now locally extinct, for example, is still better than having no threatened species there at all. Cluster fenced areas could play a significant role in the recovery and enduring conservation of threatened species even if they are ‘not as good’ as reserves in some cases.

### **3.6 Conclusion**

Cluster fences are rapidly being erected in Queensland and across Australia, and several species that also present threats to wildlife conservation are being either eradicated or substantially reduced across spatial scales not seen in decades. Some clusters already anecdotally report the absence or near-absence of all these species. Declines in such pest animals are yielding economic and environmental benefits to livestock producers, and could yield benefits for threatened fauna conservation as well, in the following ways:

- Creation of new locations where it is possible to change the trajectory of extant threatened species populations;
- Creation of new locations where it is possible to reintroduce locally-extinct species;



- Creation of new locations suitable for improving antipredator defences and overcoming prey naivety issues;
- Addition of new biomes not currently represented in the national fenced reserve system;
- Opportunities to develop private-public partnerships to share the costs of constructing high-security conservation fences (i.e. new, large conservation fences can be erected for a fraction of current costs);
- Reduction or shifting of ongoing fence maintenance costs to sources non-reliant on government, philanthropy or public donations; and
- Alleviation of threatened species overpopulation in some of the current conservation reserves.

Cluster fenced agricultural lands may represent a stepping stone in the effort to take threatened species from high-security reserves and re-establish free-ranging and self-sustaining populations of them in unbounded areas. Though we have described a positive and optimistic opportunity for threatened fauna conservation, almost all of this remains invalidated *in situ*. We therefore recommend (1) on-ground monitoring be conducted to establish the presence or absence of pest and threatened fauna in priority clusters, (2) increased effort be applied to assisting the recovery of extant threatened fauna within clusters, (3) studies that monitor the net outcomes for all biota in cluster fences take place, and (4) pilot studies be conducted to reintroduce threatened species expected to be resilient to the conditions found within cluster fences. Though not all threatened species will be suitable and many issues might remain unresolved, we contend that cluster fences on livestock lands should be seriously considered a key resource in the ongoing effort to conserve and recover Australia's many unique and threatened fauna species.

### **3.7 Author Contributions**

BLA, DS and KW contributed to the conception and design of the research that led to this manuscript. DS and KW acquired the data. DS analysed and interpreted the data. DS drafted the initial manuscript and all authors contributed critically to the drafts and gave final approval for publication.

### **3.8 Acknowledgements**

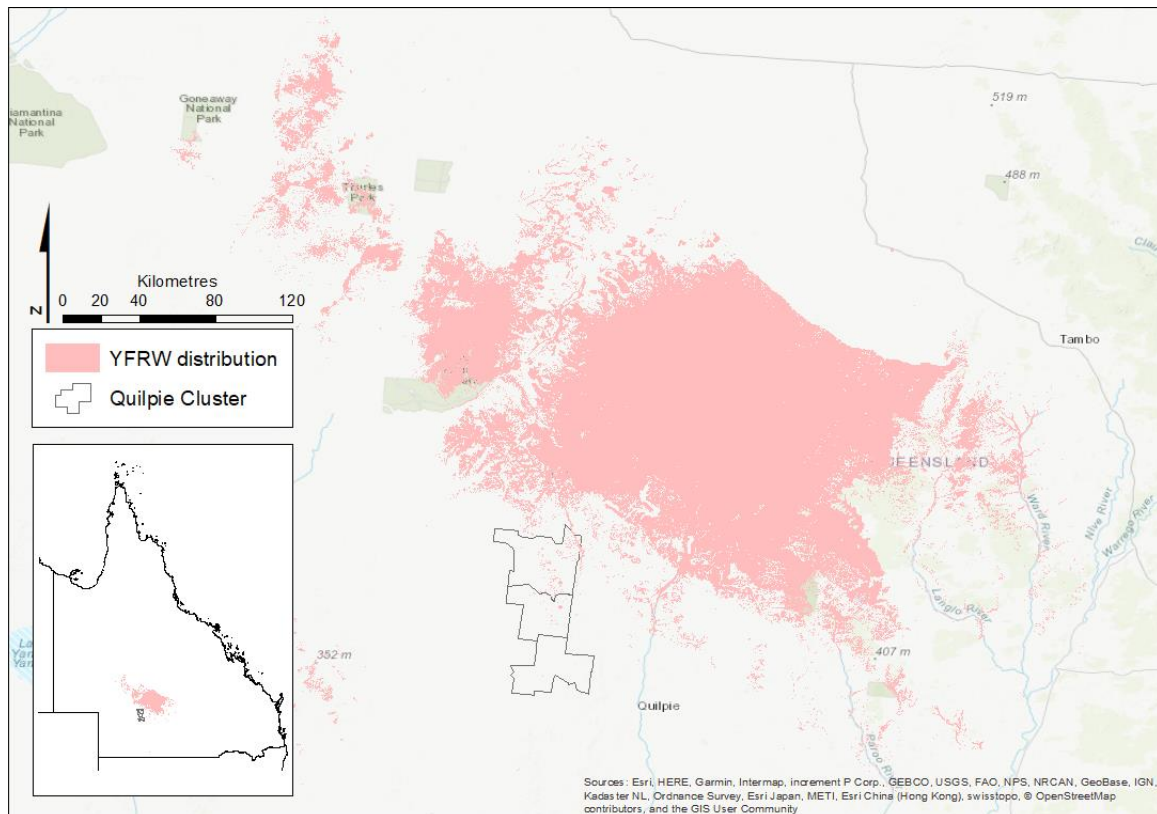
DS is funded by a Research Stipend Scholarship for the completion of his PhD, for which this manuscript forms a part. DS's research project is also funded by the Holsworth Endowment, for which we are incredibly grateful. Some sections of this manuscript were presented at the Australasian Wildlife Management Society (AWMS) conference in Darwin (2019), and the authors wish to acknowledge the contribution conference attendees made to the development of this manuscript's structure and content.

## **Chapter 4 : The yellow-footed rock-wallaby (*Petrogale xanthopus celeris*)**

This chapter presents the relevant background on the focus species of this thesis, the yellow-footed rock-wallaby. This chapter establishes a working understanding of both YFRW in Queensland and Australia-wide. As this thesis aims to explore the effects of exclusion fencing on non-target species, with a focus on YFRW, outlined in this chapter is the relevant biology and ecology, threats and conservation, and current understanding of YFRW. These areas were identified as potentially influencing a species' response to fencing or are impacted by fencing (Chapter 2). Finally, this chapter ends with an explanation of the rationale in electing to focus on YFRW for this thesis.

### **4.1 Introduction**

*Petrogale xanthopus celeris* is one of two subspecies of yellow-footed rock-wallaby, and is found exclusively in the mulga lands bioregion near Adavale, in central-western Queensland (Sharp, 2002). The other subspecies of YFRW (*P. x. xanthopus*) is found in similar bioregions across multiple populations in the Flinders, Gawler, and Olary Ranges of South Australia, and a single population in New South Wales. The Queensland subspecies has been subject to far fewer ecological surveys and studies than its southern counterpart, and much of what is reported in the literature about YFRW is assumed to be applicable across both subspecies. Both subspecies have a conservation status of Vulnerable under the *Environmental Protection and Biodiversity Conservation Act (1999)*, and the International Union for Conservation of Nature (IUCN) red list of threatened species lists *P. xanthopus* as 'Near Threatened' (Copley, Ellis, & van Weenen, 2016). The species is also mentioned in 'The action plan for threatened Australian macropods (2011-2021 WWF)'. *P. x. celeris* is also classed as vulnerable under state legislation (*Queensland Nature Conservation Act 1992*). *P. x. celeris* faces the same threats as is faced by *P. x. xanthopus* in South Australia and New South Wales (Threatened Species Scientific Committee, 2016a, 2016b).



**Figure 4.1: Potential distribution of yellow-footed rock-wallaby habitat in Queensland**  
 A map showing the potential distribution of yellow-footed rock-wallaby in Queensland. This map shows the likely location of appropriate habitat, not necessarily populated by YFRW. Polygon shows the site of the Quilpie Cluster, see Chapter 5.

## 4.2 Biology and Ecology of YFRW

Between the two subspecies of YFRW there are no obvious morphological differences, though Sharp (2002) noted that *P. x. celeris* appears to have less pronounced colouration. Both subspecies of YFRW display high habitat specificity, and rarely occur outside of a strict set of habitat criteria (Copley, 1983; Gordon et al., 1993; Lim & Giles, 1987; Roache, 2011). The YFRW typically occupies gorges, rock crevices, boulder piles and natural rocky outcrops along the low sandstone remnant hills of the Grey Range in Queensland (Copley, 1983; Gordon et al., 1993; Pope, Sharp, & Moritz, 1996; Potter, Cooper, et al., 2012; Threatened Species Scientific Committee, 2016b). These hills are usually covered in *Acacia* spp. woodlands and shrublands (Gordon et al., 1993; Threatened Species Scientific Committee, 2016b). Rock piles are considered necessary for shelter during hot and dry summers (Lim, 1987) and as refuge from predation, particularly for juveniles (Sharp, 2002). Other limitations to distribution of the species include annual rainfall, and habitat connectivity (Gordon et al., 1993). YFRW only occur in areas where rainfall exceeds 150 mm per annum, and up to 200 mm for higher density populations (Sharp, 2002).

Colonies can comprise of 10 to 100 individuals, with larger colonies made up of a number of smaller social groups (Pope, Sharp, & Moritz, 1996; Sharp, 1997a; Threatened Species Scientific Committee, 2016b). The average home range size of individual YFRW calculated in Queensland populations has been estimated at approximately 23.5 ha (Sharp, 2009). Sharp (2002) identified a number of variations to individual ranges, including distinctions between male and female home ranges, larger ranges during drier periods, and significantly smaller ranges if the movements to the closest free water points were excluded (Sharp, 2002).

Due to the lack of data prior to the early 1970s, the majority of information relating to the historical distribution of Queensland's YFRW is speculative. *P. x. celeris* were first located in the Adavale basin and surrounding Grey Range but are now known to be further south-west in the Grey Range northwest of Quilpie (Roache, 2011; Figure 4.1). Until a study by Gordon et al. (1993) the status of the YFRW population in Queensland was largely unknown, but the subspecies was considered rare or uncommon due to its cryptic nature. Gordon et al. (1993) estimated the total population to be between 5,000 and 10,000 individuals. Though a number of important limitations to the accuracy of this estimate were identified by the authors, it was still being used as the guide for discussing conservation efforts for the subspecies as late as 2016 (Threatened Species Scientific Committee, 2016b). Since the Gordon et al. (1993) study, several projects have estimated the size of various subpopulations (see Lapidge, 2001; Sharp, 2002; Sharp & McCallum, 2010) and theorised an overall decline in YFRW populations across Australia (Woinarski, Burbidge, & Harrison, 2014). There has been no recent comprehensive study of total YFRW population size across their range (Threatened Species Scientific Committee, 2016b), though some aerial surveys were conducted in 2017 (pers. comm. Tracy Watts, 2017; pers. comm. Luke Geelen, 2019). A large proportion of the known total population is located within Idalia National Park and across the Grey Range that lies south-west of the township of Blackall (Roache, 2011). However, a number of factors are continually reducing this range (Threatened Species Scientific Committee, 2016b) and the IUCN currently considers the size of the Queensland population to be unknown (Copley, Ellis, & van Weenen, 2016).

### 4.3 Threats and Conservation

YFRW numbers have been declining since the arrival of Europeans in Australia (Lim, 1987). Soon after European settlement rock-wallabies were hunted extensively for fur trading (Figure 4.2), but in more recent times their decline can be attributed to predation and competition by introduced species (Copley, 1983; Gordon, McGreevy, & Lawrie, 1978; Lim, 1987; Sharp et al., 2015). Habitat fragmentation may also be a threat to the species (Threatened Species Scientific Committee, 2016b).

The red fox is known to have contributed to the decline of the YFRW (Lim et al., 1987). Mature YFRW fall outside of the Critical Weight Range (CWR; 35g–5500g) that are typically most at risk from predation by foxes and feral cats. However, juvenile YFRW are known to be largely independent of the mother from just one kilogram in weight (Sharp, 2002). These sub-adults (weighing between 1 and 5 kg) are highly susceptible to predation from both foxes and cats (Burbidge & McKenzie, 1989), which inhibits recruitment (Lethbridge et al., 2019). Apart from foxes, the only other recorded predators of the YFRW are the wedge-tailed eagle (*Aquila audax*; Lim et al., 1992) and the dingo (Hornsby, 1997; Sharp, 2002). Eagle predation on YFRW is left unmanaged (Sharp, 2002) and the feral cat is often included as a listed threat to YFRW populations even though there is little evidence of YFRW predation by cats. There are some anecdotal reports from opal miners in the study area who have witnessed cats hunting YFRW (pers. comm. Eric Stelzer, 2019), but feral cats' aptitude for predation on CWR species is often cited where evidence is lacking (Burbidge & McKenzie, 1989). Additionally, cats are known predators of other rock-wallaby species (Read, Dagg, & Moseby, 2019). The presence of dingoes close to YFRW colonies may actually be of some net benefit to YFRW by presumably reducing the impacts of other pest animals, though these relationships remain unclear. Dingoes are known predators of feral goats, and can limit goat numbers (Gordon et al., 1993). There is also literature reporting dingoes as suppressors of fox and cat abundance through competition and predation, leading to net benefits in native species populations (Gordon, Moore, & Letnic, 2017; Letnic & Koch, 2010). However, dingoes are also known to be active predators of *Petrogale* spp. (Whitehouse, 1977) and the role of dingoes in suppressing invasive meso-predators is still debated (Allen, Engeman, & Allen, 2011; Cooke & Soriguer, 2017; Letnic et al., 2011). Dingoes have also been assessed as providing a high risk to YFRW through direct effects regardless of any indirect benefits they might provide

(Allen & Fleming, 2012). It is also worth considering the ecological pressures that confined rock wallabies to rocky habitats over the last few thousand years, which primarily included dingoes and other terrestrial predators. More research in this area is needed to elucidate whether dingo control may also be beneficial (or otherwise) to YFRW.



**Figure 4.2: Photos of *P. x. celeris* hunting (1920 CE)**

Images from the Tully Family Museum showing Tully family members with yellow-footed rock-wallaby hunted at Dillybrew (Ray Station – Figure 5.1). Dated 1920 CE (seven years before first scientific record of the Queensland subspecies). Images provided by Mark Tully (property owner, Ray Station).

A constant threat to YFRW persistence is resource competition with introduced species, particularly the feral goat (Gordon et al., 1993). Feral goats are a known threat to the biodiversity of the arid and semi-arid zone, and have been shown to have a negative correlation with macropod abundance (Russell, Letnic, & Fleming, 2011). Goats, rabbits (*Oryctolagus cuniculus*) and wallaroo (*Osphranter robustus*) were also shown to have 75%, 53% and 39% dietary overlap with YFRW, respectively, in a study of *P. x. xanthopus* (Dawson & Ellis, 1979). During dry periods goats and YFRW have the highest dietary overlap due to competition for limited food resources. YFRW rely heavily on browse (forage from low trees and shrubs) which constitutes up to 44% of their diet (Dawson & Ellis, 1979). Goats have a significant advantage over YFRW in their ability to access browse, as they can stand on their hind-legs to reach higher branches, taking the browse line (lowest available forage on all trees) higher than YFRW can access (Dawson & Ellis, 1979). The removal of goats from YFRW areas has been shown to have a positive effect on the coverage and diversity of vegetation and YFRW abundance (Hayward et al., 2011; Lethbridge, Harper, & Strauss, 2010; Russell, Letnic, & Fleming, 2011).

A large proportion of potential *P. x. celeris* habitat is on free-hold agricultural land (Figure 4.1). Historically, properties had more viable grasslands for domestic species to graze; however, as a result of historical overstocking by pastoralists (Letnic, 2000) this is no longer the case. To help maintain stock numbers pastoralists have subdivided paddocks, provided free water points and cleared extensive amounts of gidgee (*Acacia cambagei*) and mulga (*A. aneura*; James, 2003; Letnic, 2000). Each of these actions has resulted in potentially negative outcomes for the YFRW. There is evidence that higher livestock grazing intensity impacts macropod species (James, 2003), and particularly in the case of sheep, this pushes the sheep into the more rugged zones inhabited by YFRW. The same effect also amplifies competition with other native herbivores. Wallaroos (referred to as Euro in some literature) usually graze on the lower slopes of rocky hills, but are forced further up slope and into competition with YFRW in search of forage (Dawson & Ellis, 1979). Subdivision would also likely cause some fragmentation of isolated colonies, restricting potential movement behaviours between colonies. Clearing of gidgee and mulga may also affect movement and increase fragmentation given that YFRW use shade trees as shelter (Sharp, 1997b). But livestock grazing is not the only source of habitat destruction. Mining in the arid and semi-arid zones is prevalent throughout history and the present (Letnic, 2000), and habitat for YFRW has been cleared to make way for open-cut opal mining (pers. obsv.), disrupting the species.

Connectivity between populations is an important determinant of a species' long-term persistence (Gyllenberg & Hanski, 1997; Sharp, 2002) and gene flow has been presented as a requirement for the persistence of isolated populations in the environment (Trakhtenbrot et al., 2005). The concepts of habitat fragmentation and movement behaviours are concomitant. The more fractured a landscape, the more movement is required to connect all populations. Records of rock-wallaby movements between colonies have been historically lacking (see Hazlitt, Goldizen, & Eldridge, 2006; Lapidge, 2001; Piggott, Banks, & Taylor, 2006; Sharp, 1997a), though not entirely absent. Sharp (1997a) observed one dispersal event in a study of 120 tracked YFRW, and several recorded and inferred movements were documented for captive-released YFRW (Lapidge, 2001). Movement has also been inferred through genetic data, for both *P. x. celeris* (Pope, Sharp, & Moritz, 1996) and *P. x. xanthopus* (Potter et al., 2020). In areas of connected habitat, YFRW, like other rock-wallaby species, are thought to follow metapopulation theory



(Hazlitt, Eldridge, & Goldizen, 2004; Lethbridge et al., 2019; Pearson & Kinnear, 1997) where populations consist of multiple demes (colonies) and individuals utilise suboptimal habitat when moving between better quality sites.

#### **4.4 YFRW genetics**

The only relevant Queensland-based study of YFRW genetics occurred in 1996, which used four microsatellite loci and mitochondrial DNA (mtDNA) to assess questions of population structure and dispersal distance across suitable and unsuitable habitat (Pope, Sharp, & Moritz, 1996). No other studies since have specifically addressed questions about the population genetics of the YFRW in Queensland. However, analogous investigations of rock-wallabies including *P. x. xanthopus* have occurred (Piggott, Banks, & Taylor, 2006; Potter et al., 2020) and there is a large body of research dedicated to ordering the phylogeny of the *Petrogale* genus (Eldridge & Close, 1997; Potter et al., 2017; Potter, Cooper, et al., 2012).

Eldridge et al. (1999) showed that there is potential for negative genetic effects if a population of rock-wallabies is isolated for an extended period of time. For this reason, a number of *Petrogale* spp. genetic studies have focused on the movement of individuals between colonies (see Eldridge, Kinnear, & Onus, 2001; Hazlitt et al., 2006; Piggott et al., 2006; Piggott, Banks, & Taylor, 2006; Pope, Sharp, & Moritz, 1996; Potter, Eldridge, et al., 2012; Spencer et al., 1995). Many of these studies identified barriers to dispersal as a risk factor for the persistence of individual colonies, and the movement between colonies is essential for the maintenance of effective metapopulations (Hanski, 1991).

The *Petrogale* genus's high habitat specificity has been a suggested driver of their rapid diversification (Potter et al., 2017; Potter, Cooper, et al., 2012), with the recent and swift radiation of the genus likely occurring between 3.7 and 0.5 million years ago (Potter, Cooper, et al., 2012). Research on chromosomal rearrangement, radiation and speciation has been repeatedly built upon (see Eldridge & Close, 1993; Eldridge, Close, & Johnston, 1990, 1991; Eldridge & Johnston, 1993; Eldridge, Johnston, & Close, 1992; Eldridge et al., 1989; Eldridge, Johnston, & Lowry, 1992; Hayman, 1989; O'Neill et al., 1999; Potter et al., 2017; Potter, Cooper, et al., 2012; Rofe & Hayman, 1985; Sharman, Close, & Maynes, 1989). Most recently, genetic analysis of all 23 chromosomal taxa of *Petrogale* has supported earlier work placing rock wallabies into 4 chromosomal groups; *brachyotis*,

*xanthopus*, *lateralis*, and *penicillata* (Potter et al., 2017). This study resolved several phyletic relationships within *Petrogale*, including showing support for *P. xanthopus* and *P. persophone* as a monophyletic group. Within the *P. xanthopus* species, phylogenetic research shows the divergence of *P. x. celeris* and *P. x. xanthopus* to have occurred around 180,000 years ago (Eldridge, 1997). Pope, Sharp, and Moritz (1998) indicated that the populations in Queensland, New South Wales and South Australia are genetically distinct through phylogenetic analysis; though, as had previously been theorised, the South Australian and New South Wales population were genetically more closely related. Eldridge (1997) showed the average sequence divergence to be 0.72% between the two subspecies of *P. xanthopus*, which is greater than the divergence between some distinct rock-wallaby species. Pope, Sharp, and Moritz (1998) concurred with this assessment and recommended that the populations from each state need be managed as separate units and that the New South Wales population should not be genetically supplemented with translocated individuals from South Australia. Understanding of YFRW genetics has also recently been built upon from a conservation perspective, with a recent study of microsatellite loci across the South Australian subspecies showing the sampled populations to be fragmented, with little gene flow (Potter et al., 2020). The results of that study led the authors to suggest translocation of individuals to augment the populations.

#### **4.5 Rationale**

In addition to its vulnerable status, the YFRW is an ideal species for a case study to assess the effects of exclusion fencing as it fulfils three key criteria; (1) The species occupies an area that is fragmented by both modern and historical exclusion fencing, (2) it is unlikely to be able to permeate the exclusion fences, and may rely on movement of individuals between colonies for the maintenance of metapopulation dynamics and genetic diversity, and finally, (3) it faces threats that may be alleviated within the cluster (predation and dietary competition). As a result, comparisons of yellow-footed rock-wallaby behaviour, genetics and competition should be a useful example to inform land managers about the wider risks and benefits of cluster fencing to non-target species.

## **Chapter 5 : Study Site, environmental conditions and other relevant background**

The entirety of this study took place on seven adjacent livestock properties that share common exclusion fence boundaries. Ray, Bunginderry and Canaway Downs Stations are fenced to form the Quilpie Cluster, and are collectively surrounded by an exclusion fence. Outside the fence are 15 Mile, Alaric, Yeenunga and Trinidad Stations (Figure 5.1). This group of properties is situated approximately 75 kilometres north-west of the township of Quilpie in the Mulga Lands Bioregion (Environmental Resources Information Network, 2016) of Central-western Queensland (Figure 4.1).

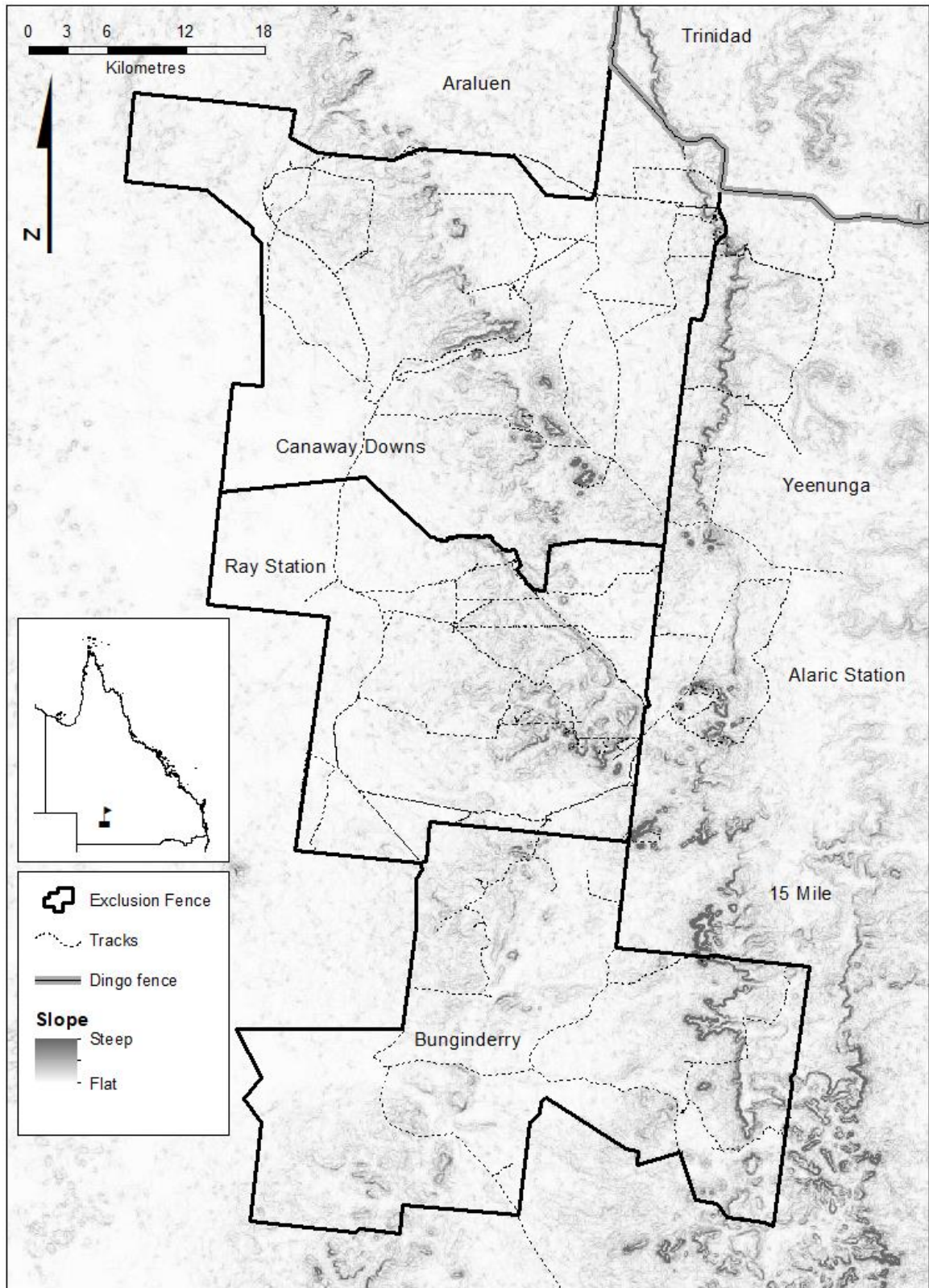
### **5.1 Topography and vegetation**

The Quilpie cluster is approximately 230,390 hectares in size (ArcMap v10.5.1) and forms part of the Grey Range, which is characterised by tertiary sandstone mesas and hills running 700 km south-west from Mount Grey (which is ~90 km WSW of Blackall, Queensland) to just beyond the New South Wales border (Knibbs, 1910; Silcock & Fensham, 2014). The landscape is comprised of predominantly flat areas of rocky ground covered in *Acacia* spp.. Mulga (*Acacia aneura*), bendee (*A. catenulate*), lancewood (*A. petraea*), and mountain sandalwood (*Eremophila oppositifolia*) are the predominant tree species on the mesas and slopes, with *Acacia*, *Cassia* and *Eremophila* spp. forming the shrub-layer. Grasses and forbs do not occur at high densities but sidas (*Sida* spp.), lovegrass (*Eragrostis* spp.), and *Sporobolus* spp. are present in these areas (Gordon et al., 1993). *Eucalypt* spp. are not uncommon in areas with higher water flow, predominantly river-red gum (*E. camaldulensis*) and Coolabah (*E. coolabah*) in areas of permanent or semi-permanent water. Rocky tabletops or mesas are broken up by large flats of grass typically in a sandier soil, with brigalow (*A. harpophylla*) and gidgee (*A. cambagei*) trees (Gordon et al., 1993; Sharp & McCallum, 2010). The ground vegetation in these areas is dominated by invasive burr (*Xanthium* sp.) and Mitchell-grasses (*Astrebla* spp.), kangaroo grass (*Themeda triandra*) and Queensland blue grass (*Dichanthium sericeum*) are also present (pers. obsv.).

The Canaway Fault runs the length of the eastern edge of the study site (Figure 5.1). This fault forms a north-south running cliff line of connected, continuous complex topography. Apart from the fault, two distinct groupings of mesas and complex topography occur in the study site. The first is a series of mesas and hills that form a line running NW to SE through

the centre of Canaway Downs. The second is a grouping of cliffs, valleys and gullies that form a complex (and largely impassable and dense) landscape a few kilometres from the eastern border of Ray Station. This section of habitat is known locally as The Matrix (pers. comm., Mark Tully - Ray Station, 2017).

All the properties have at least one active or unused opal mine, and The Matrix and several other sections of properties have been explored for other natural resource mining opportunities, as recently as early 2019. While these mining operations do not directly provide any income for the property owners there are several indirect benefits, including the upkeep of access roads through what is typically the more difficult to traverse sections of properties, and the provision of water points used for opal mining that might be used by livestock. Opal miners in the area have also unearthed dinosaur remnants, including fossils and footprints, though these artefacts are not sought after in the Quilpie Cluster area (pers. comm. Eric Stelzer - Stelzer Mine, 2018).



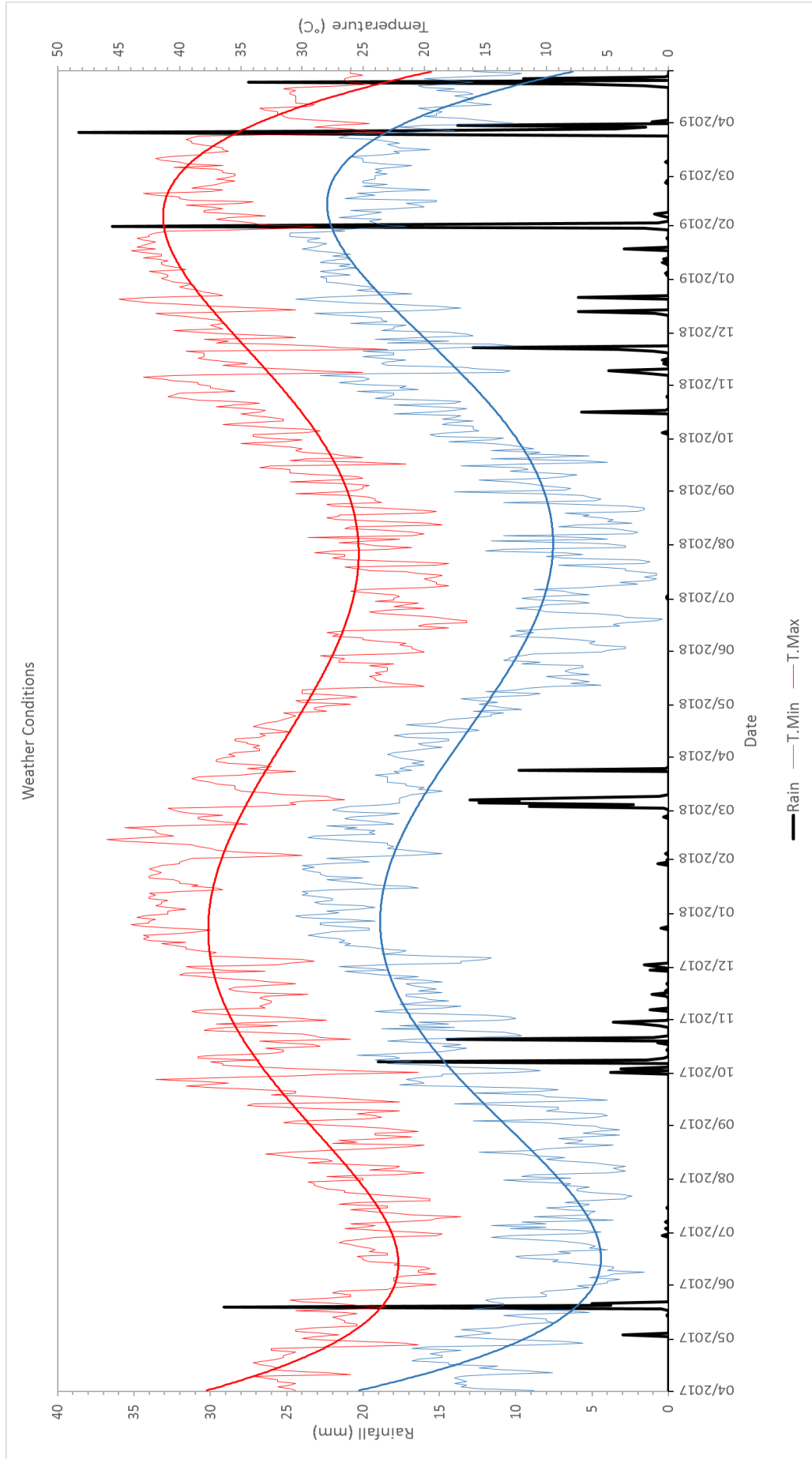
**Figure 5.1: Map of Quilpie Cluster**

An elevation map of the Quilpie cluster, showing the location of the exclusion fences (bold black line), and the DBF (bold grey line). Dashed lines show the location of unsealed property tracks/roads. Map is colour ramped to the steepness of ground slope from white (flat ground) to black (cliffs). A broader scope of this location can be seen in Figure 4.1.

## 5.2 Climate, weather and water

South-west Queensland typically has hot summers with relatively high rainfall, and cool winters with lower levels of rainfall (Department of Environment and Heritage Protection, 2019; Sharp, 2011). Data on minimum and maximum temperature and rainfall for the study site was interpolated from point observations recorded by the Bureau of Meteorology (Scientific Information for Land Owners, SILO). The minimum and maximum temperatures calculated for the entire study period were 0.5°C and 46°C, with daily minimum and maximum averages of 16.6°C and 31.5°C, respectively. The total rainfall at the study site for the entire study period (2 years) was 414 mm (Figure 5.2), which is below average and should have been ~678 mm over that period (average 339 mm per annum; [http://www.bom.gov.au/climate/averages/tables/cw\\_045015.shtml](http://www.bom.gov.au/climate/averages/tables/cw_045015.shtml)). For the duration of the study period the area was considered to be in drought.

Surface water is scarce and natural water points are uncommon throughout the study area. Historically, the land owners tapped artesian water which was allowed to flow freely onto the surface creating oases of grasses, also known as bore drains. However, due to the drastic increase in density of native pest species in the areas surrounding bore drains and the rapid depletion of local groundwater, free-flowing bore drains were capped or removed and are now uncommon (Department of Agriculture, Water and the Environment, 2019). None presently exist within the study site. Water points are now maintained by the land holders for livestock in the form of troughs and turkeys-nest dams (built up rings of dirt that hold large quantities of water at depth to reduce the loss of water to evaporation). These water points are still a hub for both native and domesticated species. Natural water points are also present within the study site. There are several billabongs and rock-holes across the properties. Billabongs are non-flowing sections of river that rarely dry out during dry seasons or drought, and some within the study site are believed to be spring fed (pers. comm. Mark Tully – Ray Station, 2017). Rock-holes are small pools of water (~2-20 m<sup>2</sup>) in diameter and of varying depth that form along rocky plateaus or cliff lines where water erodes a depression as it flows over the rock. Rock holes can last for several months or years following rain but do dry out in prolonged periods of dry weather.

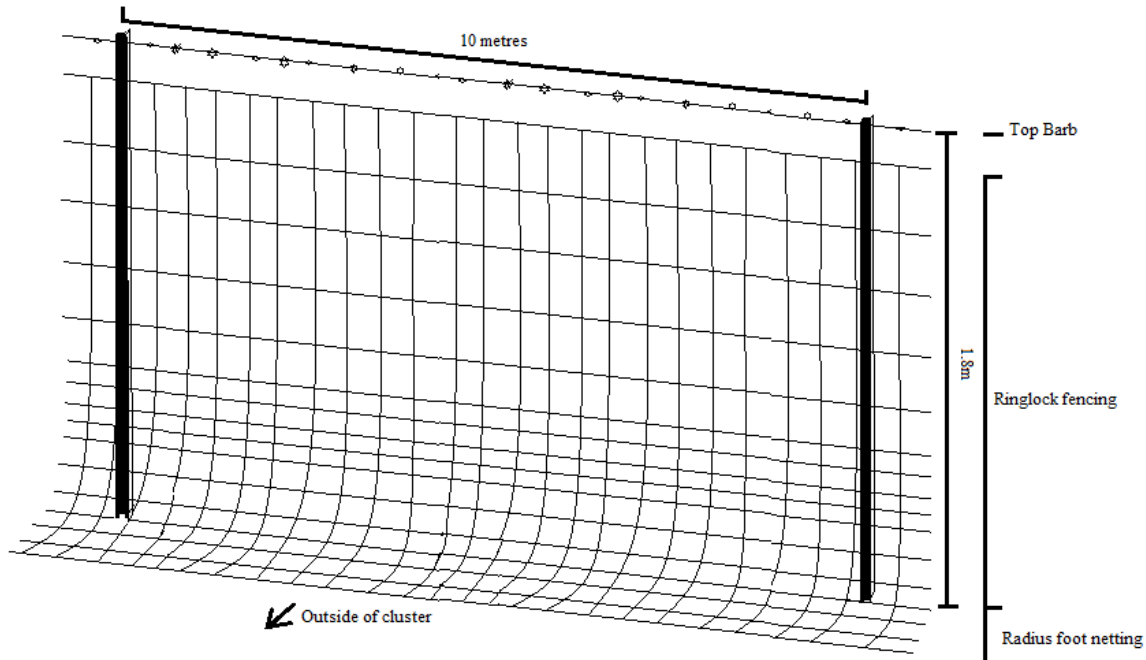


**Figure 5.2: Graph of weather conditions at study site**

Graph depicting the weather conditions at the Quilpie Cluster for the duration of the study period (x-axis). Black line shows the rainfall for each day (primary y-axis). Light red shows the maximum temperature recorded for each day. Light blue shows the minimum recorded temperature for each day. Trend lines for the maximum and minimum temperatures are shown in dark red and dark blue, respectively (secondary y-axis). Data retrieved from SILO (Scientific Information for Land Owners).

### 5.3 Exclusion Fencing

The exclusion fence surrounding the Quilpie cluster is approximately 1.8 metres high, with a barbed top wire and a radius apron, i.e. the mesh that makes the body and the apron of the fence are a single piece (Figure 5.3). The apron is on the outside of the fenced area with the exception of locations where the fence crosses inflowing waterways where the apron could hinder the movement of debris downstream and lead to damming and more extensive damage to the fence during floods. This design of fence is common to many cluster fenced properties in western Queensland and is continuous around all three properties in the Quilpie cluster except in the most north-west corner where Canaway Downs is separated from Trinidad by the dingo barrier fence (DBF; Figure 5.1). This section of the DBF is made from two sections of wire netting stapled or wired to wooden posts and buried under the soil at the bottom to avoid burrowing. Due to the age of the DBF (components may be up to 130 years old) and how the fence has been mended over these years, the exact design of this fence deviates in some sections. A section of the DBF at the southern end of this part of the boundary has been recently upgraded to resemble the cluster fence design.



**Figure 5.3: Diagram of exclusion fence design**

Diagram showing the design of exclusion fencing used around the Quilpie cluster. Fence stands at approximately 1.8m tall. Steel posts are spaced at 10 metre intervals. Strained radius netting (apron) extends approx. 30cm out from fence base.



More recently (late 2018 to early 2019), the land holders of Bunginderry, Ray and Canaway Downs reduced the size of the cluster to individual property sizes by erecting exclusion fences along their internal borders within the cluster. This reduces the management areas for wild dog control which allows for more effective management. These fences follow the same design as the perimeter fence, except with the entirety of the radius apron optimised for the direction of water flow.

#### **5.4 Livestock and native species**

Within the exclusion fence all properties run both cattle and sheep. However, Bunginderry and Ray more commonly identify as sheep grazing properties while Canaway Downs identifies as predominantly running cattle. Outside the fence Alaric and Yeenunga run only cattle, and Trinidad and 15 mile run sheep and cattle. All properties also have feral goats. While these goats are not counted as livestock assets for the properties, irregular harvesting of the goats does provide some income for the property owners which has resulted in a reluctance from landholders to totally remove the pest species. The site has a diverse range of native species. The most often seen mammal species are the large macropods; red kangaroo, eastern grey kangaroo (EGK) and euro. Exclusion fencing would very likely impede the immigration of these species into the cluster fence, and they are also managed inside the clusters to a certain extent (mostly through shooting for harvest). Other often observed native species include goanna (*Varanus* spp.), echidna (*Tachyglossus aculeatus*), yellow-footed rock-wallaby, wedge-tailed eagle and emu (*Dromaius novaehollandiae*). A full list of vertebrate species observed during field work and data collection at the study site has been included in Appendix III (listed alphabetically by class).

## **Chapter 6 : Habitat use by yellow-footed rock-wallabies in predator exclusion fences**

**Reference:** Smith, D., & Allen, B.L. (2021). Habitat use by yellow-footed rock-wallabies in predator exclusion fences. *Journal of Arid Environments*, 184, xx-xx.

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### **6.1 Abstract**

Predator density changes can alter behaviours of prey, and removal of predators is expected to free prey from predation risks. Due to pest management activities within predator exclusion fences in central-western Queensland, Australia, dingoes (*Canis familiaris*) have been actively controlled in places also inhabited by endangered yellow-footed rock-wallabies (YFRW, *Petrogale xanthopus*). These pest management practices are expected to enable YFRW to utilise otherwise riskier habitats, though the occurrence of these processes remain unclear. Here, we assessed the responses of YFRW habitat use to pest species management in exclusion fences. In congruence with previous work, we find the likelihood of YFRW presence is positively correlated with increasing habitat complexity ( $r = 0.39$ ). Greater coverage of ground and shrub vegetation also correlated positively with YFRW presence ( $p < 0.05$ ). However, we found no difference in YFRW habitat selection inside and outside exclusion fences, which was considered to be an artefact of populations of predators still surviving inside the fence, along with insufficient elapsed time to adequately assess behavioural responses by YFRW since the construction of the fence. These results support the view that even small numbers of predators can have a limiting effect on threatened prey and/or that researchers must allow sufficient time to observe prey responses to changes in predation risk.

### **6.2 Introduction**

Habitat selection is an active behavioural process by which animals seek habitat features that are either directly or indirectly associated with their ability to survive and reproduce (Krausman, 1999). Understanding the factors influencing a species' habitat selection is critical to understanding its ecology and informing its appropriate management. However, these factors are dynamic, and changes in the abundance of sympatric species can change

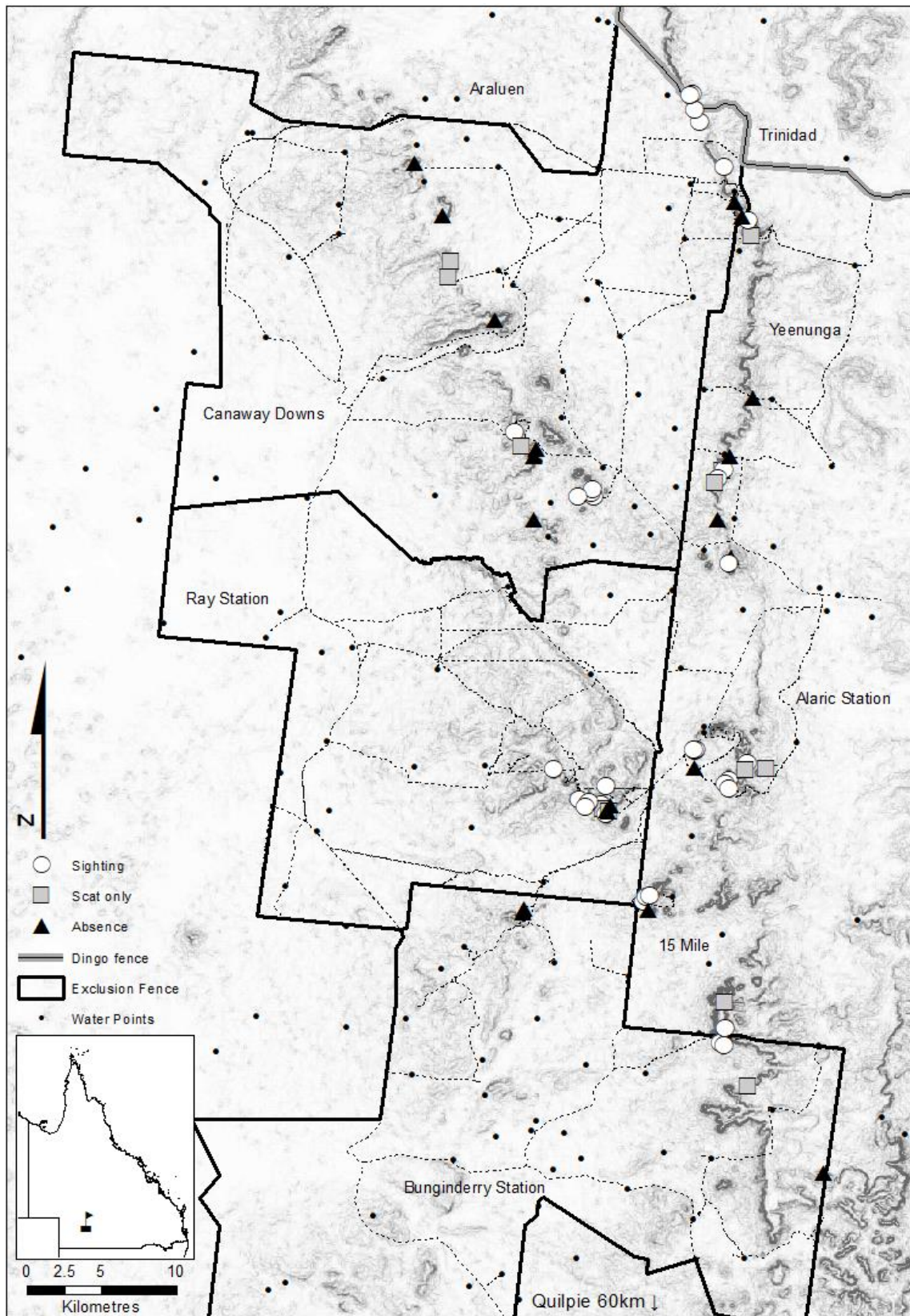
both demographics and behavioural traits, including how other species use habitat (Creel & Christianson, 2008). For many species, two primary variables influencing habitat use are predator density and resource availability (Gilliam & Fraser, 1987). Where predators are found in high densities, prey may select habitats with greater options for refuge; where competitors are in high densities, individuals may forage in riskier areas in search of nutriment; and where both are present, species must trade-off between forage quality and predation risk (Anholt & Werner, 1999; Gilliam & Fraser, 1987; Hughes, Ward, & Perrin, 1994). Changes in predator or competitor densities may therefore alter the habitat use of other species. Understanding this dynamic process is of particular importance for endangered species of conservation concern, especially in systems where human activities can have strong effects on wildlife (Allen et al., 2017).

Animal exclusion fencing occurs ubiquitously around the world, with variable effects (Smith, King, & Allen, 2020). In central-western Queensland, Australia, many livestock producers have recently erected such pest-proof fences for the purpose of excluding several pest species including dingoes (*Canis familiaris*) and kangaroos (e.g. *Osphranter rufus*); over 66,000 km<sup>2</sup> of land has been fenced in this way since 2013 (Smith, Waddell, & Allen, 2020). Dingoes cause negative economic impacts through the predation of livestock, primarily sheep (*Ovis aries*) (Allen & West, 2013, 2015). In the absence of dingoes, kangaroo populations can grow to unsustainable levels and cause severe overgrazing (Hacker, Sinclair, & Pahl, 2019; Waters et al., 2019). The fences allow farmers to better manage both predation pressure and total grazing pressure (TGP) by preventing the reestablishment of these species following their removal (Clark, Clark, & Allen, 2018). As a result of these pest management practices, habitat selection behaviours of extant native species may change, particularly species that are threatened by predation and competition.

The yellow-footed rock-wallaby (YFRW) is a medium-sized, marsupial macropod found in a few discrete populations across semi-arid Australia (Copley, Ellis, & van Weenen, 2016), and the Queensland subspecies (*Petrogale xanthopus celeris*) is distributed across the same region where pest exclusion fences are being erected at a rapid rate (Smith, Waddell, & Allen, 2020). YFRW are a listed ‘vulnerable’ species, and face a number of threatening processes including predation and dietary competition with introduced mammals (Threatened Species Scientific Committee, 2016b). The YFRW, like other rock-wallabies (see Murray et al., 2008), occupy naturally fragmented and rugged landscapes.

Previous studies of YFRW have indicated that they display high habitat specificity, and rarely occur outside of a strict set of habitat criteria (Copley, 1983; Gordon et al., 1993; Lim & Giles, 1987; Roache, 2011). YFRW typically occupy gorges, rock holes, boulder piles and natural rocky outcrops along the low sandstone remnant hills of the Grey Range in central and central-western Queensland (Copley, 1983; Gordon et al., 1993; Pope, Sharp, & Moritz, 1996). This proclivity for complex, difficult-to-access habitats is likely (at least in part) an evolutionary response to predation (Sharp, 2002); predators of rock-wallabies included thylacines (*Thylacinus cynocephalus*) prior to their extinction, but now primarily include dingoes, wedge-tailed eagles (*Aquila audax*), and the introduced European red fox (*Vulpes vulpes*) and feral cat (*Felis catus*) (Lapidge & Henshall, 2001; Lim et al., 1992; Sharp et al., 2002). Canid predators are expected to have the most severe impacts on YFRW (Allen & Fleming, 2012). Given YFRW use topographical complexity as refuge from predators, they might be expected to utilise a broader range of habitats in response to a human-induced decrease in predation risk. Changes in use of available habitat by rock-wallabies as a result of pest species control has been observed in the past (Hayward et al., 2011; Kinnear, Onus, & Sumner, 1998).

We investigated the responses of this threatened marsupial to an assumed reduction in predation risk, comparing the habitat use of YFRW inside and outside a large area fenced where dingoes and foxes are actively controlled as pests. Our aims were to (1) identify correlates of YFRW site occupancy and (2) assess whether the recent advent of exclusion fencing and the subsequent control of key predators affected YFRW habitat selection. We further describe a simple and reliable method for locating YFRW in the field.



**Figure 6.1: Map of the study site**

A map of the Quilpie cluster fence showing locations of sampling sites and water points. The increasing slope (degrees) of the ground is colour ramped from white (flat) to black (steep). Black points (●) show the location of water sources. White circles indicates both scat and sightings of YFRW at site, grey squares indicates scat only, and black triangles indicates an absence of scat and sightings. Marker point on map of Queensland shows broader location.

## 6.3 Methods

### (i) *Study area*

The study area is located approximately 70 km northwest of the township of Quilpie in central western Queensland, Australia. The exclusion fencing erected around the perimeter of three local pastoral properties (Canaway Downs, Ray Station and Bunginderry Station) is known as the Quilpie cluster, intended to facilitate the removal of dingoes, foxes and other pest animals and enable greater livestock production (Figure 6.1). Fencing was completed around the entire cluster in late 2016 and additional internal fences separating individual properties were erected in late 2018. The properties are at the southern end of the Grey Range, a system of rocky highlands and sandstone mesas covered in open mulga (*Acacia aneura*) woodlands (Figure 6.2). The region is semi-arid, and receives a mean of 339 mm of rainfall each year ([www.bow.gov.au](http://www.bow.gov.au)). The properties also have large washout areas or flood plains and Mitchell grass (*Astrebla* spp.) downs useful for sheep and cattle grazing.

### (ii) *Sample site selection*

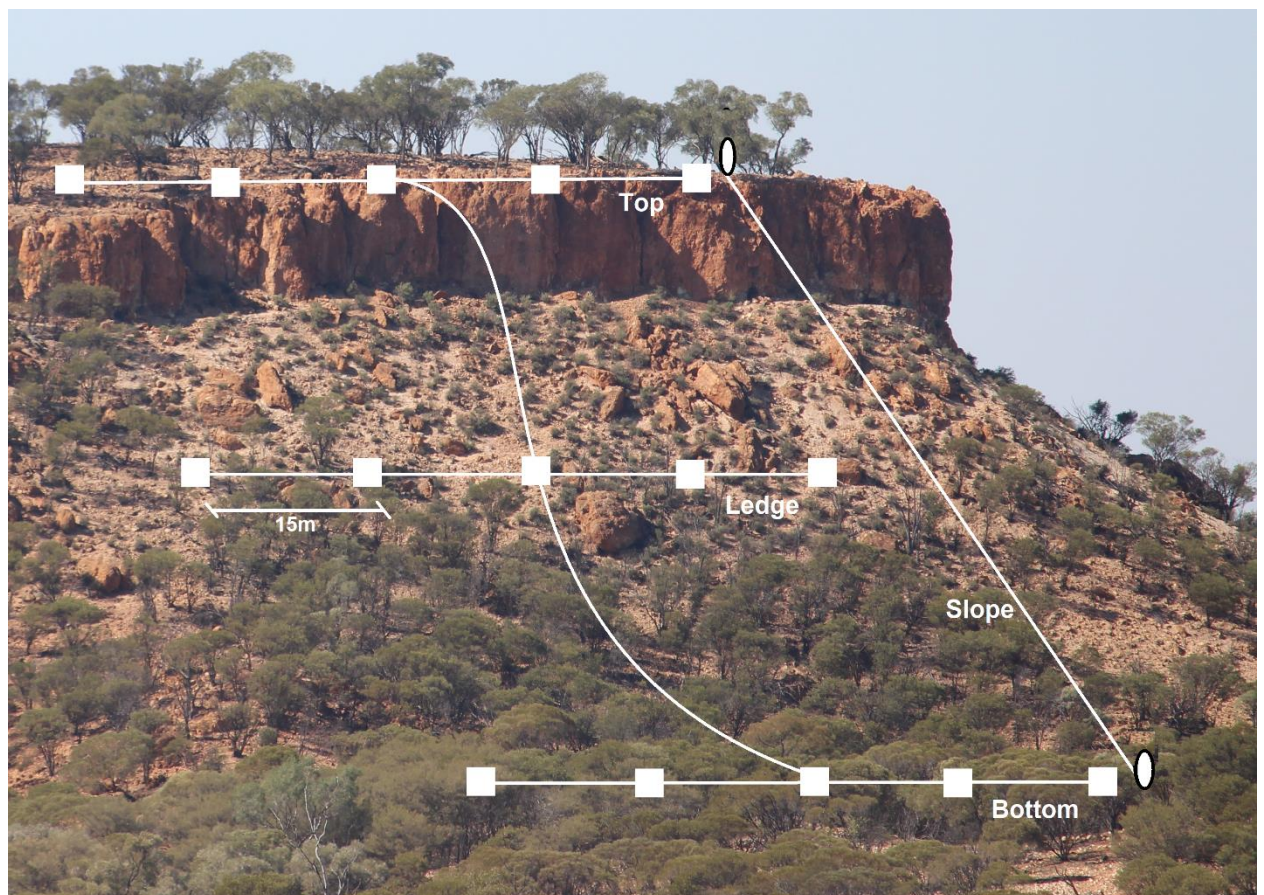
Due to the proclivity of YFRW to occupy only complex, rocky habitats, ArcMap v10.5.1 was first used to develop a map showing the slope of the terrain (Figure 6.1) to guide our sampling efforts. Once in the field, specific sampling sites were chosen at random, though an attempt was consciously made to (1) distribute the sites across the entire research area, (2) assess a similar number of sites both inside and outside of the exclusion fence, and (3) assess a similar number of sites where YFRW were or were not expected to be present. These stipulations were deemed the most appropriate to ascertain the strongest correlates of YFRW presence, and also determine whether habitat selection differed inside and outside of the exclusion fenced zone.

### (iii) *Habitat scoring*

All sampling sites were scored based on the same set of 36 criteria (Table 6.1). Initially, the GPS location and whether the site was inside or outside an exclusion fence was recorded, before the vegetation was then scored. Vegetation was scored on canopy height (metres), canopy cover (%), shrub cover (%), ground cover (%), and height of ground cover (centimetres). At each sampling site, vegetation was scored in this manner at five locations



~15 metres apart, for the top of the cliff or slope, on the ledge or middle of the slope, and also at the bottom of the slope, yielding 15 measurement locations in total for each sampling site (Figure 6.2). While scoring vegetation, the presence of spoor from YFRW, other native species (*Macropodidae* spp.), goats or cattle was also recorded. We then recorded if YFRW were known to currently occupy the site based on camera trap data (D. Smith, unpublished data), or were sighted during the scoring. Sampling sites were then further categorized as cliffs, gullies, slopes or rock-holes. The rock forms scored to determine habitat use of *P. x. xanthopus* by Lim and Giles (1987) were also recorded, along with the aspect of the sampling site. These rock form variables were the presence of steep cliffs, outcrops, gullies, terraces, caves, and rock piles, a score of boulder size, and a score of surface texture (for boulder size and texture scoring, see Lim and Giles, 1987). The slope (in degrees) was then estimated (see Figure 6.2). Finally, the distance from the sampling site to the closest known water point was later calculated in ArcMap.



**Figure 6.2: Design of vegetation scoring**

Shows the sampling design used for the collection of vegetation coverage measurements. Estimates of canopy coverage, shrub coverage and ground coverage were taken at each point (□). Estimates were recorded every ~15 metres (paced), at 5 measurement locations for each the top, ledge (middle) and bottom of the sampling site. Average slope was estimated (nearest 5°) from the shortest path between ~0° slopes. The search for spoor was also completed during this process.

(iv) *Intensity of search*

The search for the presence of YFRW at sites was conducted over 108 individual field days between the 10<sup>th</sup> of January, 2018 and the 26<sup>th</sup> of April, 2019. Sampling sites were only scored at the first visit, although all but one site (HS68) were visited on at least 2 and often 3 occasions. If YFRW were sighted or scat identified at sites on subsequent visits, the record for that sampling site was updated (all sampling sites that were later updated with ‘sightings’ already had YFRW scat present).

(v) *Quality control*

All vegetation coverage estimates were taken by the same observer. Sampling sites identified as less than <150 m apart (in ArcMap) were removed to avoid pseudoreplication. Additionally, scat identification was subjected to three levels of scrutiny. Firstly, scats were identified by the lead researcher in the field, who has experience in YFRW scat identification. Secondly, if the scat was somewhat fresh (not dried or weathered), scat pellets were collected and compared to standards of YFRW scat (Triggs, 2004). YFRW, cattle, goats, sheep and other macropods are found in the general areas we searched for scats. YFRW scat was distinguished from scats of these other species based on their size and shape (Triggs, 2004). Camera trap data (not presented here) indicated that cattle and sheep rarely (if ever) venture up into the rocky slopes and cliffs where we worked. Goats, euros or wallaroos (*Osphranter robustus*) and red kangaroos do rest in the rocky slopes, but their scat is usually distinguishable from YFRW. For completeness, however, a selected number of scats were DNA tested to confirm our visual assessment of YFRW scat identification DNA using 14 macropod specific microsatellite markers to confirm their origin as YFRW (D. Smith, unpublished data).

(vi) *Data analysis*

Habitat Scores (HS) were calculated using the variables and methods advanced by Lim and Giles (1987), who proposed a score from 0–13 based on topographical variables (see Table 6.1, variables 28-36). This score was used to assess the correlation between habitat score and YFRW occupancy. Sampling sites were given an occupancy rank based on absence of YFRW indicators (0), dry YFRW scat only (1), and YFRW presence (sightings) and fresh scat (2). A comparison of HS results was then made to the results of Lim and Giles (1987), before a bivariate Pearson’s correlation and two-tailed t-tests were used to assess the correlation of all variables to occupancy rank (Table 6.1). Surface texture (variable 35) did



not vary across any of the sites, so surface texture was removed from further analysis and chimneys (variable 27, a path similar to a ramp that allows movement between terraces) were added to make a new habitat score (0–12). This new habitat score was used for all further analyses.

Variables measured at the 15 locations at the top, ledge (middle) and bottom of each sampling site (Figure 6.2), such as ground cover percentage, were averaged across the whole sampling site prior to analysis. Comparisons were also made between HS and vegetation and occupancy on both sides of the exclusion fence to assess whether habitat selection was affected by broader canid control activities occurring across the study area. These analyses were performed in R v3.6.2. Finally, an automatic linear regression of best subsets was performed in SPSS statistics 25 to determine which variables from these data best explained the presence/absence of YFRW at a site.

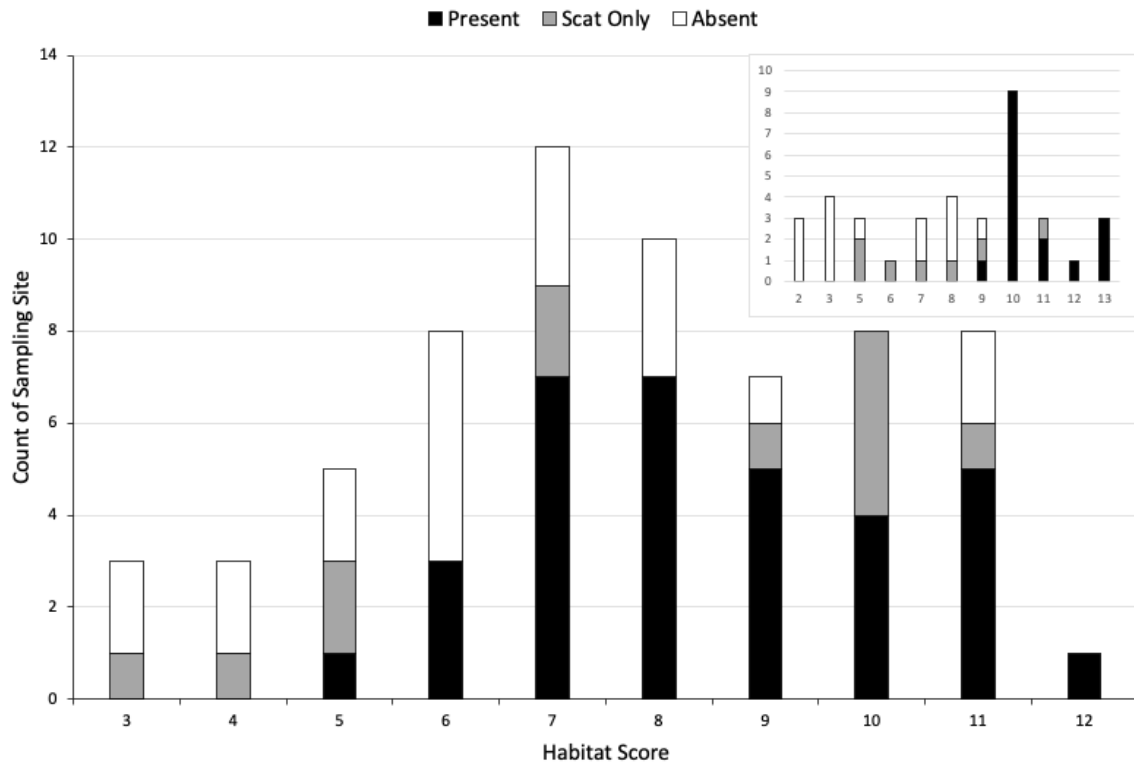
## **6.4 Results**

Most of the steep or rocky areas identified in the slope map (Figure 6.1) were previously known to observers from earlier exploratory trips to the study area, though several unknown areas of complex topography were identified from the desktop assessment and marked for later inspection. In total, 68 sampling sites were scored; 26 of these sites were associated with camera traps used to assess the activity of YFRW in the area (Taylor, 2018) and these gave rise to the shortest distances between sites (~70–100 m apart). YFRW are known to occupy home ranges larger than this distance (Sharp, 2009), and a preliminary comparison of scores amongst the closest sites revealed similar estimates of vegetation coverage and near-identical assessments of other variables. As a result, three of these 68 sampling sites were removed from the analysis to further avoid pseudoreplication. Distances between sites then varied from 153 to 7,341 m, with an average of 1,001 m (Figure 6.1).

**Table 6.1: Correlation of habitat variables to YFRW presence**

Bivariate Pearson's correlations of occupancy rank to all habitat variables. (\*) mark rows show significantly correlated habitat variables ( $p < 0.05$ ). Significance assessed using two-tailed significance test.

	<b>Variable</b>	<b>r</b>	<b>Description</b>
1.	Canopy Height - Top (m)	-0.092	Mean height of canopy at top of the hill
2.	Canopy Height - Ledge (m)	0.209	Mean height of canopy on ledge/slope of the hill
3.	Canopy Height - Flat (m)	0.095	Mean height of canopy at the bottom of the hill
4.	Ground Height - Top (cm)	-0.012	Mean height of grass/forbs at top of the hill
5.	Ground Height - Ledge (cm)	0.199	Mean height of grass/forbs on ledge/slope of the hill
6.	Ground Height - Flat (cm)	-0.089	Mean height of grass/forbs at the bottom of the hill
7.	Canopy Cover - Top (%)	0.034	Mean percentage of canopy cover at the top of the hill
8.	Canopy Cover - Ledge (%)	0.076	Mean percentage of canopy cover on the ledge/slope of the hill
9.	Canopy Cover - Flat (%)	0.150	Mean percentage of canopy cover at the bottom of the hill
-	Mean Canopy Cover (%)	0.118	Overall average canopy cover of the site
10.	*Shrub Cover - Top (%)	0.294	Mean percentage of shrub cover at the top of the hill
11.	Shrub Cover - Ledge (%)	0.239	Mean percentage of shrub cover on the ledge/slope of the hill
12.	Shrub Cover - Flat (%)	0.167	Mean percentage of shrub cover at the bottom of the hill
-	*Mean Shrub Cover (%)	0.347	Overall average shrub cover of the site
13.	Ground Vege Cover - Top (%)	0.159	Mean percentage of grass/forb cover at the top of the hill
14.	*Ground Vege Cover - Ledge (%)	0.425	Mean percentage grass/forb cover on the ledge/slope of the hill
15.	Ground Vege Cover - Flat (%)	-0.043	Mean percentage grass/forb cover at the bottom of the hill
16.	*Mean Ground Cover (%)	0.251	Overall average grass/forb cover of the site
-	Mean of all Vege (%)	0.234	Overall averaged vegetation cover of the site
17.	*Goat spoor (+/-)	0.328	Goat spoor recorded as present or absent during HS
18.	Cattle dung (+/-)	0.043	Cattle dung recorded as present or absent during HS
19.	Native species spoor (+/-)	0.133	Other macropod spoor recorded as present or absent during HS
20.	*Slope (degrees)	0.323	Angle between bottom and top records (see Fig. 2)
21.	Distance to known water (m)	-0.085	Distance from known water to site, calculated in ArcMap.
22.	Aspect	0.088	Aspect of cliff (North, North-east etc.)
23.	Clearing (+/-)	0.162	Presence of evidence of tree clearing by land holders
24.	Mining (+/-)	0.169	Presence or absence of mining activities (within 150m)
25.	Other Ag. Use (+/-)	0.114	Presence or absence of other disturbance to site (e.g. roads)
26.	*Altitude (m)	0.305	Height of cliff (at top of hill)
27.	Chimneys (+/-)	0.180	Presence or absence of ramps between cliff levels
28.	*Steep Cliff (+/-)	0.431	Presence or absence of vertical cliff faces
29.	Outcrops (+/-)	-0.092	Presence or absence of rock-piles or cliffs away from main site
30.	Gullies (+/-)	-0.043	Presence or absence of wash areas, forming gullies
31.	Terraces (+/-)	0.155	Presence or absence of multiple levels of cliff
32.	Caves (+/-)	0.156	Presence or absence of caves
33.	*Boulder size (S/M/L)	0.574	Size of boulders (small, medium or large), see Lim & Giles, 1987
34.	Rock piles (+/-)	0.227	Presence or absence of piles of rock forming refuges
35.	Surface Texture (S/I/R)	-0.028	Roughness of rock (smooth, intermediate, rough)
36.	Water (+/-)	0.162	Presence or absence of a water point close to site
-	*Habitat Score (Lim & Giles, 1987)	0.384	Score of var. 28-36 (ranges from 0-13, see Lim & Giles, 1987)
-	*New HS (w/o ST, w/ chimneys)	0.397	Score of var. 27-36 (exc. 35, ranges from 0-12)

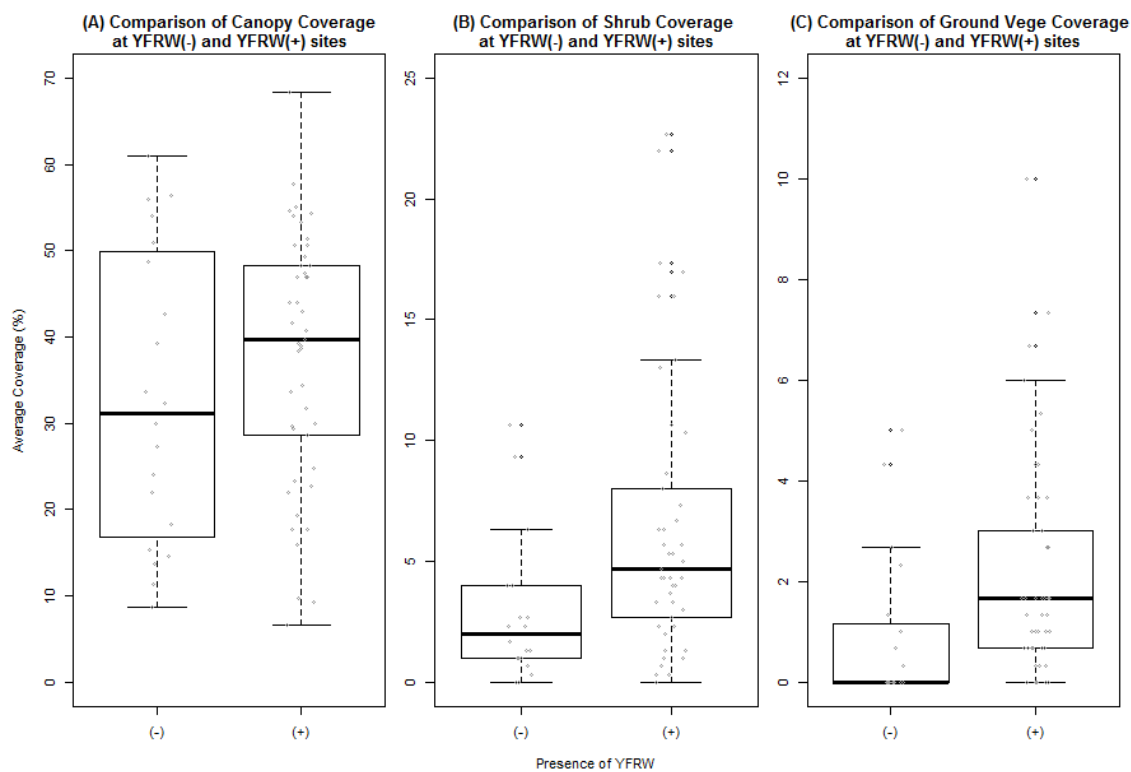


**Figure 6.3: Relationship between Habitat Score and YFRW presence.** Stacked column graph showing the relationship between habitat score and YFRW presence. Y-axis shows the number of locations. X-axis shows the habitat score. Columns divided by absence (white), presence of scat only (grey) and sighting and scat present (black). Inset graph shows results presented in Lim and Giles (1987), whose methods were followed.

Of the remaining 65 sites, 69.2% yielded YFRW scats and 50.8% yielded YFRW sightings (Figure 6.1). Goat and cattle dung, and other native species (e.g. red kangaroo, euro, or grey kangaroo *Macropus giganteus*) scat was recorded at 92.3%, 60.0% and 95.4% of sites, respectively. Of the 65 sites, only two had evidence of land clearing, six of opal mining, and one of ‘other human/agricultural disturbance’. Most sites (44.6%) are slopes, followed by cliffs (41.5%), gullies (7.7%), and rock-holes (6.2%). The mean distance from each site to known water was 1,645.6 metres (min =16.7 m, max =5,159.0 m).

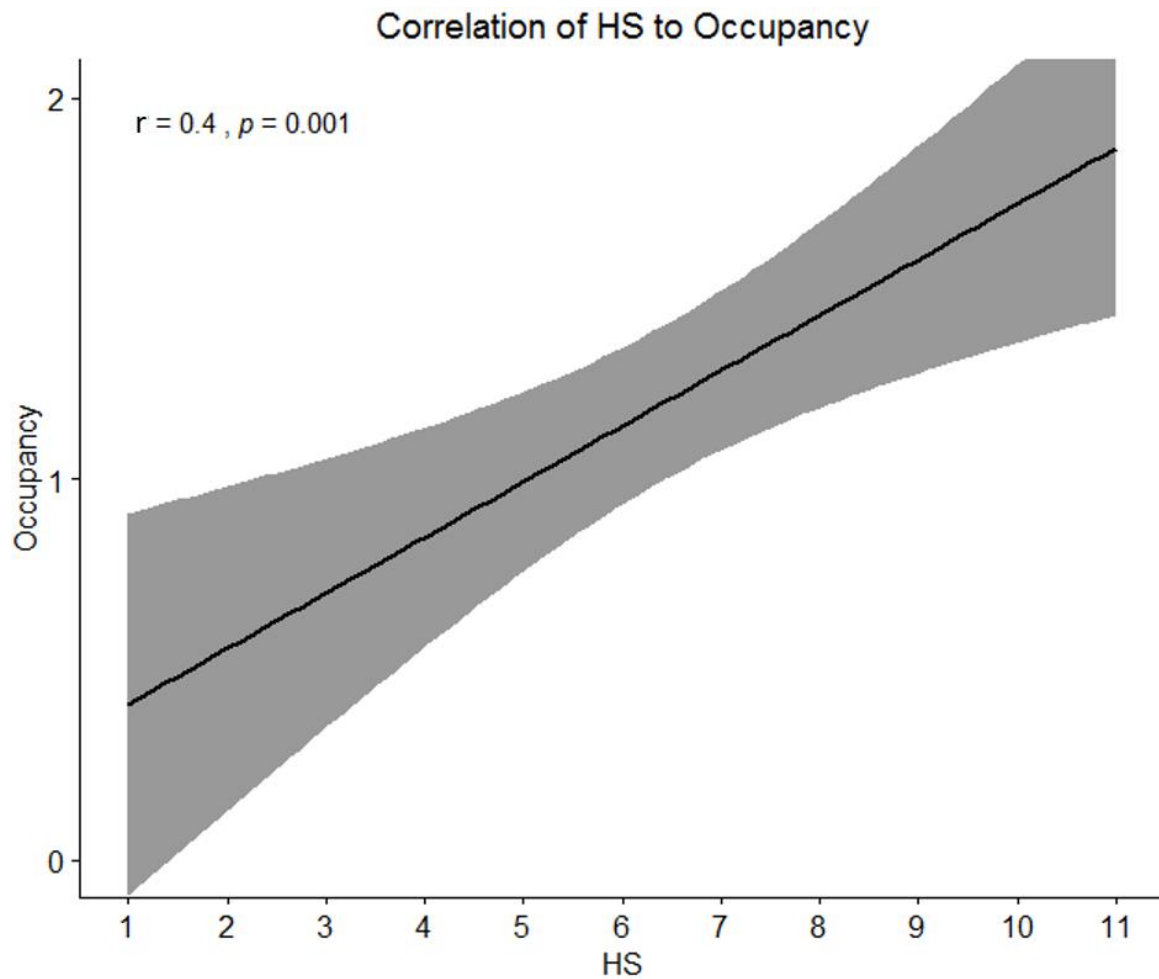
A comparison of sample sites across the exclusion fence showed no difference in HS use ( $t = -1.67$ ,  $df = 42.23$ ,  $p = 0.10$ ), or vegetation coverage (all t-tests resulted in  $p > 0.05$ ) across the treatment for sites with either scat or sightings. Narrowing the previous result to only those sample sites with sightings also revealed no demonstrable differences ( $t = -1.04$ ,  $df = 31.00$ ,  $p = 0.31$ ). As such, there was no differences between YFRW habitat use inside and outside the exclusion fence. Comparing our results to that of Lim and Giles (1987) resulted in similar YFRW occupancy across HS (Figure 6.3) and confirmed the finding that increasing HS, and therefore increasing habitat complexity, had a positive correlation with

YFRW presence (Table 6.1). Significant positive relationships were observed between the coverage of shrubs and ground vegetation, and YFRW occupancy; as well as the presence of goat scat and YFRW occupancy (Table 6.1). This was also supported by t-tests which showed significantly greater coverage of both shrubs and ground vegetation (though not canopy cover) at sites with YFRW occupancy (Figure 6.4). Automatic linear regression of best subsets revealed the best predictors of YFRW occupancy to be boulder size and the presence of steep cliffs and rock piles, as well as shrub cover on the top and slope, and ground vegetation cover on the slope. A model including these vegetation variables explained 57% ( $r = 0.567$ ) of the variation in occupancy, compared to the HS of habitat complexity alone, which accounted for 40%, ( $r = 0.397$ , Figure 6.5).



**Figure 6.4: Comparisons of vegetation coverage in YFRW(-) and YFRW(+) sites**  
 Boxplots comparing average coverage of (A) canopy, (B) shrubs, and (C) ground vegetation across sights with and without signs of YFRW activity. (A) Mean(-)=33.0, mean(+)=37.8, df=31.97, p=0.29. (B) Mean(-)=2.8, mean(+)=6.5, df=61.67,  $p < 0.05$ . (C) Mean(-)=0.9, mean(+)=2.2, df=51.9,  $p < 0.05$ .

Amplification of scat DNA was successful using *Macropodidae* and *Petrogale* microsatellite primers, showing identification of YFRW scat by researchers to be a reliable method in the absence of sightings to determine occupancy (positive and negative controls included; D. Smith, unpublished data).



**Figure 6.5: Correlation of Habitat Score to YFRW occupancy**  
 Figure shows the correlation of HS (w/o surface texture, w/ chimneys) to occupancy. YFRW occupancy has three levels, Absence = 0, Dry scat only = 1, and Scat and Sighting = 2.  $r=0.397$ ,  $p=0.001$ .

## 6.5 Discussion

Predation risk is predicted to affect prey populations in diverse ways, including constraining their habitat use to less risky places (Creel & Christianson, 2008). Accordingly, reductions in predation risk brought about by the control of predators is expected to alter habitat use by prey, enabling them to forage further away from refuge and occupy otherwise riskier habitats. We found that YFRW site occupancy was strongly linked with habitat characteristics that enable greater refuge from predation (Table 6.1, Figures 6.3, 6.4 and 6.5), such as steep cliffs and vegetation cover, but this pattern of habitat use was similar in places where predators were and were not controlled.

To assess habitat selection in YFRW we amalgamated sections of previously used generic and rock-wallaby specific habitat scoring methods (Department of Environment and

Heritage Protection, 2014; Lim & Giles, 1987; Murray et al., 2008; Telfer, Griffiths, & Bowman, 2008) and compared site variables to YFRW occupancy. Determining occupancy in cryptic species can be difficult, particularly when the species occupies remote or hard-to-access habitat. Detections of such species often relies on reliable identification of spoor, such as scats. Scat identification in macropods is a reliable and consistent method of determining their presence due to variations in species ranges and diagnostic scat characteristics (Triggs, 2004), and scat has been used to identify presence successfully in other studies of rock-dwelling macropods (Lim & Giles, 1987; Telfer, Griffiths, & Bowman, 2006). Previous studies of rock-wallaby habitat requirements have estimated the age of dry pellets and used their density as an index of rock-wallaby abundance. This was not attempted during this study. The age and quantity of dry pellets can be difficult to determine, and as a result, such studies can be unreliable (Block et al., 2001; Cristescu et al., 2012). As such, we simplified the results by categorising the sites into three ordinal data groups that indicate the absence (0), presence of scat (1), and presence of scat and sightings of YFRW (2). This system uses dry scat but no YFRW sightings as an indicator that the site may not be a permanent YFRW population, but rather an area of sub-optimal habitat, used only when resource availability allows it, or as temporary refuge habitat for dispersing rock-wallabies. YFRW in Queensland have very distinctive scat in comparison to other macropod species in the same region (see Triggs, 2004, pp. 131–143), but conflation and misidentification with goat scat is sometimes possible due to the similar size and shape. For this reason, DNA microsatellite analysis was used to confirm the correct identification of YFRW scat, and we were confident that we were indeed assessing YFRW and not other species.

We found that the distribution of YFRW across varying habitat scores is similar to those presented in Lim and Giles (1987) (Figure 6.3). While dry YFRW scat was present at HS as low as 3 in our study, sightings were only recorded at locations with a HS of 5 or higher, congruent with earlier findings. Increasing HS was also positively correlated with YFRW occupancy (Figure 6.5). The requirement for complex topography across *Petrogale* species has been firmly established (Murray et al., 2008; Telfer, Griffiths, & Bowman, 2008), so this finding is predictable. We found, that the strongest correlated variables within the HS to be the presence of steep cliffs and large boulders. Whilst these requirements are consistent with Lim and Giles (1987), that study also reported the presence of surface water and smooth rock surfaces to be major determinants of YFRW presence which were not

supported in our results. Our research took place during a period of sustained drought, in which the greater study region received very little rainfall and surface water. The distances to known surface water points was therefore mapped in ArcMap, and we investigated its relationships to YFRW occupancy, but found none. YFRW are known to obtain sufficient water from their diet and dew (Lim, 1987) and travel up to 1.5 km to water sources when needed (Sharp, 2011).

The view that YFRW prefer smooth rock surfaces was harder for us to confirm. The sites we assessed in Queensland consist of sandstone tablelands, and while the sandstone layers are smooth, the ironstone cap-rock that facilitates the formation of the mesas, and allows formation of the large boulders as the mesas break away, has a consistent rough surface texture across all sites. As such, surface texture was an uninformative variable and therefore removed from the HS model, and chimneys were added (Figure 6.5). The addition of chimneys increased the strength of the correlation to YFRW occupancy, but only marginally. The strongest determinants of YFRW occupancy were identified through best subset automatic regression modelling. This process resulted in boulder size, the presence of steep cliffs and rock piles, as well as increasing shrub cover on the top and slope, and ground vegetation cover on the slope to be the greatest predictors. Overall shrub and ground cover were also significantly correlated to the presence of YFRW (Table 6.1). These results are also previously established in the literature (Gordon et al., 1993; Lim & Giles, 1987; Sharp, 2002). Also, the diet of YFRW has been shown to consist mostly of forbs and browse (Dawson & Ellis, 1979), and rock-wallabies are known not to move far from the slopes and cliffs of their home range in search of dietary resources (Sharp, 2002). These observations support the concept that ground vegetation would positively correlate with YFRW occupancy.

We found no significant differences in habitat use across exclusion fencing treatments. A comparison of HS across inside and outside sites occupied by YFRW revealed no difference and the vegetation present at these locations did not significantly vary either. In other words, available habitat was similar on both sides of the fence and YFRW occupied the same habitat types on both sides. Risk effects are known to influence habitat use by prey (Anholt & Werner, 1999; Creel & Christianson, 2008); however, this does not appear to have occurred here. Our data cannot show why this occurred, but at least three reasons are possible. First, habitat use by YFRW might not be strongly influenced by predation

risk. Second, predator densities or predation risk might not have been substantially reduced as a result of pest management inside the exclusion fence. Third, predator densities might have been lower on the inside of the fence, but sufficient time since predator control might not have elapsed for YFRW to recognise the lowered predation risk and respond accordingly. All three of these reasons might be contributing to our failure to observe a difference in YFRW habitat use between treatments, but these processes require further exploration.

## **6.6 Conclusions**

We have shown that YFRW prefer to occupy sites with complex, rugged habitat and vegetation cover, typical of species that are highly sensitive to predation risk. However, we were unable to demonstrate that predator control altered YFRW habitat use. These findings have important implications for those involved in YFRW conservation, and those interested in prey responses to predation risk more broadly. The habitat sampling methods we employed proved to be a powerful and informative way to identify the presence of YFRW in this region, and future studies might seek to apply to these techniques at broader scales to investigate the distribution and abundance of YFRW. Our failure to observe a treatment difference in YFRW habitat use also suggests that even small numbers of primary predators can have a limiting effect on threatened prey, and that researchers must allow sufficient time to observe prey responses to changes in predation risk. More broadly, we have identified some of the challenges involved in assessing the responses of threatened fauna to management intervention, and we encourage continued interest in this important topic.

## **6.7 Author contributions**

The concept of this study was developed by BLA and DS. DS collected all data and conducted all data analysis. DS created the first draft of the manuscript. Both DS and BLA contributed to the editing of the manuscript for submission.

## **6.8 Acknowledgements**

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Australia's First Peoples, for their past and continuing care of the land and waters, and their contribution to modern Australia. Livestock producers also granted access to the site.

## **Chapter 7 : Pest management effects and diel activity of yellow-footed rock-wallabies and sympatric pest animals**

**Reference:** Smith, D., Taylor, M., & Allen, B.L. (in review). Pest management effects and diel activity of yellow-footed rock wallabies and sympatric pest animals.

### **7.1 Abstract**

Due to the continuing pressure from invasive or pest animal species in central and central-western Queensland, Australia, livestock producers have constructed exclusion fences in an attempt to better control pest species on their properties. Anecdotally these fences (colloquially called ‘cluster fences’) have yielded promising results for pest management in the few years since they have been completed, but how these fences may alter the behaviours and population viability of extant threatened fauna is unknown. Here, we assess the diel activity and behaviours of both a native at-risk macropod, yellow-footed rock-wallaby (YFRW; *Petrogale xanthopus celeris*), and relevant pest species (dingoes *Canis familiaris* and feral goats *Capra hircus*) at a site fenced in late 2016 in western Queensland. We used 26 camera traps deployed for a total of 15,092 camera trap days over two years to compare species’ activity and behavioural differences inside and outside the cluster fence. YFRW behaviours and age structures were no different inside and outside the fence. YFRW display a bimodal distribution of daily activity, with most activity occurring around 08:00 and 22:00. Yearly YFRW activity peaked between April and November, with very little activity observed during summer months. YFRW daily activity inside and outside the fence revealed an 84% overlap, with differences in activity deemed significant. Our results also show that YFRW and feral goats had a daily activity overlap of 80%. There has not been any quantifiable positive or negative effect of pest management practices within cluster fencing on YFRW activity in the three years since the completion of the cluster fence. Feral goat competition may be preventing YFRW from realising the benefits of reduced predation pressure inside the fence. Our findings imply that the benefits of cluster fencing to threatened fauna like the YFRW may be limited in places where herbivory is not managed in association with predation.

## 7.2 Introduction

Predator-prey dynamics take place in both the spatial and temporal dimensions (Eriksen et al., 2011), and time is a factor by which species can separate to avoid interspecific confrontations (Carothers & Jaksić, 1984). While prey species attempt to avoid predators spatially, they may also attempt to minimize temporal overlap with predators and competitors (Brown, Laundré, & Gurung, 1999). Changes in the distribution and densities of one species therefore have the potential to alter other species' behaviours (Anholt & Werner, 1999). For example, an increase or decrease in the density of predators can result in a behavioural change in prey (Creel & Christianson, 2008). These behavioural changes can include vigilance behaviours, changes in habitat preferences, or changes in the timing of animal activity (Brown, Laundré, & Gurung, 1999; Monterroso, Alves, & Ferreras, 2013). The resultant diel activity of a species is therefore often influenced, at least in part, by local predation and competition pressure (Monterroso, Alves, & Ferreras, 2013).

In Australia, livestock producers have erected exclusion fencing around groups of similarly operating properties to prevent the incursion of dingoes (*Canis familiaris*) and pest herbivores including Red Kangaroo (*Osphranter rufus*), feral goats and feral pigs (*Sus scrofa*). Dingoes cause negative economic impacts through the predation of livestock, predominantly sheep (*Ovis aries*; Allen & West, 2013, 2015). There is a long history of dingo control in Australia, and exclusion fencing as a management tool to control their activities is not new either (McKnight, 1969); Australian land managers have utilised fencing as a tool against pest species since at least the late 1800s (Agriculture and Environment Committee, 2017). This early control resulted in the dingoes being largely eradicated from the south-east portion of the country (about 25% of Australia) by the early 20<sup>th</sup> century and the subsequent formation of the Dingo Barrier Fence (DBF) to inhibit reinvasion (Allen & West, 2013). The DBF is a >5, 000 km exclusion fence that stretches from the south coast of Australia to south-east Queensland. Despite this early success, dingoes have recolonised much of this area and are again perceived to be a threat to farm productivity and, as a result, broad scale exclusion fencing of properties (known as 'cluster fencing' when the fences surround a group or cluster of properties) has again arisen over the last 10 years (Smith, Waddell, & Allen, 2020; Chapter 3). Aside from dingo control, cluster fences also allow land managers to better manage total grazing pressure (TGP; Waters et al., 2019), mostly through the control of red kangaroos and feral goats. While the

potential benefits of cluster fencing to agriculture may be readily understood, less clear are the effects on non-target fauna also present within the fences. Other species may experience positive and/or negative effects of fencing, both directly as a result of the barrier to movement, and indirectly, such as changes in species composition or behaviour that result from pest species management and greater control over TGP (Smith, King, & Allen, 2020; Chapter 2).

The yellow-footed rock-wallaby (YFRW; *Petrogale xanthopus celeris*) is a medium sized macropod found in central-western Queensland in the area that is now being segmented through increasing cluster fencing practices (Smith, Waddell, & Allen, 2020; Chapter 3). This threatened species occupies complex, rocky habitat formed by the erosion of low-lying remnant sandstone hills (Gordon et al., 1993). They are known to experience predation pressure from canids (Pearson & Kinnear, 1997; Sharp, 2002) and the subspecies found in South Australia (*P. x. xanthopus*) has been shown from to benefit from both predator and competitor control (Lethbridge, Harper, & Strauss, 2010; Lethbridge et al., 2019). A change in density of competitors may also result in a behavioural change in affected colonies of the species, similar to those documented for other *Petrogale* species (Kinnear, Onus, & Sumner, 1998); several species are known to have high dietary overlap with the YFRW, including feral goats (Dawson & Ellis, 1979). The YFRW might therefore be a species that alters its behavioural patterns in response to the predator and herbivore management occurring within cluster fences.

Here we used camera traps to investigate potential changes in YFRW ecology that result from pest management practices, where pest species were actively controlled inside cluster fencing, but not outside. Based on our understanding of predator-prey interactions and previous assessments of similar species (described above) we hypothesised that, compared to outside the fence:

1. YFRW will exhibit a broader or different temporal range inside the fence;
2. YFRW will express different behaviours inside the fence, primarily a greater proportion of foraging or resting behaviours, and;
3. YFRW populations will have different age structures inside the fence.

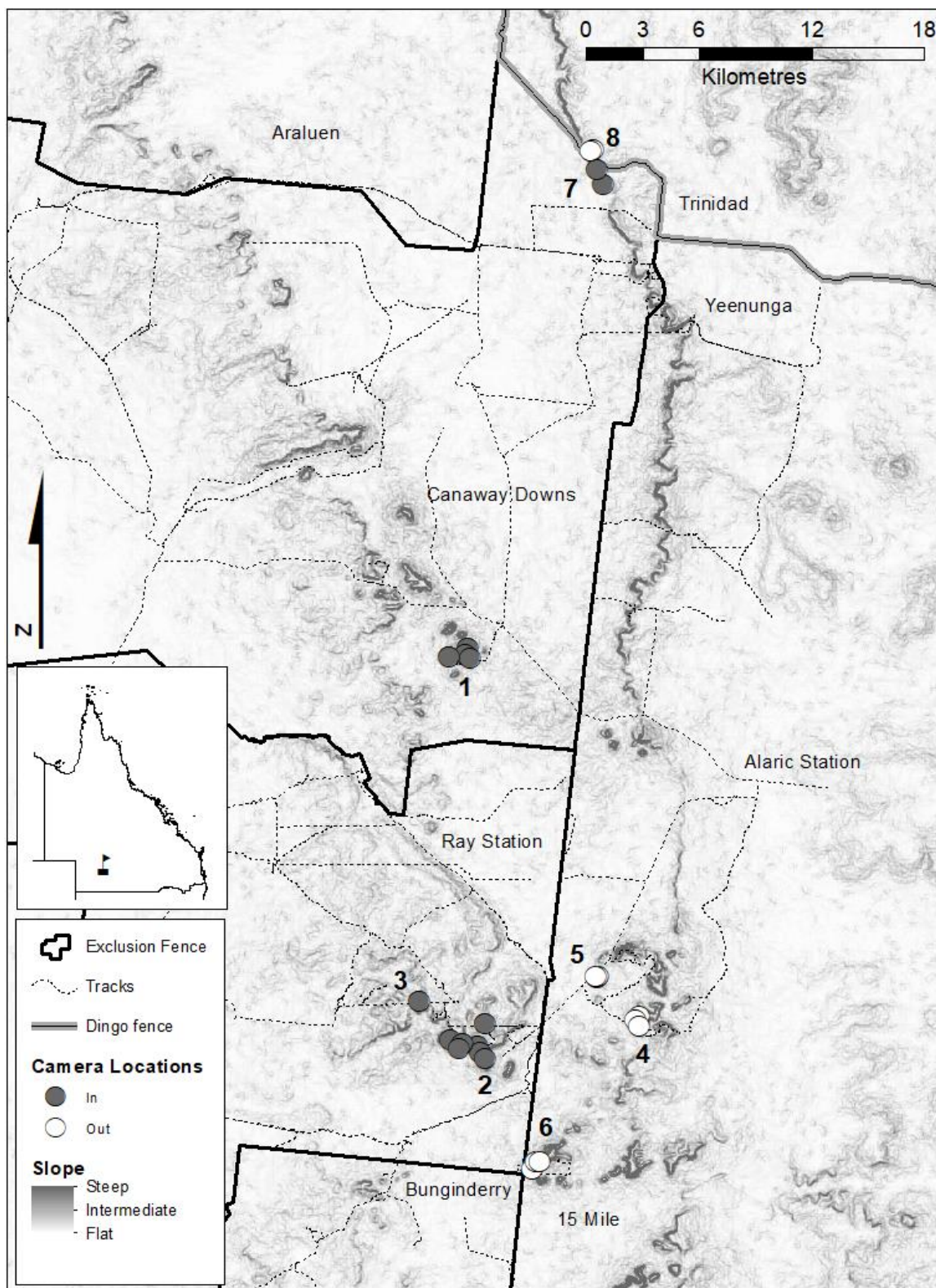
We also report the activity of other key species, primarily the potential predators and competitors of YFRW. Through this study we aimed to develop a greater understanding of

the early effects of exclusion fencing and pest species management on non-target wildlife of interest.

### 7.3 Methods

#### (i) *Study location and sampling sites*

The study site is approximately 70 km north-west of the township of Quilpie in the semi-arid zone of central-western Queensland (Figure 7.1). The cluster fenced area is 2,247 km<sup>2</sup> in size, with a perimeter of 327 km. Camera traps were placed at multiple sites inside and outside the fence that appeared to have substantial YFRW activity, as determined during preliminary assessments of scat density and presence of necessary habitat requirements for YFRW (Figure 7.1; Smith & Allen, 2021; Chapter 6). These sites were typically rocky mesas or cliff lines surrounded by lightly wooded plains and tabletops dominated by mulga (*Acacia aneura*) and other *Acacia* and *Eucalyptus* species. Cameras were placed at four sites inside and four similar sites outside the cluster fence, with a total of 15 cameras placed inside and 11 outside the cluster fence. Initially, 19 cameras were placed at locations as part of an earlier project (Taylor, 2018), the final seven were placed when this project began. The placement of these final cameras was (in part) to rectify uneven allocations between treatments. The cluster fence is approximately 1.8 m tall, with a 30 cm radial apron on the outwards facing side of the fence to prevent animals burrowing in, although the apron was buried in a small section of the fence in the north-east corner of the cluster.



**Figure 7.1: Map of camera placement**

A map of camera locations inside (dark marks) and outside (light marks) the Quilpie cluster fence, showing the location of eight numbered sub-sites or colonies of yellow-footed rock wallabies. A black marker has been placed on the inset map of Queensland to show the approximate location of the study site.

(ii) *Camera placement*

We used Reconyx™ HC600 Hyperfire™ and Reconyx™ XR6 UltraFire™ cameras attached to sturdy trees at approximately 65 cm above the ground and positioned to optimise triggers of animal movement and reduce false triggers (Meek, Ballard, & Fleming, 2015). This was done by placing cameras in areas with animal pads, tracks or trails, or areas with a high density of YFRW faecal pellets (Triggs, 2004). Cameras were placed at both the top and bottom of cliffs, and at a variety of locations within each site to optimise records of YFRW, while also ensuring that the array of locations were representative of each site. Sites were separated by a minimum distance of 750 m, and cameras within sites were set a minimum of 70 m apart (Figure 7.1; Table S7). These distances were based on the understanding that YFRW occupy a home range of ~25 hectares, or for reference, a circular range with a ~285 m radius (Sharp, 2002). Cameras were set to their minimum intervals between photos with no delay between triggers. Photos per trigger was standardised across cameras to five photos per trigger. Though there are two models of camera, under these conditions the camera function was the same. The cameras per site can be seen in Figure S6.

(iii) *Data collection and scoring*

Data collection took place over two years, from the 26<sup>th</sup> of April 2017 until the 22<sup>nd</sup> of April 2019. Data were collected from cameras on multiple occasions over this period. This allowed for the batteries of each camera to be changed and SD cards swapped to ensure they did not reach capacity, and to stagger data entry labour requirements. Over the study period there were several gaps in data (Table S8) due to changes in alignment of cameras after being bumped by animals, and some batteries failing (see Discussion).

Photographs were scored based on all animals present in the photograph, and the number of each species present in the frame was also recorded (i.e. if two dingoes were present in a single photo this results in two dingo records). If no animals were present, the photograph was scored as 'Bush'. When an animal was present but it could not be identified, these were scored as 'Unknown'. A 'Blank' field was also recorded when the photograph was not clear enough to reliably score due to rain, dust, or vegetation blocking the lens. When YFRW were present in the photograph, the sex, age class (pouch young, juvenile-at-foot, or adult)

and behaviour was recorded. Feral goat age class was also recorded (i.e. kid, adult). Photo scores were recorded in Microsoft Excel.

YFRW behaviours were classified as Resting, Crouching, Crouching and Looking, Standing, Standing and Looking, Foraging, Hopping, Pentapedal Locomotion, Grooming, Affiliative Interaction, Aggressive Interaction, Other Interaction, Other Behaviour, and Behaviour Unknown (Figure S7). Due to ambiguity between some of these behaviours (e.g. 'Crouching' and 'Crouching and Looking'), some categories were later grouped to create mutually exclusive and ecologically relevant behavioural classes identified as 'Resting', 'Foraging', 'Hopping', 'Interaction' and 'Other Behaviour' which were used for all analysis (diagrams of each behaviour are shown in Figure S7).

#### *(iv) Analyses*

Activity was calculated as the count of each animal per camera day (Engeman, 2005). For example if 10 photos with a single red kangaroo in each were counted over 10 days, red kangaroo activity would be one per camera day for that period. This activity metric was used as it standardises activity across sites with different lengths of camera deployment, and different numbers of cameras per site. Nested analysis of variance (ANOVA) was used to check for differences in activity by site within each treatment, before Welch's t-tests were used to assess activity by camera placement and treatment. Although previously found to be insignificant by Taylor (2018), the effect of camera placement at the top and bottom of cliffs on raw YFRW activity was reassessed here for completeness. Nested ANOVA and chi-square tests of proportion were also used to assess behaviour of YFRW and age data of both YFRW and feral goats. Coefficients of overlap were determined for diel activity using the `overlap` v0.3.3 (Meredith & Ridout, 2014; Ridout & Linkie, 2009) and `camtrapR` v2.0.3 (Niedballa et al., 2016) packages in R v3.6.2. Watson-Wheeler tests for homogeneity of angles were used to test significant differences between activity plots using the R package `circular` v0.4 (Jammalamadaka & Sengupta, 2001; Lund et al., 2017).

## **7.4 Results**

### *(i) Sample Sizes*

A total of 335,268 images were captured over the study period. Removing all images that did not contain any identifiable animals (i.e. photos scored 'Bush', 'Unknown' or 'Blank')

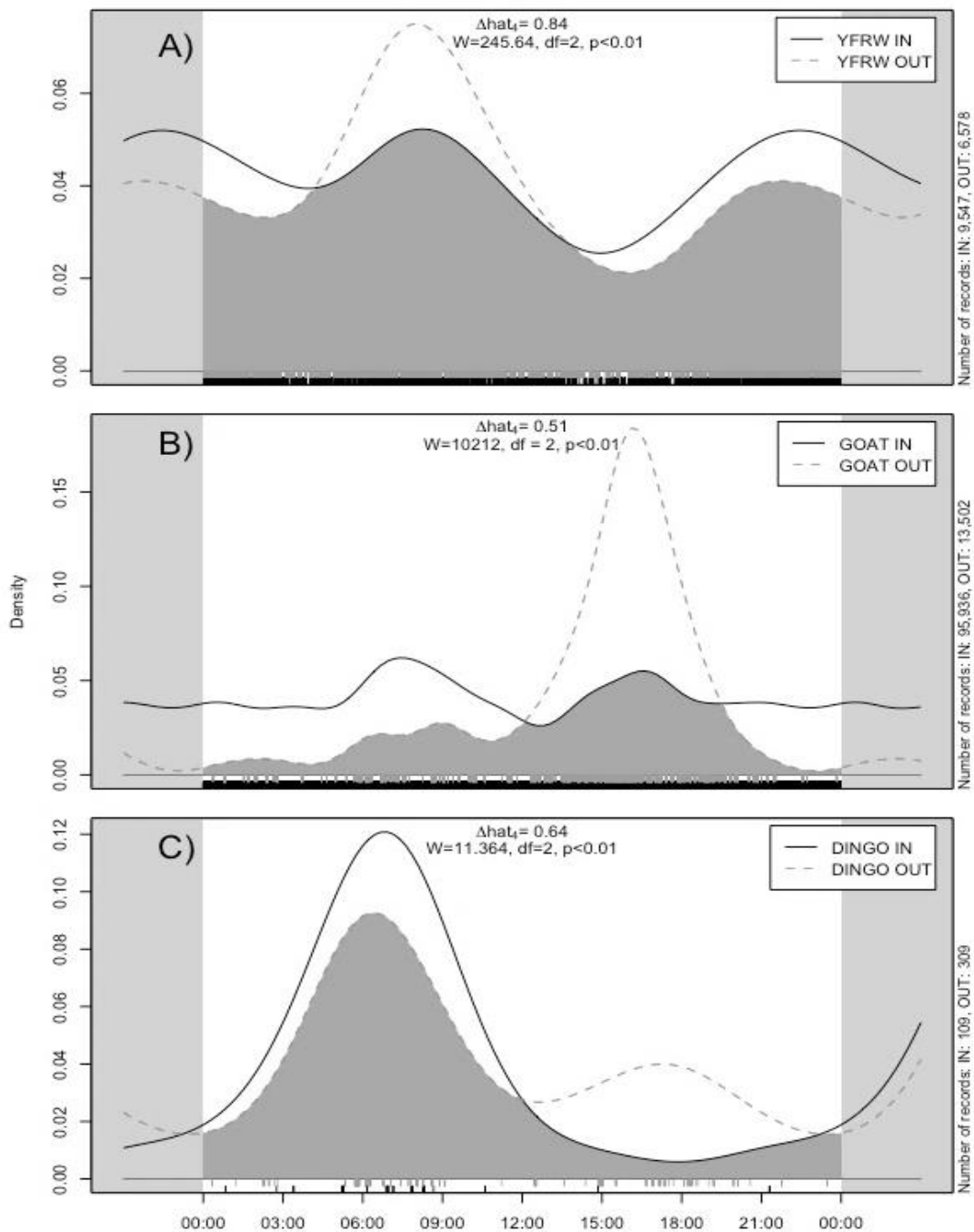


resulted in a total of 79,568 useful images showing 144,796 counted animals over 15,092 camera days (Table S9; Table S10). Cameras recorded an overall average of 9.1 animals/camera-day (SE = 2.50; Table S11). Differences in animals/camera-day were insignificant across treatments ( $F(1,6) = 2.72, p = 0.2$ ) or between camera placements at the top or bottom of cliffs for animals/camera-day ( $t = -1.1, df = 20.6, p = 0.3$ ) or YFRW/camera-day ( $t = 0.3, df = 21.7, p = 0.8$ ).

The 26 cameras deployed each took between 31 and 3,253 photos of YFRW ( $\mu = 555.3, SE = 141.3$ ), confirming the presence of YFRW at each site. This resulted in a minimum recorded sum of 32 and a maximum of 3,402 individual YFRW counted across all photographs at the respective sites ( $\mu = 618.9, SE = 155.2$ ). YFRW were recorded in groups of up to 4 individuals (on more than one occasion), but were most often recorded alone, with an average of 1.11 YFRW per photo (SE = 0.02) in photos where YFRW were recorded. Cameras recorded an overall average of 1.13 YFRW/camera-day (SE = 0.26), with 1.14 YFRW/camera-day (SE = 0.27) recorded inside and 1.12 YFRW/camera-day (SE = 0.41) recorded outside the cluster fence. One-way ANOVA showed no difference in YFRW/camera-day between sites in each treatment (inside:  $F(3,11) = 0.482, p = 0.7$ ; outside:  $F(3,7) = 0.249, p = 0.9$ ), and as such they were pooled into treatment for all further analysis of YFRW. Additionally, there was no difference in YFRW/camera-day across treatment from nested ANOVA ( $F(1,6) = 0.003, p = 0.96$ ).

In total, 418 records of dingoes were collected over the entire study period, resulting in low numbers of dingoes/camera-day across treatments (Inside:  $\mu = 0.01, SE = 0.003$ ; Outside:  $\mu = 0.06, SE = 0.02$ ). There is an insignificant difference in dingoes per camera-day between treatments ( $F(1,6) = 1.78, p = 0.23$ ). Cameras recorded an average of 6.7 goats/camera-day (SE = 2.5) across the study site; 9.9 goats/camera-day (SE = 4.01) for inside, 2.2 goats/camera-day (SE = 1.3) outside the cluster fence. Goats were recorded at seven out of the eight sites (Site 8 had no goat records), and 22 out of the 26 cameras. Other than zero records, the minimum goat count was one goat at Camera 23 (Table S9), which also represented the only goat record for the site (Site 7). The maximum number of goat records was 35,775 at Camera 6 ( $\mu = 4203.1, SE = 1503.1$ ). No significant difference in goats/camera day was found between inside and outside the fence ( $F(1,6) = 2.94, p = 0.14$ ).

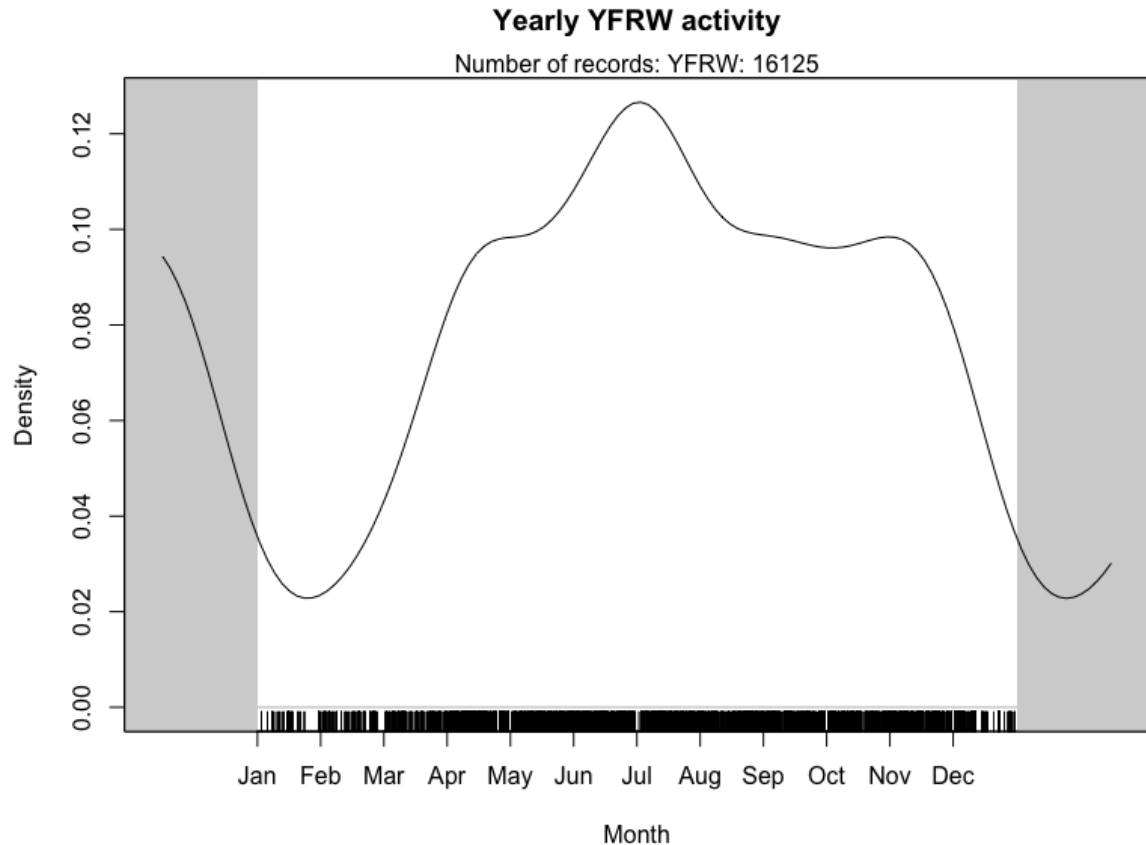
(ii) Activity and Overlap



**Figure 7.2: Kernel density overlap plot of diel activity of A) YFRW, B) Feral Goats and C) Dingoes**  
Plot shows the kernel density overlap of: **A)** Diel YFRW activity for all 16,125 records of YFRW across the treatment (Inside = 9,547, Outside = 6,578).  $\Delta\hat{\alpha}_4$  (coefficient of overlap) = 0.83, with significant variation,  $p < 0.01$ . **B)** Diel Feral Goat activity for all 109,438 records of Feral Goats across the treatment (Inside=95,936, Outside=13,502).  $\Delta\hat{\alpha}_4$  (coefficient of overlap) = 0.51, with significant variation,  $p < 0.01$ . **C)** Diel Dingo activity for all 418 records of Dingoes across the treatment (Inside = 109, Outside = 309).  $\Delta\hat{\alpha}_4$  (coefficient of overlap) = 0.64, with significant variation,  $p < 0.01$ . For all graphs the solid line shows the activity of Inside and the dotted line shows Outside. Dark grey shading shows overlap of density distribution.

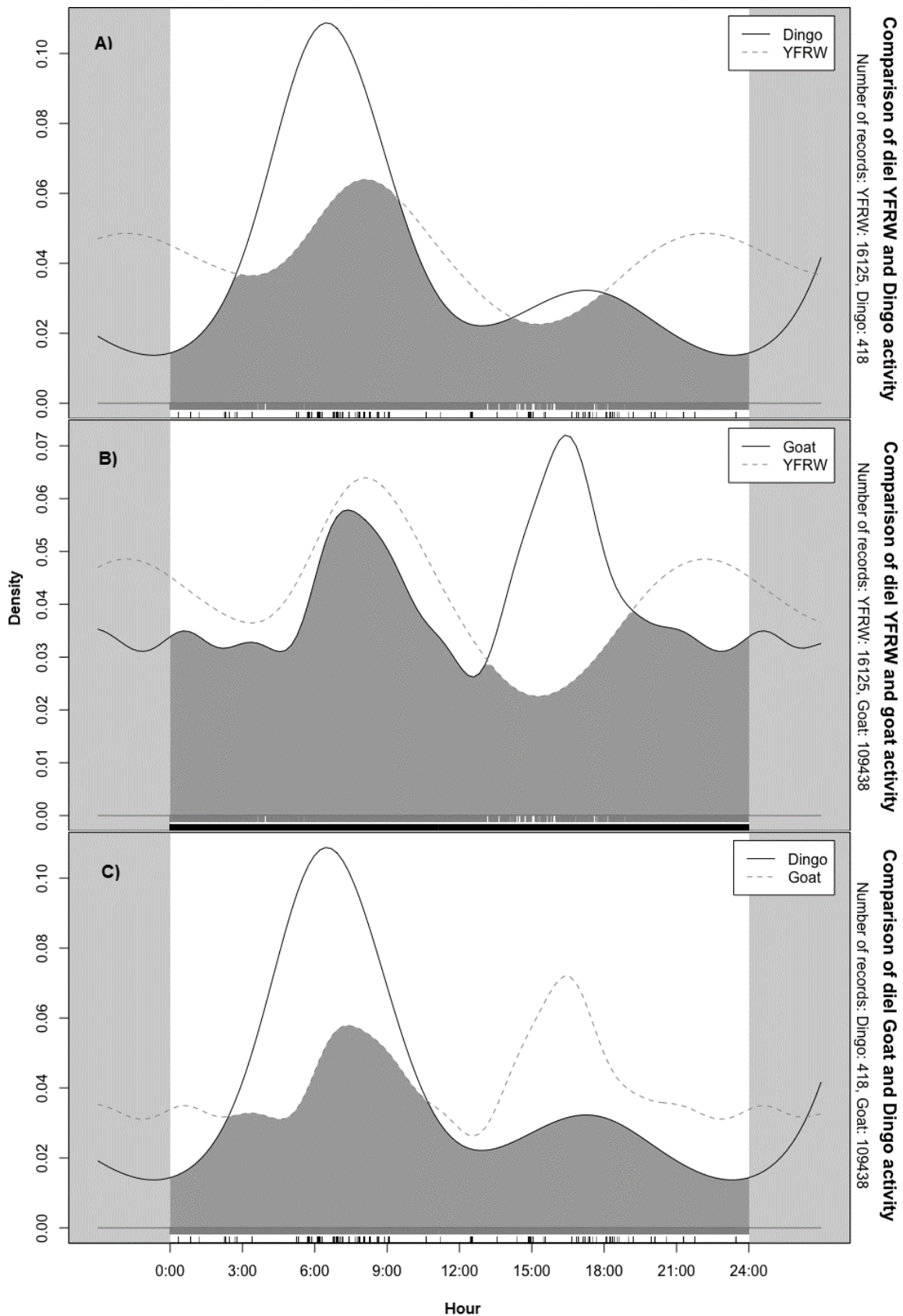
Overall, YFRW at the study site were most active between the hours of 08:00 and 09:00 of a morning and between 22:00 and 23:00 of a night (Figure 7.2-A). YFRW daily activity peaked between 08:00 and 9:00 on the outside of the fence; however, YFRW activity

peaked around 22:00 inside the fence. Estimates of plot kernel density overlap produced a daily activity overlap coefficient ( $\Delta\hat{a}_4$ ) of 0.84 when comparing inside and outside YFRW activity. Watson-Wheeler tests revealed the density curves to be significantly different ( $W = 245.64$ ,  $df = 2$ ,  $p < 0.001$ ). Yearly activity peaked between April and November each year (Figure 7.3).

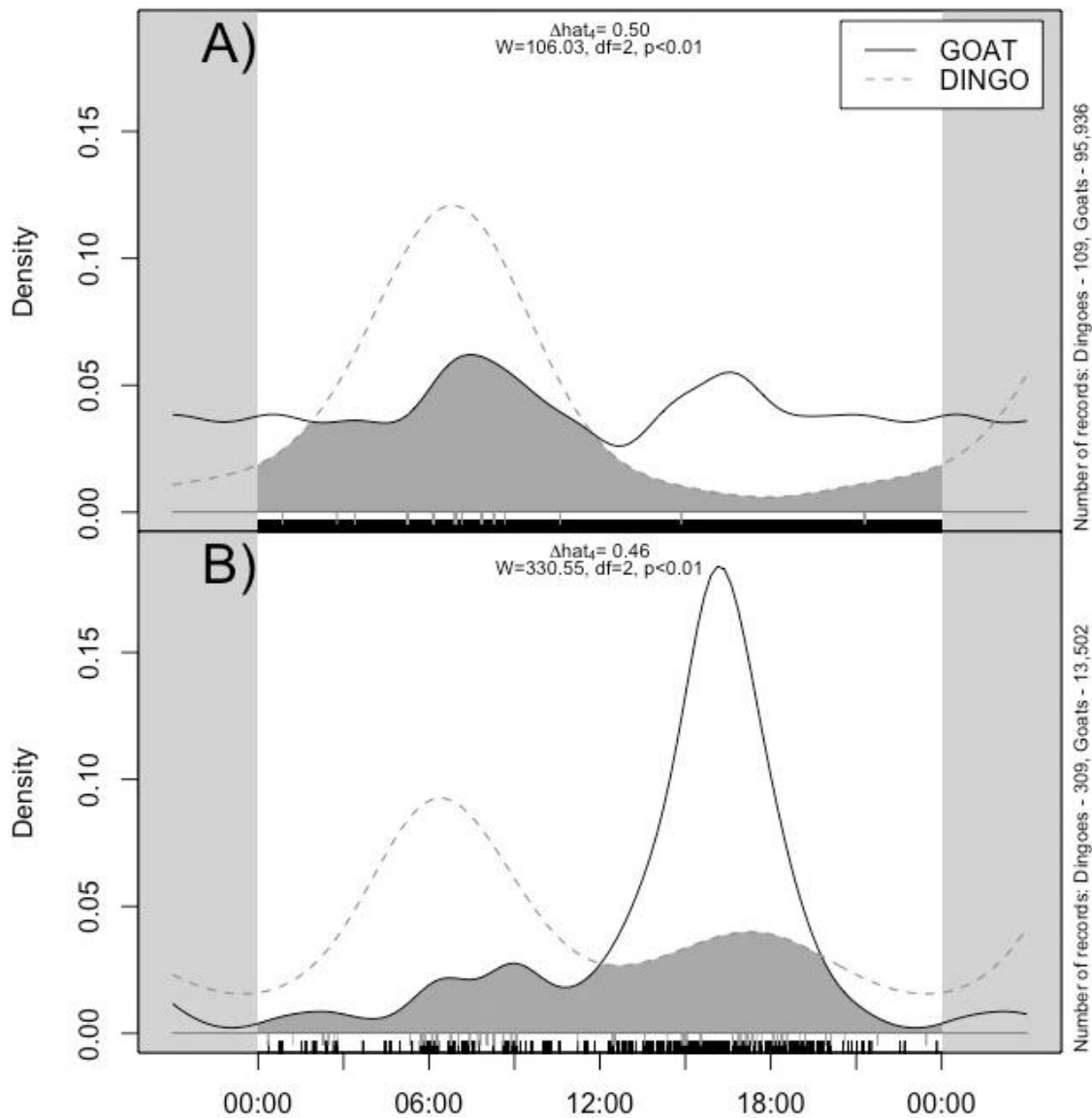


**Figure 7.3: Kernel density overlap plot of yearly activity of YFRW**  
Plot shows the yearly activity YFRW activity for all 16,125 records of YFRW, x-axis shows the month.

Most dingo activity occurred in the morning, with the main peak of activity at approximately 07:00 (Figure 7.2-C). Dingo activity also had a second smaller peak at approximately 17:00 ( $W = 11.36$ ,  $df = 2$ ,  $p < 0.001$ ). Estimates of plot kernel density overlap produced a diel activity overlap coefficient ( $\Delta\hat{a}_4$ ) of 0.59 between YFRW and dingoes (Figure 4-A). Goat activity was crepuscular with peaks at approximately 07:00 and 17:00. Watson-wheeler tests showed feral goat diel activity was significantly different across the treatment also ( $W = 10212$ ,  $df = 2$ ,  $p < 0.001$ ). Estimates of plot kernel density overlap produced a diel activity overlap coefficient ( $\Delta\hat{a}_4$ ) of 0.80 between YFRW and feral goats (Figure 7.4-B). Feral goat and dingo activity had an overlap coefficient of 0.6 (Figure 7.4-C). Comparing dingo activity to feral goats inside the cluster fence resulted in an overlap of  $\Delta\hat{a}_4 = 0.50$  and  $\Delta\hat{a}_4 = 0.46$  outside (Figure 7.5).



**Figure 7.4: Kernel density overlap plot of diel activity between YFRW and feral goats**  
 Plot shows the kernel density overlap of diel (A) YFRW and Dingo (B) YFRW and goat, and (C) Goat and Dingo activity for all records of YFRW, dingoes and feral goats. Dark grey shading shows overlap of density distribution.  $\hat{\Delta}_4$  (coefficient of overlap) = (A) 0.59, (B) 0.80, and (C) 0.60. All the activity curves varied significantly from each other,  $p < 0.01$  from Watson-Wheeler test.



**Figure 7.5: Kernel density overlap plot of feral goats and dingoes A) inside and B) outside cluster fencing**  
 Plot shows the kernel density overlap of diel feral goat and dingo activity A) inside and B) outside cluster fencing. Solid line shows goat activity and dashed line shows dingo activity. Dark grey shading shows overlap of density distribution.  $\Delta\hat{\alpha}_4$  (coefficient of overlap) = (A) 0.5 and (B) 0.46. All the activity curves varied significantly from each other,  $p < 0.01$  from Watson-Wheeler test.

(iii) *Behaviour, sex and age class*

Tests of proportion showed individual behaviour categories as a proportion of all behaviours recorded were different across treatments (Table S12). However, tests assessing whether activity (count of behaviour/camera-day) differed across treatments or not found no differences (all tests  $p > 0.05$ , see Table S13). Tests of equal proportions showed that a greater proportion of YFRW records inside the cluster fence had pouch young ( $\chi^2 = 9.74$ ,  $df = 1$ ,  $p < 0.01$ ) or were juvenile ( $\chi^2 = 122.12$ ,  $df = 1$ ,  $p < 0.01$ ). These proportions of records were also significantly different when pooled ( $\chi^2 = 128.64$ ,  $df = 1$ ,  $p < 0.01$ );

however, again these proportions were not further supported by a difference in counts/camera-day (Table S13). Additionally, a test of equal proportions comparing the age class ratio (kids/adults) of feral goats inside (3.7%) and outside (1.2%) the fence showed significantly fewer kids outside the cluster fence ( $\chi^2 = 233.09$ ,  $df = 1$ ,  $p < 0.01$ ) with an average of 0.37 kids/camera-day (SE = 0.13) inside and 0.03 kids/camera-day (SE = 0.02) outside the cluster.

## 7.5 Discussion

### (i) *Key findings*

Removing strongly interactive predators from ecosystems has been predicted to restructure faunal and floral communities by alleviating top-down limitations on prey (Estes et al., 2011). In our study, the pest management activities occurring inside cluster fences should enable populations of YFRW living inside the fence to increase, exhibit a broader or different temporal range, express different behaviours (e.g. increased foraging or reduced vigilance), and exhibit different age structures (e.g. a greater proportion of pouch young in the population). However, the results of our study were mixed. Overall camera trap rates of YFRW were similar inside and outside the fence (Table S13), suggesting that YFRW abundance was no greater as a result of pest management practices within the cluster. Diel activity patterns of YFRW and goats were different inside and outside the cluster fence, with YFRW exhibiting higher activity peaks at dawn outside the fence (Figure 7.2). YFRW activity fluctuated less inside the fence, with a less distinct peak in the morning when compared to outside the cluster. Proportions of behaviours and ages of YFRW recorded were different between treatments, but there was no difference in age structures or the expression of YFRW behaviours per camera-day (Table S13). Differences in YFRW and goat activity patterns and goat age structure aligned with our predictions, but the similarity in overall YFRW camera trap rates, behaviour and age structure did not align with our predictions. Diel activity showed dingoes were most active in the morning on both sides of the fence. Goat activity mostly occurred in the evening outside the cluster (Figure 7.2). There was also a greater number of goat kids inside the fence than outside as a proportion of all goats, consistent with previous assertions that dingoes suppress feral goats (Allen et al., in press; Allen, Goullet, & Palmer, 2012). However, no difference was found between goats and dingoes across the fence (Table S13).

(ii) *Activity and Overlap*

As expected, an analysis of YFRW activity revealed the species to be broadly crepuscular (Figure 7.2-A); the evening activity peak occurs outside the twilight hours, as it does for related species (Caughley, 1964; Morgan et al., 2019). YFRW activity also peaked in the coolest months of the year, with very little activity on camera over the hottest summer months (Figure 7.3). Many mammal species adjust their activity, at least in part, to avoid unfavourable temperatures (Caughley, 1964; Diете et al., 2017). Based on our results YFRW appear to avoid unfavourable heat at the daily and yearly levels. Our results suggest both goats and dingoes are also crepuscular; both peak in the morning at 06:00-07:00 and 16:00-17:00, though the activity peak for dingoes was higher in the morning both inside and outside the cluster (Figure 7.2-C). Higher morning activity levels by dingoes has been observed previously (e.g. Brook, Johnson, & Ritchie, 2012).

There was a significant difference in temporal YFRW activity (Figure 7.2-A) across the cluster fence. Both inside and outside, YFRW still displayed a bimodal (crepuscular) distribution; however, YFRW occurring inside have lower activity in the morning and a slightly higher peak of a night in comparison to YFRW outside the fence. Inside the fence, YFRW activity also fluctuated less. Though our hypothesis predicted differences in activity, our observed differences are difficult to attribute to pest management. Firstly, overlaps of activity of dingoes with YFRW produced one of the lowest overlapping coefficients observed in this study ( $\Delta\text{hat}_4 = 0.59$ ; Figure 7.4). Intuitively, a change in predator density would result in behavioural shifts that inversely correspond to that change (Creel & Christianson, 2008). In this case, a peak in dingo activity in the morning might suggest that YFRW living in the presence of a higher density of dingoes would reduce their activity at this time, and due to dingo management within the fence, YFRW may be more active at this time. However, opposite patterns to this were observed (Figure 2-B). This raises several other hypotheses that need further exploration to fully explicate the results. Primarily, (1) other unknown variables across the treatment, rather than pest management practices, may be the source of the observed differences in YFRW activity. For example if ground water or vegetation was more available YFRW may be less or more active at those sites. Secondly, (2) dingo management may result in a counter intuitive response in YFRW activity. A decrease in overall dingo activity, might have resulted in a decrease in predator aversion behaviours and therefore activity in YFRW (Creel & Christianson, 2008). This

hypothesis was also partially explored though the behaviours recorded for YFRW, though there was no significant differences in sedentary behaviours per camera-day (such as foraging and resting) or active behaviours per camera-day (such as hopping) across the treatment which may indicate this change. Lastly, (3) the extreme overlap with feral goat diel activity may have an effect on YFRW activity. Competitors exert an influence on the timing of sympatric species habitat use (Carothers & Jaksić, 1984) and feral goats are known to compete heavily with YFRW for resources (Dawson & Ellis, 1979). However, feral goats/camera-day was not significantly different across the treatment either. As a result of these hypotheses and an inability to further explore the causal factors behind the differences or absence of differences in YFRW across treatments, we are unable to confidently attribute pest management to these changes in YFRW activity at this time.

While pest management practices have not appeared to have had any measurable effect on YFRW activity, it may be contributing to differences in feral goat activity. Figure 7.4 shows the timing of dingo activity peaks to be closer to the timing of goats rather than YFRW overall. Comparing the activity plots of goats inside the cluster fence with dingoes resulted in an activity overlap of 0.50, while feral goats outside the fence have an overlap of 0.46 (Figure 7.5). Goats outside the treatment clearly have much less activity in the morning (when dingoes are most active) and concentrate their activity in the evening. However, to reliably attribute these differences in feral goat activity to pest management we would need to show that dingoes were indeed at lower densities within the cluster. While the greater proportion of dingo records did occur outside the treatment, our data showed only weak or insignificant differences in dingoes per camera day across the cluster fence. This lack of a difference may be an artefact of the sampling methods, or may reflect true density similarity. Camera traps were placed to optimise detection of YFRW, not dingoes, and whilst we would expect dingo density within the entire cluster fence to be lower due to active dingo management, dingo density at YFRW colonies may actually be the same given that YFRW provide a source of food for dingoes (Hornsby, 1997). Given greater time and funding, more appropriate methods to compare predator densities would have also been used (such as passive tracking indices generated from sand-plot data); however, this was outside the scope of our study.



(iii) *Behaviour and population structure*

The behaviour and age of each YFRW was recorded (where possible) during photo scoring. Behavioural data were collected in order to evaluate whether a reduction of predation risk inside the fence resulted in differences in behavioural expressions across the treatments. Previous studies have shown that in the absence of a predator, rock-wallabies may express behaviours differently, such as utilising a broader range of habitat (Kinnear, Onus, & Sumner, 1998). While proportions of observed behaviours did differ, comparisons of behaviours/camera-day between sites and across the treatment revealed no significant differences (Table S13). Testing equal proportions revealed that a greater proportion of YFRW inside the cluster were pouch young or juveniles. However, differences in recorded YFRW ages/camera-day also showed no significant differences across treatment. These mixed results do not align neatly with our predictions; however, the lack of an observed treatment effect may not be so surprising when it is remembered that the Quilpie cluster fence was completed only in the last 3 years (2016), and changes in behavioural expression and population structures may only reveal themselves after longer periods (Begon, Harper, & Townsend, 1996; Moseby, Hill, & Read, 2009).

This relatively short timeframe does not appear to have slowed the impact of dingoes on goats. Though total goats per camera day did not differ, the fraction of goat kid records as a proportion of total goat records was demonstrably less outside the fence, and differences in kids/camera-day supported this finding. Dingoes have been demonstrated to swiftly suppress and eradicate goat populations when given the opportunity (Allen et al., in press; Allen, Goullet, & Palmer, 2012; Parkes et al., 1996; Whitehouse, 1977).

(iv) *Summary and recommendations*

This study explored three main hypothesis; that pest management within clusters would (1) result in a change in YFRW temporal activity, (2) a change in YFRW behaviours, and (3) a change in population age compositions. We found mixed results that did not always align with our predictions. These results have important implications for managers, particularly in relation to feral goats, and suggest that pest management practices may release feral goats from predation pressure. Increases in feral goat activity would likely have negative outcomes for both native species, and livestock production as a result of increases in TGP.

Part of the reason we could not attribute changes in YFRW to pest management was that we could not demonstrate dingoes had been significantly impacted by management inside cluster fences using our sampling methods. This could be due to our use of sampling methods that were targeted towards YFRW and not dingoes, but it might also be due to a true absence of sufficient dingo removal inside the cluster fenced area. An alternative explanation is that insufficient time may have elapsed for YFRW to express consistent changes in activity, behaviour and population constitutions. To more reliably assess the responses of non-target and native prey to the removal of predators inside fences, we recommend that future studies utilise a variety of fauna sampling methods capable of detecting low predator densities and population change if it occurs, and conducting studies for periods long enough to observe any prey responses.

## **7.6 Author contributions**

The concept of this study was developed by BLA. DS and MT collected and entered all data (with the help of volunteers) and DS conducted all data analysis. DS created the first draft of the manuscript. All authors contributed to the editing of the manuscript for submission.

## **7.7 Acknowledgements**

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## **Chapter 8 : Movement between yellow-footed rock-wallaby (*Petrogale xanthopus*) colonies, inferred from estimates of kinship**

**Reference:** Smith, D., Lethbridge, M.R., Allen, B.L., & Andrew, R.L. (in review).  
Movement between yellow-footed rock-wallaby (*Petrogale xanthopus*) colonies, inferred  
from estimates of kinship.

### **8.1 Abstract**

Understanding the exchange of individuals between wildlife populations, particularly those with naturally fragmented habitats, is important for effective management. This is of particular consequence when the species is of conservation concern, and isolated populations may be lost due to predation or competition pressure, or catastrophic events such as wildfire. Here we use kinship and population structure analysis to highlight potential recent movement between colonies of yellow-footed rock-wallaby (*Petrogale xanthopus* Gray, YFRW) at two locations in the Grey Range of Queensland, and at four sites in the Gawler Ranges of South Australia. These sites are also compared to a single site from the Flinders Ranges, a connected landscape of YFRW habitat. Using reduced representation next-generation sequencing (NGS), we acquired and filtered a set of ~17,000 single-nucleotide polymorphisms (SNPs) to examine population structure, examine genetic relationships within populations and identify putative migrants of YFRW. Pairwise comparisons of individual relatedness indicated several contemporary movement events between colonies within both the Gawler and Grey Ranges. These results are of particular note in South Australia, where threat abatement may facilitate dispersion, and in Queensland, as the colonies are separated by anthropogenic barriers: predator exclusion fencing designed to exclude dingoes (*Canis familiaris*) from grazing land. This work also highlights the need for threatened species management at the landscape level, as isolated colonies may require movement events to mitigate the genetic effects of prolonged isolation, and insure against colony extinction.

## 8.2 Introduction

An exchange of individuals between spatially or genetically discrete populations of the same species is known as metapopulation (Wells & Richmond, 1995). For species in such systems, movement behaviours are essential, as they insure against risk of extinction from negative pressures (such as predation, competition and catastrophic events; Holyoak & Lawler, 1996), and protect against the negative genetic effects of consanguineous mating (Olivieri, Michalakis, & Gouyon, 1995; Perrin & Mazalov, 1999). Movement behaviour also gives rise to potential recolonization of “empty” sites or the formation of new populations at suitable sites (Hanski, 1998). Understanding such movement is therefore critical for effective species management (e.g. Lee & Bolger, 2017; Seward et al., 2019; Sielezniew et al., 2019; Srivathsa et al., 2019).

For cabalistic species, it can be hard to determine whether individuals are moving between isolated colonies. In these species, the use of genetic techniques to identify the relationships between individuals of a population can be used to infer recent movement events, and is extremely useful to understanding barriers to dispersal (Escoda, Fernández-González, & Castresana, 2019). Further to this, advancements in genetic technologies such as Next-Generation Sequencing (NGS), and the development of more robust methods of determining kinship values provide greater confidence in the results of kinship analysis. This confidence allows for inferences to be drawn between kinship values and the probability of a contemporary movement between colonies (Escoda et al., 2017).

The yellow-footed rock-wallaby (*Petrogale xanthopus* Gray, YFRW) is a threatened macropod found in the semi-arid zone of Australia. There are two subspecies of YFRW: *P. x. xanthopus* is found in the Flinders, Olary and Gawler Ranges of South Australia (SA; Threatened Species Scientific Committee, 2016a), and the Gap and Coturaundee Ranges in New South Wales (NSW; Lim & Giles, 1987), and *P. x. celeris* is found in the Grey Range in Queensland (QLD; Threatened Species Scientific Committee, 2016b). The distribution of both subspecies is assumed to have significantly decreased since European settlement (Copley, 1983). Rock-wallabies (*Petrogale* spp.) have high habitat specificity, only occupying complex rocky habitats (see Gordon et al., 1993; Lim & Giles, 1987; Telfer, Griffiths, & Bowman, 2008). As a result of this habitat specificity, suitable rock-wallaby habitat is naturally fragmented, leading to metapopulation structure (Lethbridge et al.,

2019; Lethbridge & Strauss, 2015; Murray et al., 2008; Ruykys & Lancaster, 2015). Primary evidence of movement between colonies of YFRW is surprisingly scarce given the quantity of literature describing the species, particularly in SA. A study using tracking collars and ear-marked YFRW in Queensland found evidence of a single movement event over 36 months (Sharp, 2002), and another (also with collars) revealed several long distance transient movements of released captive-bred rock-wallabies (Lapidge, 2001). The latter also inferred several dispersal events from wild rock-wallabies that were trapped at the previously empty reintroduction sites.

The limitations of radio tracking and capture-mark-recapture to detect all movement behaviours is evident; both rely on the captured individual moving during the study period. With the exception of GPS and remote capture data, the ability to detect or recapture individuals that have moved is also further limited by the distance of the movement. Detecting long distance movements, such as natal dispersal, with such techniques is understandably infrequent for YFRW. As long as two related individuals are sampled, the use of genetic data mitigates both these shortcomings, as it theoretically detects any contemporary movement event between colonies at any distance. To this end, a recent study assessing YFRWs' genetic health identified 13 putative first generation migrations of between 2 and 60 km over connected habitat in the Flinders Ranges using population assignment methods based on the individuals' genetic structure gained through microsatellite analysis (n = 194, Potter et al., 2020). Potter et al. (2020) built on earlier genetic analysis of dispersal (also using microsatellite analysis), that had concluded that YFRW rarely move between colonies, even within connected habitat (Pope, Sharp, & Moritz, 1996). Although the methods employed were reliable, this earlier Queensland based study was limited by sample size. These early studies were also performed without or with limited threat abatement practices in place, which may be critical to allow YFRW movement behaviours.

While the recent publications on the same species are informative, a greater understanding of YFRW inter-colony movement is needed to understand potential metapopulation dynamics and inform management of the species. The distribution of *P. x. celeris* has recently been heavily subdivided by agricultural exclusion fences (Smith, Waddell, & Allen, 2020; Chapter 3). These fences were constructed to limit the movement of dingoes/wild dogs onto livestock properties where they have a negative economic impact

on livestock production, but may also impact non-target species such as *P. x. celeris*. While the fences may ultimately be of a benefit to the species' long-term persistence (Smith, Waddell, & Allen, 2020; Chapter 3), exclusion fencing has the potential to isolate colonies, which may lead to deleterious genetic consequences (Smith, King, & Allen, 2020; Chapter 2). The conservation of *P. x. xanthopus* may also be reliant on an understanding of inter-colony relations. Predation by red foxes (*Vulpes vulpes*), competition with feral goats (*Capra hircus*) and catastrophes can all contribute to extinction risk of the entire metapopulation. Understanding the interchange of the individuals between sites would potentially give insight into the effect of pest species control on metapopulation dynamics and the utility of broad-scale pest management in the effective conservation of YFRW.

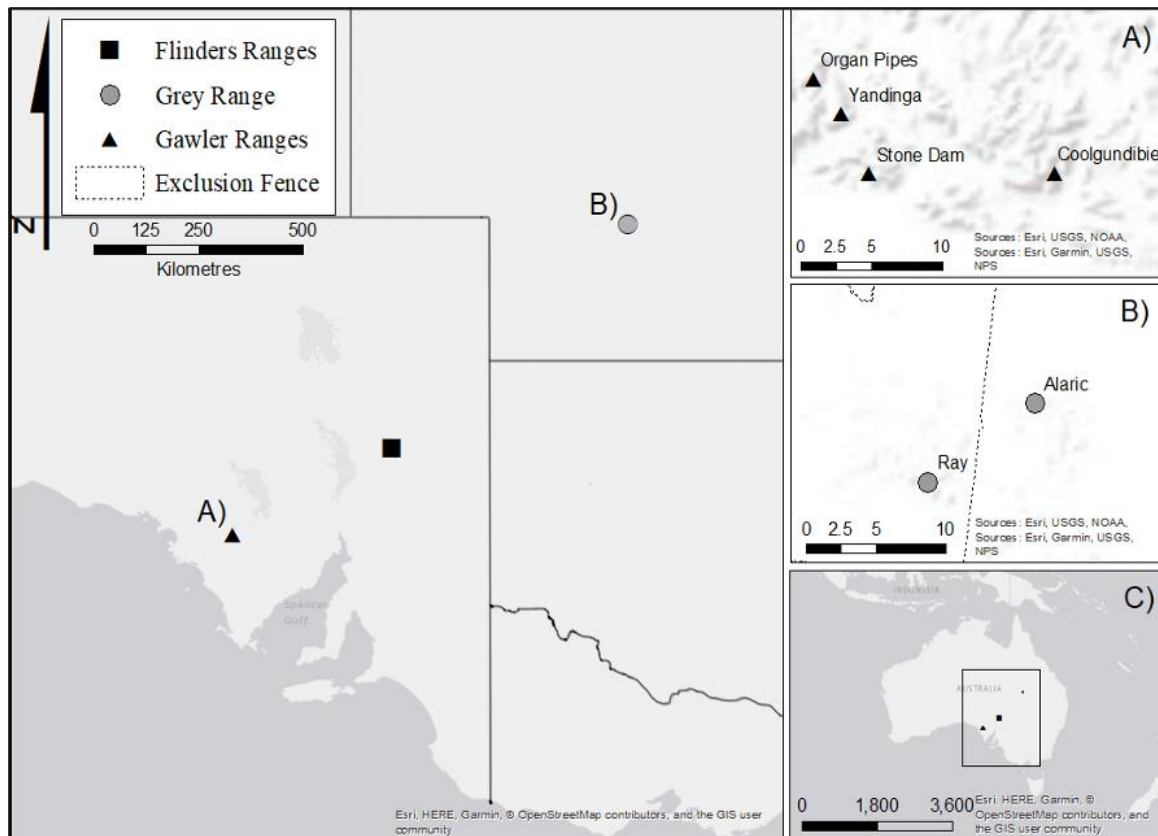
Given the cryptic nature of the species and its disjunct distribution, we used reduced representation next-generation sequencing (Kilian et al., 2012) to study movement and population structure of YFRW. Our objective was to assess if there had been recent movement of YFRW between geographically close colonies, using population structure analysis, pairwise relatedness coefficients and identification of first generation migrants. We also discuss the value of kinship analysis to infer species movement within potential metapopulations, and how this analysis affects the management of YFRW, both in South Australia and Queensland.

### 8.3 Methods

#### (i) Study sites

YFRW were trapped and ear biopsies were obtained at four sites in the Gawler Ranges, and one site in the Flinders Ranges (South Australia) by Lethbridge and Andrews (2014) and later Lethbridge (*unpublished*), under a S.A. Wildlife Ethics approval: S23997-19, and at two sites in the Grey Range, near Quilpie in central-western Queensland, under a University of Southern Queensland Animal Ethics approval: USQ-17REA011 (Figure 8.1). The Gawler Ranges (GWL), the Flinders Ranges (FLD) and the Grey Range (GRY) will hereafter be referred to as 'populations'. Trapping sites within populations will be referred to as 'sites' or 'colonies'. Trapping in South Australia took place from August 2012 to September 2016, and in Queensland in 2018 and 2019. The two sites in Queensland are separated by 8 km, including approximately 5 km of unsuitable habitat. Historically this gap between the two populations was traversable by YFRW (no obvious obstructions), but

an exclusion fence was erected two years prior to sampling to alleviate the pressures of wild dog predation on sheep properties. The sites in the Gawler Ranges are not disrupted by fencing but vary in distance from Yandinga, a theorised source/refuge population (Figure 8.1). The Gawler Ranges populations were also historically disrupted by exclusion fencing (circa 1920s), but spatial and temporal information on this fencing is minimal/non-existent.



**Figure 8.1: yellow-footed rock-wallaby trapping sites**

The main map displays the locations of the Gawler Ranges, Flinders Ranges and Grey Range. Inset A) shows the locations of the 4 colonies trapped in the Gawler Ranges (SA). Inset B) shows the location of 2 colonies trapped in the Grey Range (QLD). Inset C) shows the context of the main map within the broader global region. Waukawoodna Gap (SA; the only trap site within the Flinders Ranges) is marked by a solid square (■) on the main map.

(ii) *Trapping, sampling, extraction and sequencing*

YFRW were trapped using established methods in soft-walled treadle cage (M. Lethbridge, unpublished). A tissue biopsy was removed from each rock-wallaby's ear using a 3 mm punch. The sample is taken from the opposite ear to an ear-tag employed for potential future visual identification in the field. Morphometric data (tail length, length of both feet, weight and sex) were also collected before, finally, each YFRW was microchipped for identification on recapture. Tissue samples were placed in Longmire's buffer solution (Queensland samples - Longmire, Maltbie, & Baker, 1997) or 100% ethanol (South Australian samples) and stored in a cool place until extraction. DNA was extracted from

95 YFRW samples (25 from Queensland and 70 from South Australia) using a salt-based ethanol extraction and quantified on a Qubit™ Fluorometer and quality controlled using a NanoDrop™ 3000. One sample from the Flinders Ranges site was not of high enough quality for accurate sequencing (based on quality control steps) and therefore was removed from the samples sent for sequencing. Samples were then sent to Diversity Arrays Technology (DArT P/L) in Canberra, ACT for sequencing via their DArTseq service (Kilian et al., 2012). The protocol involves technical replicates, which provides an empirical measure of the repeatability of the resulting loci. DArT conducted filtering and reference-free clustering of reads, followed by genotyping using their proprietary analysis pipeline. SNP data were returned for 92 of the 94 samples. Counts of rock-wallabies samples per colony can be seen in Table 8.1.

### *(iii) Data filtering*

The SNP data returned by DArT was first explored in R (version 3.6.2) using the `dartR` v1.1.11 package and following the workflow suggested in the package documentation (Gruber et al., 2019a, 2019b). The reports generated by this initial analysis were used to inform filtering thresholds. The unfiltered dataset was later subset into populations based on population structure of the complete dataset (see Results) for further analysis. Monomorphic loci are automatically removed during this sub-setting. These subsets were numbered Dataset 2-4, with Dataset 1 as the total, primary dataset (Table 8.1). Data were first filtered for the average repeatability of each locus (loci with repeatability  $>0.95$  were kept), and the call rate by locus ( $>0.95$ ) and by individual ( $>0.90$ ). The data was then filtered to remove over-split loci ( $<0.2$  Hamming distance), and for observed heterozygosity greater than 0.6. Finally, loci with minor allele frequencies lower than 0.05 were removed before all metrics were recalculated. Filtering thresholds were chosen based on the recommendations of Gruber et al. (2019b) and O'Leary et al. (2018).

During initial data exploration, four further samples were removed from the dataset, leaving 88 of the original 95 samples. Relatedness estimates range from 0 (totally unrelated individuals) to 1 (clones). Preliminary data exploration identified four samples with high pairwise relatedness ( $> 0.85$ ) to other samples, which is not consistent with outbreeding and random mating. These values may have arisen in a number of ways: monozygotic twinning, duplicate sampling or pipetting error prior to sequencing. The source populations and demographic data of the duplicate samples did allow for either duplicate sampling or



twinning to be the potential causes of these data. Twins are known to occur in *Petrogale assimilis* (Spencer & Marsh, 1997), and the twinning rate in *Macropus* is low, but non-zero (Inns, 1980; Norbury, 1986; van Oorschot & Cooper, 1989). Nevertheless, as these duplications would skew population statistics, and pipetting error cannot be excluded, we removed samples with high pairwise kinship estimates prior to all analyses. The sample (in each pair) of higher quality (greater call rate and average reproducibility) was kept and the sample of lower quality was removed prior to filtering and final analysis.

**Table 8.1: Table of datasets**

Summary of each numbered *Petrogale xanthopus* DArTseq dataset. Sample sizes, the subdivision of populations into colonies, and the number of samples for each colony are shown. The Loci pre-filtering column shows the total number of loci (17,864) minus monomorphic loci removed automatically in subsetting.

Dataset	Population	Colonies	<i>n</i>	Loci pre-filtering	Loci post-filtering
1.	All	-	88	17,863	9,037
2.	Gawler Ranges	Coolgundibie (10) Organ Pipes (11) Stone Dam (6) Yandinga (19)	46	6,354	3,578
3.	Flinders Ranges	Waukawoodna Gap (17)	17	12,358	7,979
4.	Grey Range	Alaric (18) Ray (7)	25	8,538	5,481

(iv) *Population structure, statistics, genetic distances, and PCoA*

Population structure analysis was performed using STRUCTURE (v2.3.4; Pritchard, Stephens, & Donnelly, 2000). Parameter settings varied by dataset. Dataset 1 was first analysed from for all clusters (*K*) from 1 to 10, with 10 independent repeats of 100,000 MCMC (Markov Chain Monte Carlo) iterations after a 10,000 iteration burn-in. Based on these results, Datasets 2-4 (subsets of Dataset 1) were analysed, using more robust methods. Each dataset was analysed from *K* = 1-10, after 50,000 burn-in and 500,000 MCMC iterations with sampling populations as priori. *K* values were estimated through an assessment of both Pritchard's model likelihood method (Pritchard, Stephens, & Donnelly, 2000) and Evanno's  $\Delta K$  method (Evanno, Regnaut, & Goudet, 2005) implemented in Structure Harvester (Earl & vonHoldt, 2012), and scrutinised following recommendations detailed in Cullingham et al. (2020) for *K* = 2 results. The production of population structure bar plots was performed in CLUMPAK (v1.1; Kopelman et al., 2015).

Also, Principle Coordinate Analysis (PCoA) was performed using Euclidean distance in R with `dartR`. Each dataset was analysed and informative dimensions examined. Plots of the two most informative axes of each population were generated with `ggplot2` v3.3.2 (Wickham, 2006).

Mean observed heterozygosity ( $H_O$ ), expected heterozygosity ( $H_E$ ), population Allelic Richness (rarefied allelic counts, per locus and population;  $A_R$ ), and inbreeding coefficients ( $F_{IS}$ ) were reported using `hierfstat` v0.04-22 in R (`basic.stats` function) with 98% confidence intervals (1000 bootstraps). Population divergence was explored using several approaches; pairwise private and fixed alleles, Euclidean and Nei's (Nei, 1972) genetic distance matrices as well as pairwise  $F_{ST}$ . Pairwise  $F_{ST}$  values were also estimated using `StAMPP` (1,000 bootstraps, 95% CI), which follows the methods of Weir and Cockerham (1984). An unrooted neighbour joining tree was constructed from a Euclidian distance matrix before, finally, effective population sizes ( $N_e$ ) were estimated for each colony using `NeEstimator` (v2.1; Do et al., 2014) using the Linkage Disequilibrium (random mating) methods for a minimum allele frequency at 0.05, 0.02 and 0.01 with Jack-knife Confidence Intervals (Jones, Ovenden, & Wang, 2016).

(v) *Coefficients of relationship, relatedness networks*

The R package `SNPRelate` v1.16.0 (Zheng, 2013) was first used to calculate Identity-By-State (IBS) fractions for each pair of rock-wallabies in each of the Datasets 1-4. Hierarchical cluster analysis using the average link (UPGMA) method was then performed on each of the IBS matrices to produce a genetic distance trees. Kinship was estimated independently in Datasets 2-4, to ensure that later filtering was not biased by sample size, and that appropriate population-specific allele frequencies were used. Dataset 1 contains SNPs that are monomorphic in individual populations and kinship values can be inflated by these fixed alleles. Identity-By-Descent coefficients (calculated by Maximum Likelihood Estimation) were estimated in R using the `SNPRelate` package (Zheng, 2013). Pairwise kinship values were estimated based on these IBD coefficients, also using `SNPRelate`. Kinship values vary from 0 to 0.5 so, for ease of comprehension, coefficients of relationship ( $r$ ), which vary from 0 to 1, were calculated by doubling kinship values (Wright, 1922). Full siblings and parent-offspring pairs are expected to have  $r$  values of 0.5, half siblings 0.25, and first cousins 0.125, and so on. To visualise close  $r$  values

geographically, the program GEPHI (v0.9.2; Bastian, Heymann, & Jacomy, 2009) was used to produce relatedness networks with the plugin GeoLayout. Individuals were treated as nodes and relatedness estimates as edge values, weighted by increasing  $r$ . These visualisations were limited to coefficients of relationship greater than 0.0625 (i.e. 1/16<sup>th</sup>, e.g. first-cousin once removed, half-first-cousin).

(vi) *First-generation migration*

GeneClass2 (Piry et al., 2004) was used to identify presumed first generation ( $f_0$ ) migrants. This was performed using the Rannala and Mountain (1997) methods with Monte-Carlo resampling at both the 0.01 and 0.05 probability threshold and all loci. This approach follows the same methods as Potter et al. (2020). The distance between the trapping (source) colony and the putative origin colony (as well as the site map, Figure 8.1) was also then generated in ArcMap (v10.5.1; Environmental Systems Research Institute, 2019).

## 8.4 Results

(i) *Population structure, statistics, genetic distances, and PCoA*

In Table 8.2, average  $H_O$  and  $H_E$  of 0.153 and 0.152 were measured respectively across the Gawler Ranges sites (Coolgundibie, Organ Pipes, Stone Dam and Yandinga), with the lowest  $H_O$  in the Organ Pipes colony ( $\overline{H_O} = 0.143$ ). This was followed by the Grey Range population ( $\overline{H_O} = 0.197$ ), and finally the Flinders Ranges colony ( $H_O = 0.254$ ).  $F_{IS}$  (calculated as  $F = 1 - H_O/H_E$ ) showed several colonies to have heterozygote excess (negative values) and several to be deficient in heterozygotes. The highest values of  $F_{IS}$  were seen in the Yandinga and Organ Pipes colonies of the Gawler Ranges (0.065 and 0.038, respectively), and Waukawoodna Gap ( $F_{IS} = 0.017$ ) also indicated heterozygote deficiency. Confidence intervals (98%, 1000 bootstraps) indicated that  $F_{IS}$  deviated significantly from the null hypothesis value of zero in all populations except the Alaric Colony ( $F_{IS} = 0.008$ ). A table of  $H_O$ ,  $H_E$ ,  $A_R$  and  $F_{IS}$  for each colony can be seen in Table 8.2. Pairwise  $F_{ST}$  was greatest between the Coolgundibie and Ray colonies ( $F_{ST} = 0.65$ ), and the lowest value was between the geographically nearby Yandinga and Organ Pipes colonies ( $F_{ST} = 0.008$ ; Table 8.3). Average  $F_{ST}$  by population was 0.03 within Grey Range colonies, and 0.04 within Gawler Ranges. Average pairwise  $F_{ST}$  was greatest between the Grey and Gawler Ranges ( $\overline{F_{ST}} = 0.63$ ). Flinders Ranges to Grey Range comparisons

resulted in slightly higher  $F_{ST}$  values ( $\overline{F_{ST}} = 0.47$ ) than Flinders Ranges to Gawler Ranges ( $\overline{F_{ST}} = 0.35$ ). All  $F_{ST}$  values with the exception of Organ pipes-Yandinga were significant ( $p < 0.001$ ; Table 8.3).

**Table 8.2: Table of Effective population sizes ( $N_e$ ),  $H_O$ ,  $H_E$ ,  $A_R$ , and  $F_{IS}$ , for each colony**

Table shows the number of individuals in the population ( $n$ ), effective population sizes ( $N_e$ ) for each colony and the lower and upper jack-knife confidence intervals of that estimate, observed heterozygosity ( $H_O$ ), expected heterozygosity ( $H_E$ ), allelic richness ( $A_R$ ) and the inbreeding coefficient ( $F_{IS}$ ), significant  $F_{IS}$  values (98% confidence, 1000 bootstraps) are denoted by an asterisk (\*).

Colony	$n$	$N_e$	CI (lower)	CI (upper)	$H_O$	$H_E$	$A_R$	$F_{IS}$
Coolgundibie	10	8.4	5.5	35.9	0.161	0.144	1.336	-0.098*
Organ Pipes	11	31.1	28.6	$\infty$	0.143	0.156	1.359	0.065*
Stone Dam	6	4.5	2.0	8.8	0.154	0.147	1.344	-0.035*
Yandinga	19	36.6	27.2	108.6	0.154	0.161	1.371	0.038*
Waukawoodna Gap	17	21.4	13.7	39.5	0.254	0.258	1.596	0.017*
Alaric	18	21.3	18.5	395.2	0.193	0.194	1.448	0.008
Ray	7	5.1	1.9	12.8	0.201	0.184	1.427	-0.086*

The neighbour-joining tree clearly separates colonies by population (Figure 8.2). This was also evident using several other different genetic distance measures, as well as through Principal Coordinates Analysis (Figure 8.3). The first two eigenvalues in the PCoA of Dataset 1 corresponded to 47.1% and 12.0% of the variation between samples.

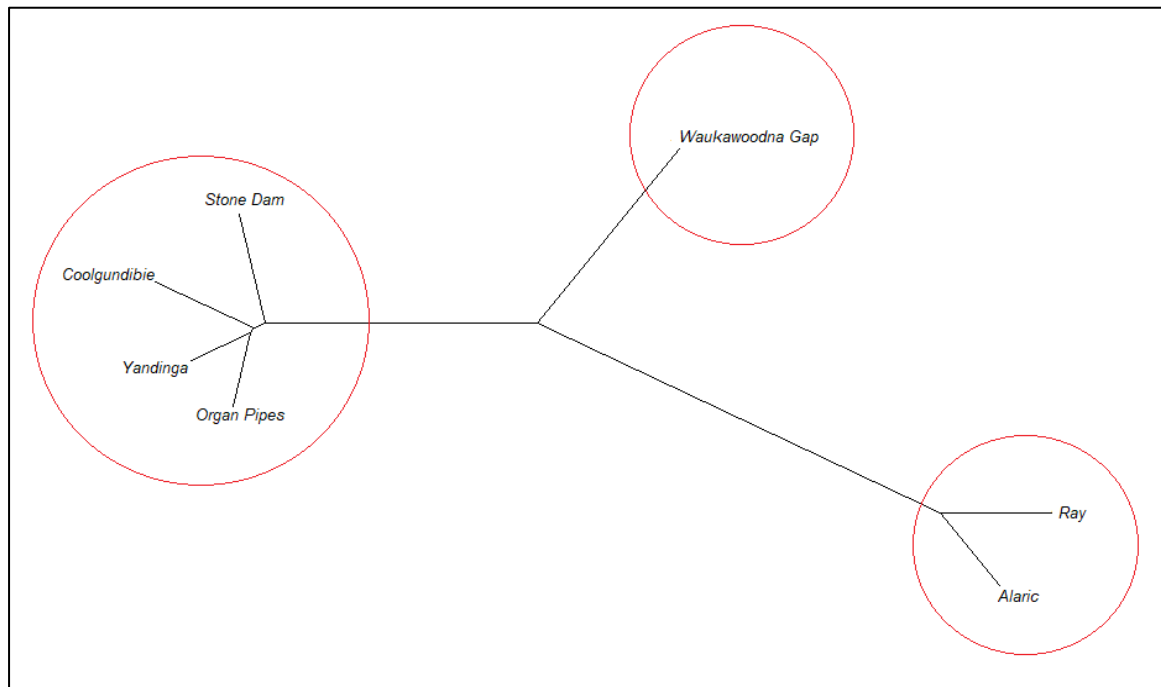
**Table 8.3: Pairwise  $F_{ST}$  and significance (from p values)**

Pairwise  $F_{ST}$  values calculated significance tested based on 1000 bootstrap repeats and 95% CI. Cell colour is scaled (green to red) by increasing  $F_{ST}$  below the diagonal. An asterisk (\*) above the diagonal indicates that the corresponding value is significantly greater than zero

Range	Colony	Grey		Gawler				Flinders
		R	A	OP	Y	SD	C	WG
Grey	Ray		*	*	*	*	*	*
	Alaric	0.0286		*	*	*	*	*
Gawler	Organ Pipes	0.6365	0.6152		-	*	*	*
	Yandinga	0.6334	0.6164	0.0083		*	*	*
	Stone Dam	0.6401	0.6142	0.0310	0.0268		*	*
	Coolgundibie	0.6514	0.6247	0.0401	0.0361	0.0769		*
Flinders	Waukawoodna	0.4643	0.4745	0.3502	0.3587	0.3405	0.3635	

Effective population size ( $N_e$ ) estimates ranged from 4.5 for the Stone Dam colony, to 36.6 for the Yandinga colony in the Gawler Ranges. In the Flinders Ranges, the Waukawoodna

Gap colony had an  $N_e$  of 21.4, and in the Grey Range, the Ray and Alaric colonies had  $N_e$  estimates of 5.1 and 21.3, respectively (Table 8.2).

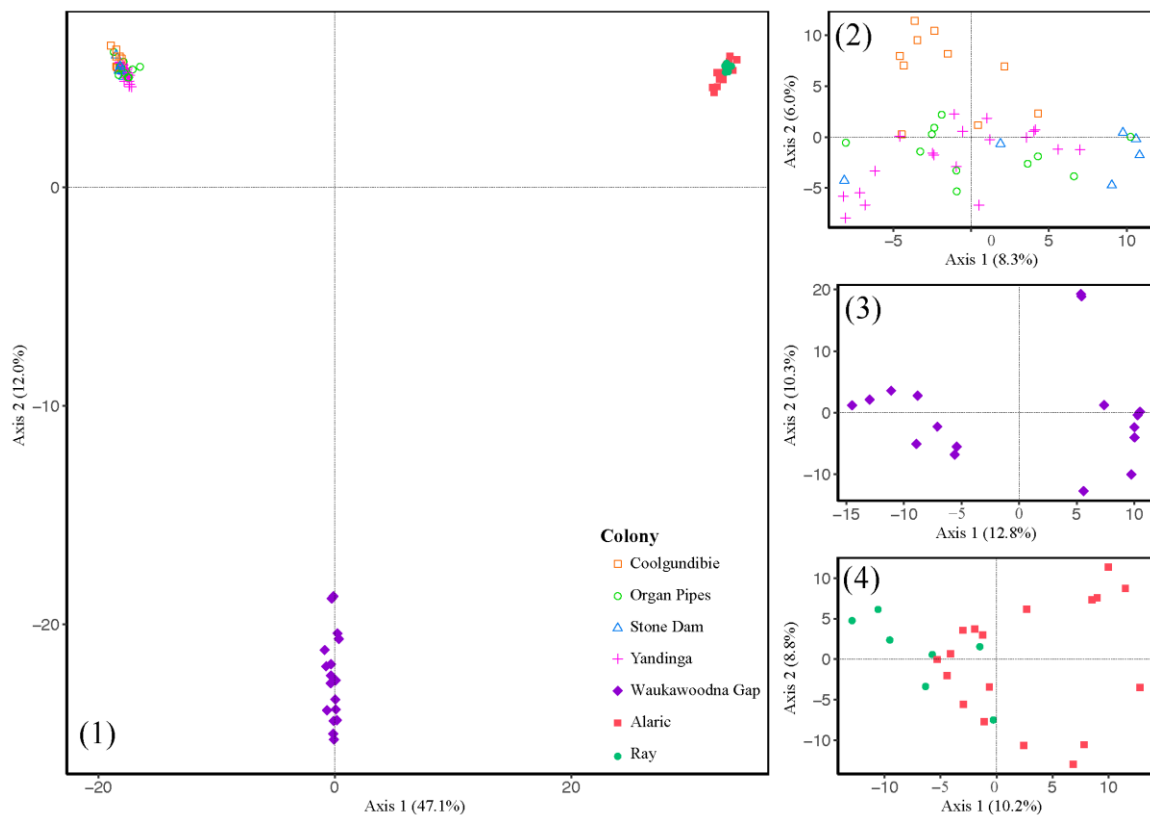


**Figure 8.2: Unrooted neighbour-joining tree of all YFRW populations**

Neighbour joining tree generated from Euclidean genetic distance matrix of YFRW. Circles indicate population groups based on mountain ranges, Gawler Ranges – left, Flinders Ranges – centre, Grey Range – right.

After STRUCTURE analysis, Evanno's  $\Delta K$  method from STRUCTURE HARVESTER indicated that  $K = 2$  was the best supported  $K$  value for Dataset 1; however, the maximum  $L(K)$ , pairwise  $F_{ST}$  values (Table 8.3), neighbour-joining trees (Figure 8.2), genetic distances, PCoA results (Figure 8.3) and other analyses all gave  $K = 3$  greater support. Therefore,

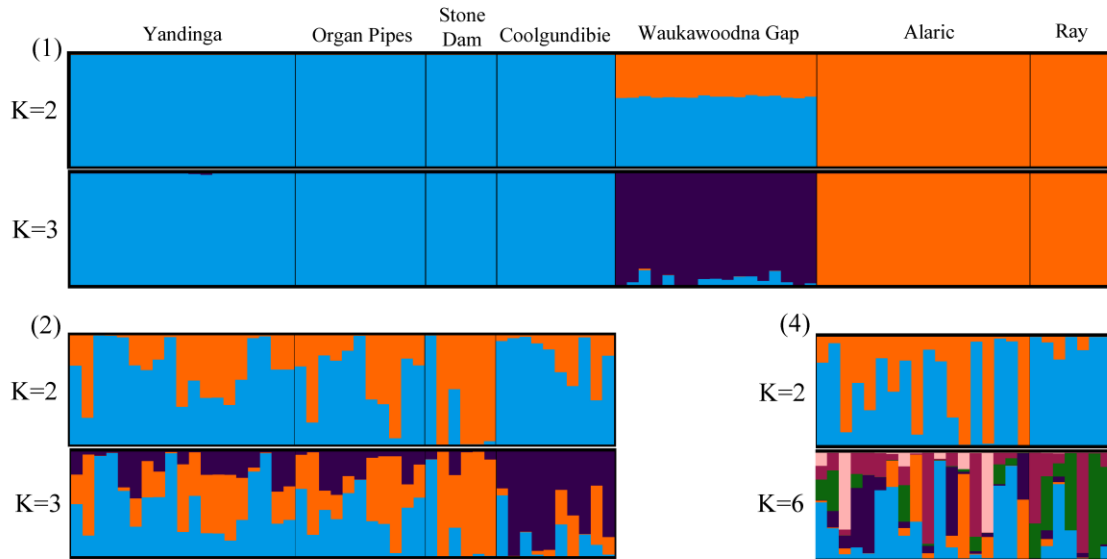
delimitations of  $K = 3$  (Figure 8.4) were used to subset the primary dataset into Datasets 2-4 for further investigation of fine population structure and kinship analysis.



**Figure 8.3: PCoA by Dataset**

Principal Coordinate analysis of each Dataset (1-4), respectively, coloured by colony. X- and Y-axes show the first and second most informative Eigen values, correspondingly, and the percentage contribution (in parentheses) of that value

PCoA analysis of the Gawler Ranges showed some potential structure (Figure 8.3) though the contributions of eigenvalues to differentiation were relatively low. Genetic distance trees showed little clear structure. Bayesian cluster analysis with STRUCTURE showed the greatest support for  $K = 2$  in the Gawler Ranges, but again  $K = 3$  (Figure 8.4), which also had a high  $\Delta K$  value, appeared to be better supported by other analysis. The colonies from the Grey Range (Dataset 4) also showed some limited genetic differentiation from PCoA, distance trees and STRUCTURE analysis ( $K = 2$  and  $K = 6$ , supported from  $\Delta K$  and  $L(K)$ ). These results were also supported by pairwise  $F_{ST}$  values, which were low, but still highly significant ( $p < 0.001$ ; Table 8.3).

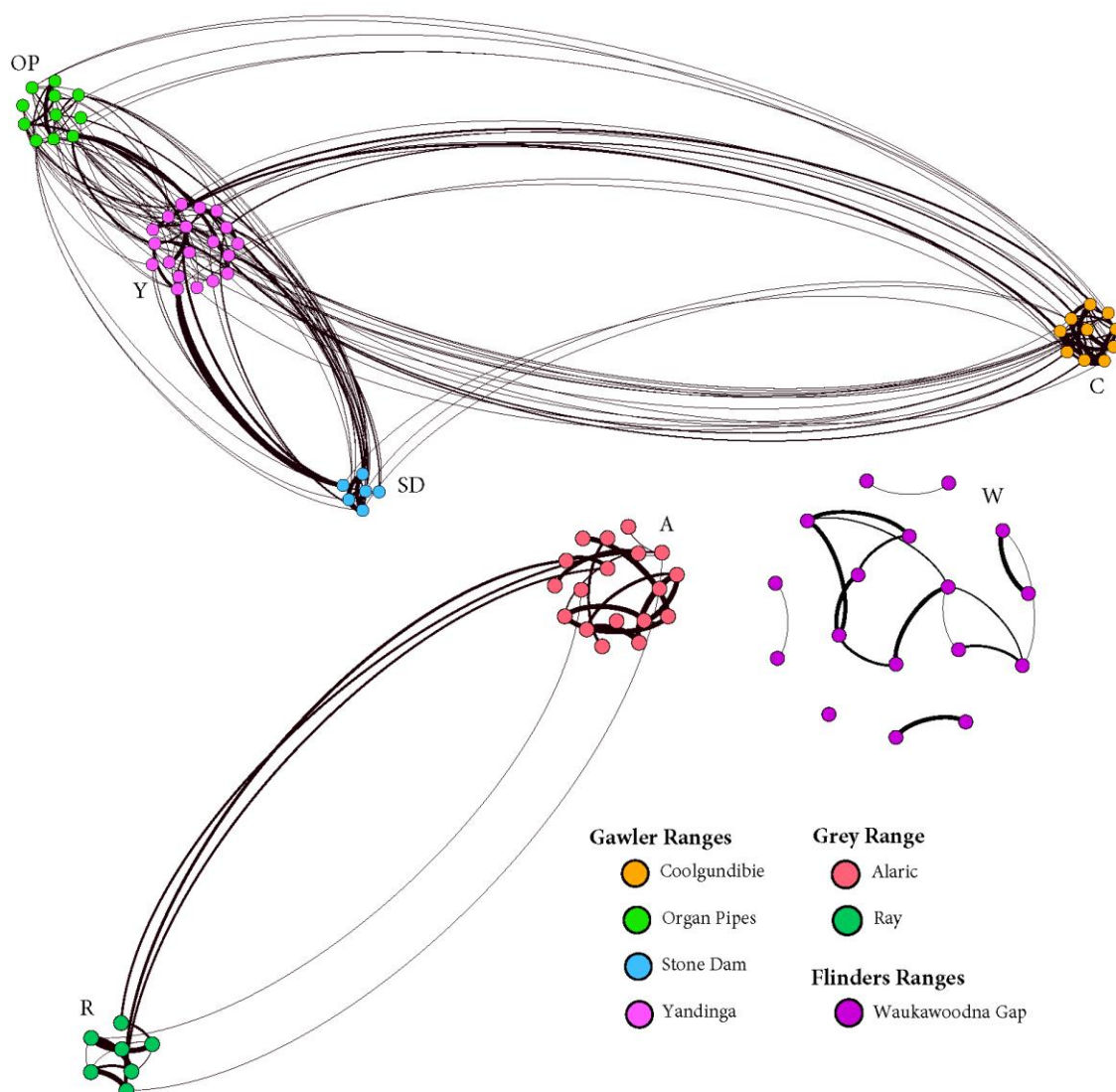


**Figure 8.4: STRUCTURE bar plots for Dataset 1, Dataset 2 and Dataset 4**

(1) Bar plots showing the major clusters for  $K=2$  and  $K=3$  (all 10/10 iterations, i.e. no minor clustering).  $K=2$  was most supported by Evanno's  $\Delta K$  method, though  $K=3$  was better supported by other methods of examining population structure. (2) Bar plots showing  $K=2$  and  $K=3$  for Dataset 2 (Gawler Ranges) and (4)  $K=2$  and  $K=6$  for Dataset 4 (Grey Range) from secondary analysis are also shown. Graphs of  $\Delta K$  and  $L(K)$  over each value of  $K$  and bar plots of all clusters ( $K=1-10$ ) can be found in Appendix VI.

(ii) *Coefficients of relationship, relatedness networks*

Average intra-population relatedness ( $\bar{r}$ ) was greatest in the Gawler Ranges (0.031). The Gawler Ranges populations had 419 relationships greater than  $r = 0$  (out of a potential 1035 pairwise relationships, 40.5%), and 161 relationships greater than the  $r = 0.0625$  threshold (15.5%; Figure 8.5). This was followed by the Grey Range where average  $\bar{r} = 0.027$ , with 67 of the 300 possible relationships greater than  $r = 0$  (22.3%), and 30 greater than the  $r$ -threshold (10.0%). Finally, in the Flinders Ranges, 25 out of a possible 136 relationships were greater than 0 (18.4%), with 15 relationships greater than the threshold (11.0%;  $\bar{r} = 0.027$ ).



**Figure 8.5: YFRW relatedness networks generated from IBD estimates**

Visual representation of relatedness estimates greater than 0.0625 generated through Identity by Descent (IBD) analysis. Coloured circles (nodes) show individuals of each population, relatedness lines (edges) are weighted by the strength of the relationship (not comparable between populations). Grouped node locations correspond to geographic locations (Figure 8.1).

(iii) *First-generation putative migration*

Putative migration here is defined as shared sequence similarities (based on genetic distance, allele frequency and Bayesian criterion; Piry et al., 2004) exhibited geographically, thus suggesting a permanent movement event from one site to another, rather than the more general definition of ecological migration, which relates to season and resource. Out of 71 individuals (46 in the Gawler Ranges and 25 in the Grey Range) 17 were identified as putative  $f_0$  putative migrants (Table 8.4). Of the 15 migrations within the Gawler Ranges, seven (41.1%) were between Organ Pipes and Yandinga. Two YFRW caught at Stone Dam appeared to be immigrants, one from Yandinga and one from Organ



Pipes, and three emigrants of Stone Dam were caught at other colonies (two at Yandinga, one at Organ Pipes). Lastly, there were three occurrences of putative migration to Coolgundibie, from the Yandinga (two) and Organ Pipes (one) colonies. These putative migrations were of ~13.32 and ~15.70 km, respectively. There were no occurrences of putative migrants from Coolgundibie caught at any other trapped site in the Gawler Ranges. Within the Grey Range population two YFRW were identified as potential  $f_0$  putative migrants; both suggest emigration from Alaric to the Ray colony.

**Table 8.4: Putative first generation migrants identified with GeneClass2**

Individuals identified as potential first generation migrants in GeneClass2. Table shows the colony the individual was captured in (Sampling colony) and the likely colony the individual emigrated from. The distance between the source and original colony is listed in kilometres. The log value of the ratio of ‘likelihood of home’ over ‘likelihood of all populations’, the log value of the ‘likelihood of home’, and the log value of the greatest likelihood (corresponding to the Emigrated from colony) were also included. All probabilities of the log-likelihood ratio were equal to 0.

Sampling Colony	Pop.	$-\log \frac{L[home]}{L[max]}$	Distance (km)	Emigrated from
<i>Coolgundibie</i>	Gawler	1.113	13.32	<i>Yandinga</i>
<i>Coolgundibie</i>	Gawler	127.519	15.7	<i>Organ Pipes</i>
<i>Coolgundibie</i>	Gawler	59.84	13.32	<i>Yandinga</i>
<i>Organ Pipes</i>	Gawler	157.855	2.89	<i>Yandinga</i>
<i>Organ Pipes</i>	Gawler	62.546	2.89	<i>Yandinga</i>
<i>Organ Pipes</i>	Gawler	99.705	2.89	<i>Yandinga</i>
<i>Organ Pipes</i>	Gawler	56.835	2.89	<i>Yandinga</i>
<i>Organ Pipes</i>	Gawler	25.571	2.89	<i>Yandinga</i>
<i>Organ Pipes</i>	Gawler	84.131	7.23	<i>Stone Dam</i>
<i>Stone Dam</i>	Gawler	176.771	7.23	<i>Organ Pipes</i>
<i>Stone Dam</i>	Gawler	505.72	4.38	<i>Yandinga</i>
<i>Yandinga</i>	Gawler	27.72	4.38	<i>Stone Dam</i>
<i>Yandinga</i>	Gawler	29.924	2.89	<i>Organ Pipes</i>
<i>Yandinga</i>	Gawler	0.5	2.89	<i>Organ Pipes</i>
<i>Yandinga</i>	Gawler	72.667	4.38	<i>Stone Dam</i>
<i>Ray</i>	Grey	149.602	8.76	<i>Alaric</i>
<i>Ray</i>	Grey	192.074	8.76	<i>Alaric</i>

Figures from reports used for filtering, distance matrices, scree plots of Eigen values of PCoA, output figures of Structure Harvester, bar plots of all values of  $K$  from CLUMPAK, histograms of relationship coefficients, outputs of GeneClass2 and other additional results can be found in the Appendix VI.

## 8.5 Discussion and Summary

### (i) *Key findings*

This study aimed to examine contemporary movement between disjunct colonies of YFRW with varying levels of connectedness in both South Australia and Queensland. We found through the examination of population structure, kinship analysis and maximum likelihood analysis of putative migration that there has likely been recent contemporary movement within metapopulations. Individuals with kinship values greater than 0.0625 were common between colonies within the Gawler Ranges with several movements greater than 13 km, and despite an approximately 5 km gap of unsuitable habitat, related individuals were present from the two sampled locations in the Grey Range. Further to this, multiple putative  $f_0$  migrations were identified within both the Gawler and Grey Ranges. From these results it is clear that YFRW are more mobile within mountain ranges than previously assumed, concurring with recently published results showing several potential  $f_0$  migration events of YFRW in the Flinders Ranges (Potter et al., 2020). Greater mobility of YFRW also fits closer with metapopulation theory, where movement between semi-isolated demes and back into locally extinct or new sites helps maintain the genetic and ecological viability of the broader population (Hanski, 1998). This new understanding is likely to affect future management of the species. Management strategies that allow for and potentially facilitate metapopulation movement are likely required for the effective conservation of the species.

### (ii) *Population structure*

The initial analysis of population structure across all samples (Dataset 1) showed that each mountain range should be considered independent of the others when considering genetic implications within the species. Populations of YFRW were shown to have an order of magnitude greater pairwise  $F_{ST}$  values of colonies between mountain ranges than within (Table 8.3), and all other methods employed for determining genetic differentiation between populations and individuals supported this (Figure 8.2, Figure 8.3, and Appendix VI). Cluster analysis implemented in STRUCTURE indicated that either 2 ( $\Delta K$  method) or 3 (maximum  $L(K)$ ) was best supported. Given the  $\Delta K$  method has the propensity to lead to the over identification of  $K = 2$  (Cullingham et al., 2020; Janes et al., 2017), and the support offered by other metrics,  $K = 3$  was determined to be the best supported  $K$  value (Figure 8.4). These results are in agreement with the literature on YFRW genetics and phylogeny

which splits the South Australian and Queensland species into subspecies, and shows that separate populations are genetically distinct (Eldridge, 1997; Pope, Sharp, & Moritz, 1996; Pope, Sharp, & Moritz, 1998; Potter, Cooper, et al., 2012; Potter et al., 2020). From the initial structure analysis there was also evidence of some within-population structure, leading us to examine fine-scale structure in data subsets based on the populations. Fine scale structure are also supported by the recent analysis of microsatellite loci, which was which showed some structure within colonies within the Flinders Ranges (Potter et al., 2020). An analysis of each of the subsets showed some genetic structure between the colonies of each population. The Stone Dam and Coolgundibie colonies in the Gawler Ranges are perceptibly separated by the first and second axis of PCoA (Figure 8.3) and for  $K = 3$  of structure analysis (Figure 8.4). The same was true of the Grey Range population, which showed some population structure (Figure 8.4), and differentiated on the primary axis after PCoA (Figure 8.3).

(iii) *Genetic diversity*

Greater genetic diversity in populations is closely linked to a population's ability to withstand genetic pressures associated with small population size and to adapt to changing environmental conditions (Frankham, 2005). Our results in YFRW showed that the smaller, less geographically connected, populations in the Gawler and Grey Ranges have lower genetic diversity ( $H_O$  and  $A_R$ ) than those of the Flinders Ranges (Waukawoodna site; Table 8.2). This outcome supports assumptions that genetic diversity should be positively correlated with population size (Frankham, 1996). Significance testing of  $F_{IS}$  also revealed all colonies, with the exception of Alaric (Grey Range), significantly deviate from zero, the null value, indicating either heterozygote deficiency (Yandinga, Organ Pipes, Waukawoodna) or heterozygote excess (Ray, Coolgundibie and Stone Dam). While the theory behind inbreeding leading to heterozygote deficiency is well established (Buri, 1956), the processes leading to heterozygote excess are less clear. Though there are several potential explanations (referenced and discussed in Stevens, Salomon, & Sun, 2007), this case is likely due to the small population sizes. Binomial sampling error can cause differences in allele frequencies of male and female breeders, leading to heterozygote excess in their progeny (Luikart & Cornuet, 1999; Robertson, 1965; Waples, 2015). This explanation of heterozygote excess in these YFRW colonies is supported by the estimates of effective population size ( $N_e$ ), which are substantially smaller in the corresponding colonies (Table 8.2).

(iv) *Inter-colony relationships and movement*

The first step employed here to examine how YFRW move between colonies was assessing pairwise relatedness ( $r$ ) between all individuals of a population. The analysis revealed relationships greater than the 0.065 threshold between all colonies within each population (excluding the Flinders Ranges), and one relationship of  $\sim 0.5$  (indicating a full-sibling or parent-progeny relationship) between two colonies in the Gawler Ranges. Relationships of greater than  $1/16^{\text{th}}$  imply a common ancestor within 4 generations. These relationships also indicate that there may be greater mobility between populations than previously recognised based on early genetic assessments (Pope, Sharp, & Moritz, 1996).

While these results indicate recent movement between the colonies, inter-colony  $r$  values that are further apart than full-sibling or parent-offspring (less than  $\sim 0.5$ ) may be a result of stepwise movement events through intermediate and unsampled colonies. To identify the likely colonies-of-origin, putative  $f_0$  migrants were identified in GeneClass2. The program identified 15 potential first generation migrants in the Gawler Ranges, and two in the Grey Range (Table 8.4). As might have been postulated from the analysis of population structure, movement between Organ Pipes and Yandinga constituted a large percentage of putative migrants. These colonies are separated by only 2.9 km of suitable habitat (Figure 8.1). As YFRW are known to move up to 1.5 km to water points (Sharp, 2011), this regular exchange of individuals was expected. Less predictable were the putative migrants into and from Stone Dam, and those from the Yandinga/Organ Pipes area, found at Coolgundibie. Interestingly, no  $f_0$  migrants were identified as having dispersed in the reverse direction, i.e. Coolgundibie to any other colony in the Gawler Ranges. Despite this exception, within the Gawler Ranges YFRW appear to move readily to areas of connected habitat, even over relatively large distances.

Within the Grey Range population, only two migrants were detected, with both indicating the individual moved from Alaric to Ray. These movements are of particular relevance, both to the understanding of species population dynamics, and to future management of the species in Queensland. The area of the Grey Range that these two colonies reside in is at the very southern end of the YFRW's Queensland distribution. The Alaric colony is located on the Canaway Fault, a line of connected YFRW habitat/cliffs that run the north-south. The Ray colony, however, is approximately 8 km distant on fragmented rocky outcrops

known as The Matrix. As previously mentioned, the shortest distance between suitable YFRW of habitat of the Canaway Fault and The Matrix is ~5 km of open, flat, farmland. Moreover, this dispersal route is now impeded by an exclusion fence (Figure 8.1).

(v) *Management implications*

Previous assessments of the Gawler Ranges had not found colonies of YFRW at Coolgundibie (Lethbridge, 2004a) or Stone Dam (Lethbridge, Andrews, & Harper, 2012; Lethbridge, Harper, & Strauss, 2010). These colonies likely represent recolonization events under threat abatement. These recolonisations and the level of movement reported here likely reflect the result of 25 years of goat and fox control (and some kangaroo management) and a major drought in the Gawler Ranges (Lethbridge et al., 2019). Integrated pest management (of feral rabbits *Oryctolagus cuniculus*, foxes, goats, cats *Felis catus* and weeds) may have driven this pulse of movement as a reduced number of predators, and also a shortage of resources in core YFRW colonies, induced density-dependant dispersal behaviours. Continued recovery of YFRW under these conditions therefore might depend on the threat abatement being widespread enough to facilitate further recolonisation events at sites that, without abatement, would be unsuitable.

For YFRW in the Grey Range, the rapid expansion of pest-exclusion fencing in the area (Smith, Waddell, & Allen, 2020; Chapter 3) potentially divides colonies that have previously relied on movement to maintain greater levels of genetic diversity, such as that of the Flinders Ranges (Table 8.2). In all cases, identified  $f_0$  migrants (Table 8.4) were also those with inter-colony ties in the relatedness network (Figure 8.5). The fence between the two colonies was completed in 2016, so either the rock-wallabies were able to pass across the exclusion fence, or the YFRW relocated colonies prior to the exclusion fences construction. The fences have proved highly effective at preventing the movement of other species (RAPAD & QFPI, 2018a), and aging the potential migrants based on tail-length data (Lethbridge, 2004b) showed that dispersal pre-2016 was possible. It would be reasonable to assume that the migrants moved colonies pre-fence, and that the population in Queensland is now fragmented by exclusion fencing (Smith, Waddell, & Allen, 2020; Chapter 3). Indications that the rapid reduction in YFRW distribution over the past century has already had negative genetic impacts on YFRW population viability make this assessment all-the-more troubling (Potter et al., 2020). This is particularly true of colonies, such as the Ray Colony, that were already not part of connected and continuous YFRW

habitat. To allay the potential negative impacts, the genetic health of isolated populations would have to be monitored into the future and mitigation strategies, such as meta-population management, be employed if/when the populations appear to be under genetic stress. Meta-population management has been successfully employed for the maintenance of genetic health issues in other mammal species caused by fencing in the past (Boast et al., 2018; Miller et al., 2015; Schroeder, 2019).

(vi) *Limitations*

While most  $F_{IS}$  values deviated significantly, they may not reflect the true value in the populations. Estimation of both  $F_{IS}$  and  $N_e$  assumes random mating and random sampling in the population; both assumptions may have been violated here. For YFRW there is evidence of social structure, dominance behaviours and philopatry (Lapidge, 2001; Potter et al., 2020; Sharp, 2002) and territorial defence (or more specifically, trap bait defence) which may result in non-random sampling. Additionally, clear delimitations between sampling populations is needed to avoid Wahlund effects (De Meeûs, 2018; Wahlund, 1928; Waples, 2015). As it appears colonies have an exchange of individuals (Figure 8.5), colonies likely represent demes of a metapopulation, and this may have altered  $F_{IS}$  estimates of colonies.

(vii) *Future research*

It is clear from the results above that for the effective conservation and recovery of YFRW, the management of the species needs to be considered at a broader scale. The long term conservation of the species relies on the ability of individuals to immigrate into neighbouring colonies for the maintenance of genetic health (Potter et al., 2020). Further to this, the recovery of the species relies on an increase in the species distribution as potentially suitable habitats become available as a result of broad scale management of threatening processes (Lethbridge, Andrews, & Harper, 2012; Lethbridge et al., 2019). Future research should seek to elucidate whether recolonised sites continue to be genetically supplemented from other colonies in the metapopulation. Additionally, as new sites arise, genetic assignment of colonising YFRW should be made a priority to establish source populations. More specifically for *P. x. celeris*, continuing genetic monitoring should be implemented alongside long term studies of behaviour, both to ensure genetic viability of the population and as a case study of the long term effects of anthropogenic barriers on species genetics and behaviour (Smith, King, & Allen, 2020; Chapter 2).

## **8.6 Author Contributions**

DS and MRL collected tissues samples from Queensland and South Australia, respectively. The methods and study design was decided on by DS and RLA as part of a broader project conceptualised by DS and BLA. DS performed all analysis. The main body of the writing was completed by DS. All authors contributed to the editing and review of the manuscript pre-submission.

## **8.7 Acknowledgements**

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## **Chapter 9 : Summary and Conclusions**

### **9.1 Key findings**

This thesis aimed to better understand the effects of exclusion fencing on non-target wildlife. An assessment of fencing literature from around the world identified several potential benefits and costs of exclusion fencing to wildlife (Chapter 2). Using the effects recorded from this literature as a guide, the potential costs and benefits of exclusion fencing were explored in the context of cluster fencing in central western Queensland. The potential benefits of exclusion fencing are; (1) separation of a disease vector from other target species, (2) the exclusion of pests and (3) predators, and (4) the redirection of species to safe passages (fence gaps and gates). In the cluster fencing context, only (2) pest and (3) predator exclusion were considered to be relevant benefits given that the fences do not aim to separate diseased species, or redirect wildlife. To explore these two relevant potential benefits, we assessed the overlap of cluster fenced properties with threatened species distributions and highlighted situations where threatening factors to these species may be wholly or partially mitigated by management practices with the clusters (Chapter 3). From this study it became apparent that the yellow-footed rock-wallaby may potentially benefit from cluster fencing given that they face a number of threats that are mitigated within the clusters and their preferred habitat is well represented within the current cluster fenced estate. As such the YFRW was selected as the study species to further assess the potential costs of exclusion fencing (Chapter 4) and a site was chosen where YFRW were present on both sides of a cluster fence (The Quilpie Cluster; Chapter 5).

The potential costs of exclusion fencing to mammals identified through the literature review were; (1) behavioural changes, (2) a barrier to movement or (3) gene flow, (4) entanglement, (5) overpopulation within the fence, (6) evolutionary restriction and (7) a barrier to migration. To assess (1) behavioural changes, YFRW habitat use was examined both within and external to the cluster fence. Though no fence effects were detected, several key factors influencing YFRW habitat use were identified, namely habitat complexity (Chapter 6). Behavioural changes were also assessed through the use of camera trap data. Observed behaviours likewise did not vary across inside and outside areas, but there was a demonstrable difference in YFRW diel activity that we could not reliably attribute to pest animal management practices within the cluster. As with habitat use, more robust



assessments of the predator and competitor densities at the site would be needed to accurately attribute the effect of fencing and management practices on YFRW behaviour. That said, camera trap data did show a potential shift in feral goat diel activity that may be more reliably attributed to a change in predator densities (Chapter 7).

To assess whether exclusion fences were a (2) barrier to movement, a genetic analysis of YFRW was performed, using samples from both the South Australian subspecies and from the study site in Queensland (Chapter 8). This assessment revealed regular movement of YFRW between colonies, in concordance with earlier suggestions the YFRW and other *Petrogale* spp. conform to a classic metapopulation structure (Kinnear et al., 2010; Lethbridge, 2004a; Murray et al., 2008). Metapopulations rely on movement between disjunct populations for the health of the greater population. As “barrier to movement” was defined as an inability for a species to move freely between areas of suitable habitat for the maintenance of natural processes (See Chapter 2, page 20), the restriction of YFRW movement between colonies within a metapopulation supports the view that cluster fences inhibit YFRW in this way. This assessment of YFRW genetics also allowed an examination of cluster fences as (3) a barrier to gene flow. While restriction of movement implies a restriction of gene flow it does not necessarily translate to negative outcomes. If the separate populations have acceptable genetic diversity and a sufficient effective population size then the genetic health of the populations might not suffer (Frankham, 1996; Méndez et al., 2014). However, parameters of genetic health from the Quilpie Cluster indicates low genetic diversity, a positive  $F_{IS}$  (the genetic signature of inbreeding) and low effective population size ( $N_e$ ) at the colony within the cluster fence. While there are several limitations to the inferences that can be drawn from these results (see Chapter 8 - Discussion), they do suggest a restriction of gene flow caused by cluster fencing may negatively impact YFRW in the future.

Though (5) entanglement as a cost of cluster fencing to YFRW was not carefully assessed in this thesis, approximately 80 km of the fence (through the most likely YFRW habitat – the western fence; Figure 5.1) was driven regularly during the collection of other data. Species observed entangled (deceased) in the fence were red kangaroos, emus, a goanna, an echidna and feral goats (always horned bucks). The fences at the study site are also checked fortnightly for breaches, which are mended. Discussions with the contracted “fence-checker” also infer that YFRW have not yet been entangled (Pers. comm. with Grant

Barkle – Ray Station 2019), though the quantity of other macropod carcasses (in this case red kangaroos) suggests that entanglement of YFRW remains a real possibility. Further systematic work is needed to better support these opportunistic observations.

The last three identified costs of exclusion fencing (5) overpopulation, (6) evolutionary restriction and (7) barrier to migration were not specifically assessed in this thesis, though they are not irrelevant in the context of cluster fencing effects on non-target wildlife, or even YFRW. Overpopulation was defined as the increase in density of a species within a fenced-off area until resource availability declines to a level that negatively impacts a species (Chapter 2, page 21). Dispersion of YFRW in response to resource depletion has been theorised to occur in *P. x. xanthopus* populations (Lethbridge et al., 2019) and if management practices within cluster results in increased YFRW abundance (see Chapter 3), then overpopulation may one day occur in the cluster unless mitigation strategies are utilised. From a YFRW conservation perspective though, this may not be such a bad thing. Properly assessing both (5) overpopulation and (6) evolutionary restriction (as with behavioural changes, see Chapter 6) would likely require both a longer term study, and also more time to have passed since the construction of the fence to accurately measure whether YFRW are affected or not. Lastly, as YFRW and most terrestrial Australian species do not perform seasonal migrations (Department of the Environment, 2019b), (7) barrier to migration was not assessed. While not relevant to YFRW specifically, this impact should not be completely discounted in the context of cluster fencing because several native species that do occupy cluster fences perform long distance movements which could result in mass mortality (see Chapter 2, page 26).

## **9.2 Recommendations and management implications**

The empirical studies presented in this thesis had several short-comings in common that should be prioritised for future similar research. Primarily, baseline data should be collected prior to the construction of fences in order to properly assess changes over time; however, this may not always be possible. As with this study, the timeline in the cluster fencing context is unlikely to allow an assessment of the site for an acceptable period pre-treatment (prior to fence construction). Historical DNA samples from the site prior to the erection of cluster fencing would have also been particularly useful for comparison. Additionally, ancillary data on ‘treatment effects’ (such as predator or competitor reductions) that quantify the influence of wildlife management practices within the fence should be

collected. In this case, my YFRW-focussed camera trap data was not well suited to demonstrate that management practices had successfully reduced the density of dingoes within the Quilpie Cluster. Passive tracking indices, collected through sand-plotting has been shown to be a useful tool for dingoes in the past (Allen et al., 2013). To this end, understanding predator-prey dynamics within and outside of the enclosure fences should remain a key priority for cluster fence research. Finally, this research took place over a two year period of drought and the lack of behavioural responses identified in Chapter 6 and Chapter 7 may be an artefact of the study length, climatic conditions, or both. Future research should be implemented over longer timeframes to account for effects that take longer to manifest and/or effects that may depend on resource availability (such as habitat use – Chapter 6) across the spectrum of wet and dry conditions. Future research should take into account these variables in order to draw the best possible inference from the results. Despite these limitations, this thesis did identify both costs and benefits of cluster fencing to non-target species that need further exploration in both in YFRW and other species in the system – a few key examples would be the effect of fencing on large distance movements made by red kangaroos and emus, and a robust assessment of wildlife entanglement in the fences.

My results raise several areas of future research that should be explored. Most notably, Chapter 3 shows that there is a potential for agricultural exclusion fences to be utilised for conservation goals in Australia. More research into the requirements of threatened species whose distributions overlap with exclusion fenced zones, and the potential for reintroductions or threatened species recovery in these zones, should be prioritised. This thesis also highlighted that a single species may experience both positive and negative outcomes. For example, in this case YFRW may benefit from exclusion fences given they are threatened with several processes that are being actively managed within clusters; however, YFRW also face potential behavioural changes, disruptions to their natural movement between colonies and potential restrictions to gene flow. Future research should also aim to develop a management decision chart for identifying potential non-target species impacts and when mitigation strategies should be implemented (an example chart is given in Figure 9.1). A framework for appropriately weighting effects should also be considered so that net outcomes can be properly judged as beneficial or costly to the species. Realistically, there will likely always be multiple species experiencing multiple fence effects, both positive and negative. While no mitigation strategies are capable of

relieving all potential issues for all species, following a decision framework brings potential issues to light and allows potential benefits to be harnessed while mitigating the costs. Confirmation of extant species prior to fence construction would assist this process. From such surveys species with behaviours, life histories or distributions that could be affected by fencing should be noted. Pre-construction mitigation strategies such as fence design and/or placement could then be employed. If these strategies are not applicable to the species or situation, post-construction mitigation strategies such as species specific gaps and gates, or translocations should be considered. In all cases, continued monitoring of potentially affected species should be considered (Figure 9.1).

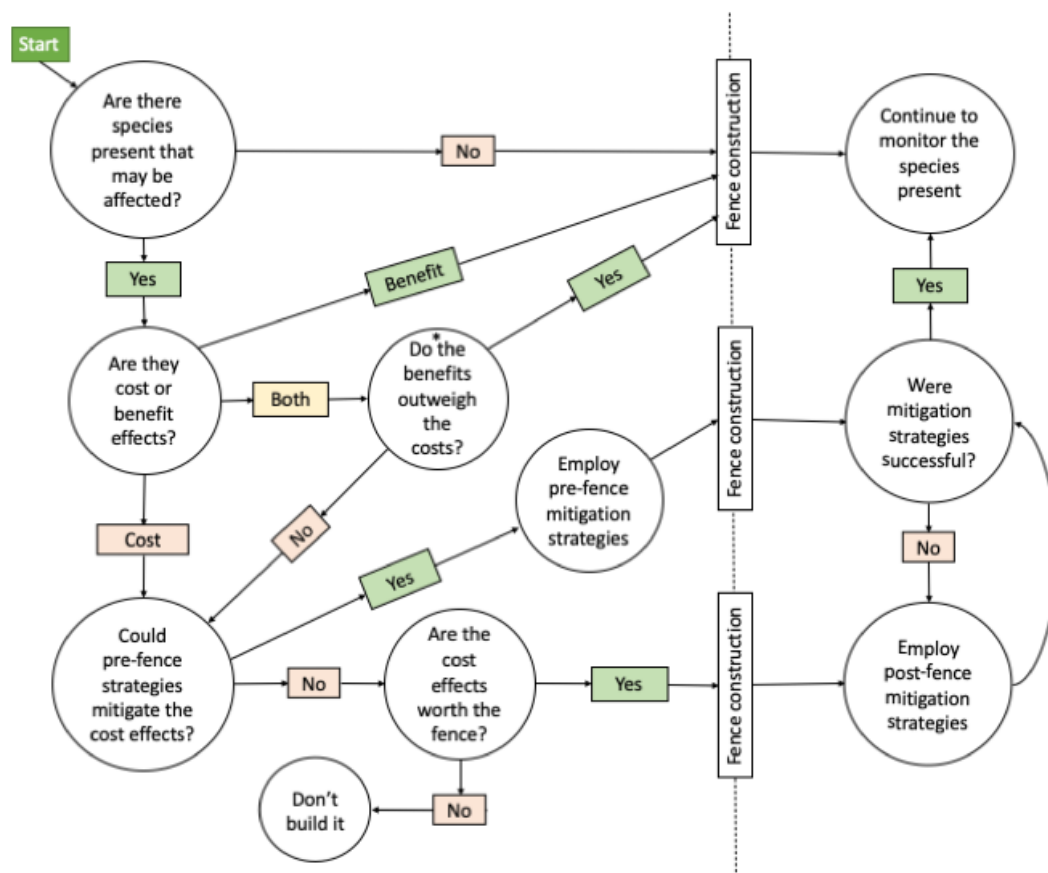


Figure 9.1: Decision chart for mitigation of exclusion fence effects

In the context of YFRW, ensuring that the identified costs (barrier to movement and gene flow) do not outweigh the potential benefits (predator and competitor exclusion) should be prioritised and if necessary mitigation strategies explored. Innovative solutions to adverse fence effects have been devised in the past (Chapter 2). A breach in the DBF was observed being exploited by YFRW (Pers. comm., Grant Barkle – Ray Station 2019) which may indicate a willingness to move under the fence. As such, the implementation of one-way

gates that allow movement out of the cluster fences could be one possible consideration. Translocations and metapopulation management across fence lines might also be considered, particularly into smaller colonies that may suffer genetically. Finally, managers should make a concerted effort to maximise the potential for cluster fences to be utilised as pseudo-conservation reserves, by effectively managing predators and TGP on their properties. Maintaining robust populations of threatened species on-farm could assist livestock producers not only by improving land condition and other ecosystem services (e.g. Palmer et al., 2020), but also through improved environmental credential marketing opportunities for livestock products. My hope is that this thesis may go some way towards achieving positive outcomes for threatened fauna and the livestock properties they inhabit.

## Chapter 10 : References

**Qualifier:** This is a comprehensive list of all citations within this thesis (for the list of references analysed in Chapter 2, please see Appendix I).

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## Appendices

This chapter compiles the Supplementary Information of each data chapter (Appendices I-VI) and an ancillary research note (Appendices VII). The Supplementary Information presented here may vary from Supplementary Material provided alongside the corresponding published manuscript, though the underlying data and analysis remains the same.

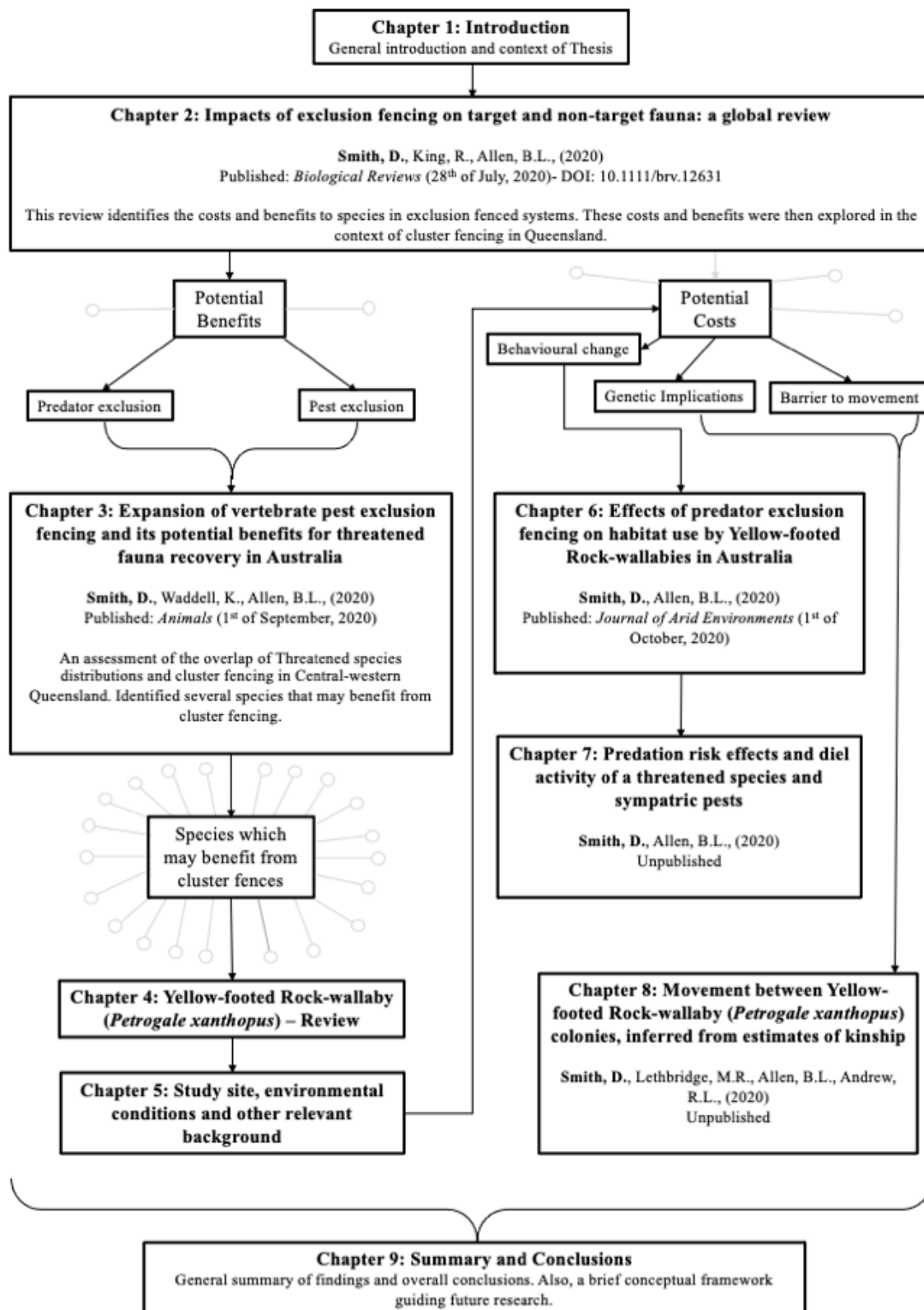
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### Keywords (alphabetically listed)

Activity	Exclusion fence
Behaviour	Feral goat
Camera trap	Fence effects
<i>Capra hircus</i>	Macropod
Cluster fence	Marsupial
Crepuscular	Metapopulation
Diel activity patterns	<i>Petrogale xanthopus</i>
DNA	Semi-arid zone
Dingo barrier fence	Threatened species
Dispersal	Yellow-footed rock-wallaby

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## Appendix I : Supplementary Information (Chapter 2)

**Table S1: List of references (alphabetical order) included in analysis of global exclusion fencing**

The references located and read to form the analysis of global exclusion fencing effects presented in Chapter 2. These references were not included in the reference list of the chapter unless referenced in the main body of the text.

#	FULL REFERENCE
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205	WILSON, R., FARLEY, S., MCDONOUGH, T., TALBOT, S. & BARBOZA, P. (2015). A genetic discontinuity in moose ( <i>Alces alces</i> ) in Alaska corresponds with fenced transportation infrastructure. <i>Conservation Genetics</i> <b>16</b> (4), 791–800.
206	WOLTZ, H., GIBBS, J. & DUCEY, P. (2008). Road crossing structures for amphibians and reptiles: Informing design through behavioral analysis. <i>Biological Conservation</i> <b>141</b> (11), 2745–2750.
207	YIU, S., KEITH, M., KARCZMARSKI, L. & PARRINI, F. (2015). Early post-release movement of reintroduced lions ( <i>Panthera leo</i> ) in Dinokeng Game Reserve, Gauteng, South Africa. <i>European Journal of Wildlife Research</i> <b>61</b> (6), 861–870.
208	YOUNG, L., VANDERWERF, E., LOHR, M., MILLER, C., TITMUS, A., PETERS, D. & WILSON, L. (2013). Multi-species predator eradication within a predator-proof fence at Ka'ena Point, Hawai'i. <i>Biological Invasions</i> <b>15</b> (12), 2627–2638.

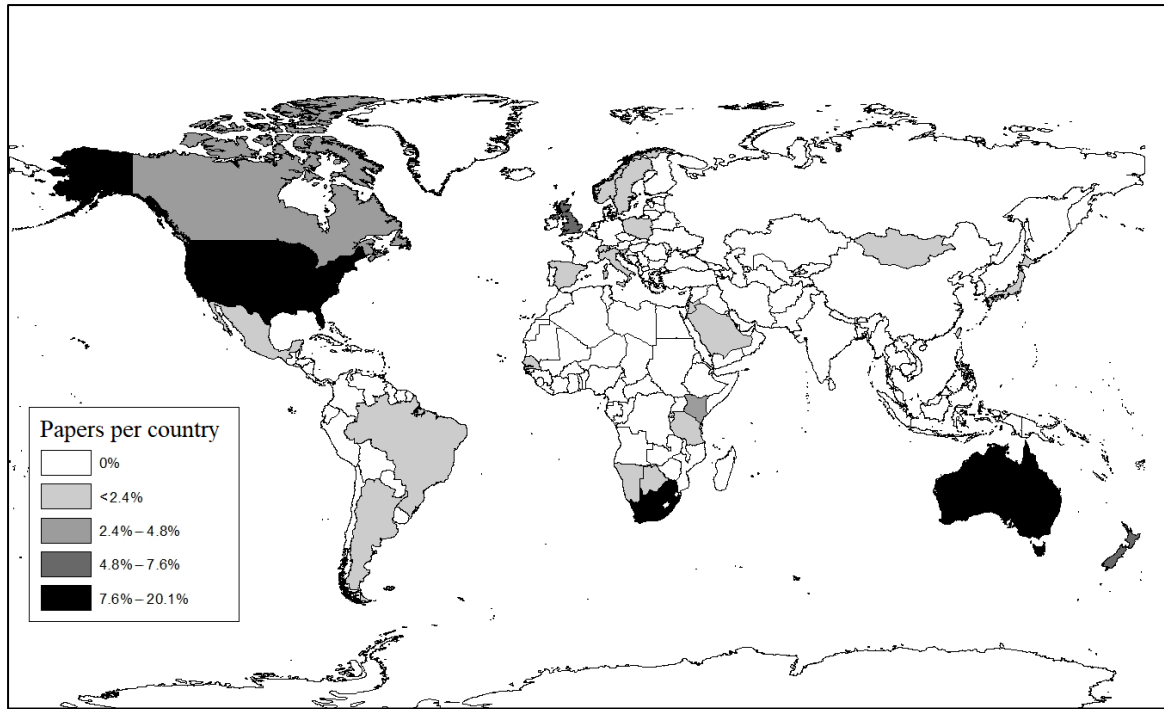
**Table S2: Count of publications each species appears in**

List of species mentioned in literature analysed to assess global fence effects. When high numbers of species or individual species were not listed, a grouping term was recorded (e.g. Birds, *Aves* spp.). Count column records the number of publications that species or group was recorded in.

#	Common name	Scientific name	Count
1	Aardvark	<i>Orycteropus afer</i>	1
2	African elephant	<i>Loxodonta</i> spp.	19
3	Albatross	<i>Phoebastria immutabilis</i>	1
4	Angulate tortoise	<i>Chersina angulata</i>	1
5	Arabian oryx	<i>Oryx leucoryx</i>	2
6	Asiatic black bear	<i>Ursus thibetanus</i>	2
7	Baboon	<i>Papio ursinus</i>	1
8	Badger	<i>Meles meles</i>	10
9	Bandicoot	<i>Perameles</i> spp.	2
10	Bearded dragon	<i>Pogona barbata</i>	1
11	Bellbird	<i>Anthornis melanura</i>	1
12	Bighorn sheep	<i>Ovis canadensis</i>	2
13	Bird	<i>Aves</i> spp.	6
14	Bison	<i>Bison bison</i>	1
15	Black bear	<i>Ursus americanus</i>	3
16	Black grouse	<i>Tetrao tetrix</i>	3
17	Black rhino	<i>Diceros bicornis</i>	4
18	Black-footed ferret	<i>Mustela nigripes</i>	1
19	Blanding's turtle	<i>Emydoidea blandingii</i>	1
20	Blue tongued skink	<i>Tiliqua scincoides</i>	1
21	Bobcat	<i>Lynx rufus</i>	1
22	Bongo	<i>Tragelaphus eurycerus isaaci</i>	1
23	Bridled naitail wallaby	<i>Onychogalea fraenata</i>	1
24	Brown hyena	<i>Hyaena brunnea</i>	2
25	Brown snake	<i>Pseudonaja textilis</i>	1
26	Brush-tail possum	<i>Trichosurus vulpecula</i>	6
27	Buffalo	<i>Syncerus caffer</i>	8
28	Burrowing bettong	<i>Bettongia lesueur</i>	4
29	Burrowing owl	<i>Speotyto cunicularia</i>	1
30	Cane toad	<i>Bufo marinus</i>	1
31	Canvasback duck	<i>Aythya valisineria</i>	1
32	Cape fox	<i>Vulpes chama</i>	1
33	Capercaillie	<i>Tetrao urogallus</i>	2
34	Caracal	<i>Caracal caracal</i>	1
35	Cat	<i>Felis catus</i>	13
36	Cattle	<i>Bos</i> spp.	15
37	Cheetah	<i>Acinonyx jubatus</i>	6
38	Cottontail	<i>Silvilagus audubonii</i>	1
39	Coyote	<i>Canis latrans</i>	10
40	Cunningham's skink	<i>Egernia cunninghami</i>	1
41	Diamondback terrapin	<i>Malaclemys terrapin</i>	1
42	Dingo	<i>Canis familiaris</i>	9
43	Dorcas gazelle	<i>Gazella dorcas neglecta</i>	1
44	Duiker	<i>Sylvicapra</i> spp. & <i>Cephalophus</i> spp.	1
45	Eastern bettong	<i>Bettongia gaimardi</i>	1
46	Eastern box turtle	<i>Terrapene carolina</i>	1
47	Echidna	<i>Tachyglossus aculeatus</i>	1
48	Eider	<i>Somateria</i> spp.	1
49	Eland	<i>Taurotragus oryx</i>	2
50	Elk	<i>Cervus elaphus</i>	8
51	Emu	<i>Dromaius novaehollandiae</i>	3
52	Euro	<i>Osphranter robustus</i>	1
53	Feral pig	<i>Sus scrofa</i>	8
54	Feral dog	<i>Canis familiaris</i>	1
55	Ferret	<i>Mustela furo</i>	1
56	Gadwall	<i>Anas strepera</i>	1
57	Genet	<i>Genetta</i> spp.	1
58	Giraffe	<i>Giraffa camelopardus</i>	2

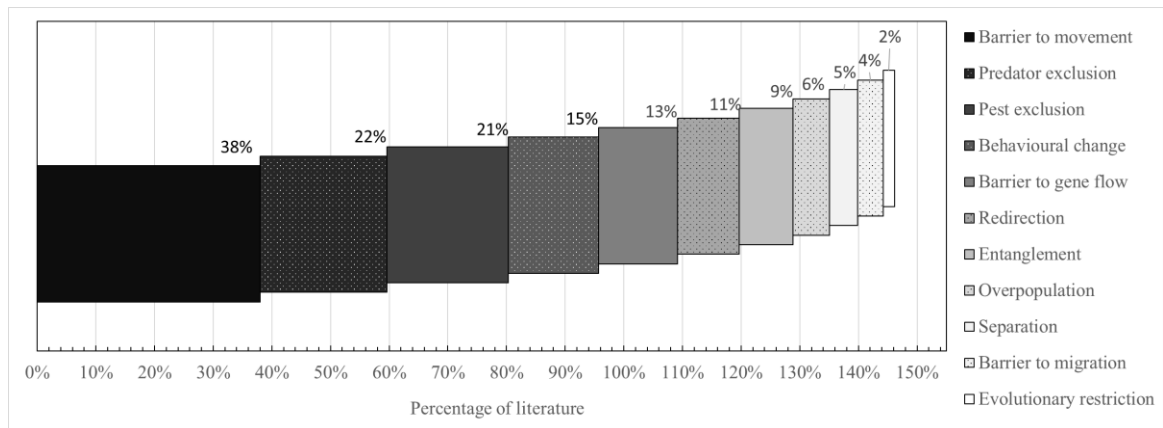
#	Common name	Scientific name	Count
59	Glaucous winged gull	<i>Larus glaucescens</i>	1
60	Golden bandicoot	<i>Isoodon auratus</i>	1
61	Grand skink	<i>Oligosoma grande</i>	1
62	Greater bilby	<i>Macrotis lagotis</i>	3
63	Greater stick-nest rat	<i>Leporillus conditor</i>	2
64	Green frog	<i>Rana clamitans</i>	1
65	Grey fox	<i>Urocyon cinereoargenteus</i>	1
66	Grey heron	<i>Ardea cinerea</i>	1
67	Grey kangaroo	<i>Macropus giganteus</i>	3
68	Grouse	<i>Tympanuchus, Centrocercus &amp; Lagopus spp.</i>	2
69	Guanaco	<i>Lama guanicoe</i>	1
70	Hamilton's frog	<i>Leiopelma hamiltoni</i>	1
71	Hare	<i>Lepus europaeus</i>	1
72	Hartebeest	<i>Alcelaphus buselaphus</i>	1
73	Hedgehog	<i>Erinaceus europaeus</i>	1
74	Hihi	<i>Notiomystis cincta</i>	3
75	Hong kong whipping frog	<i>Polypedates megacephalus</i>	1
76	Houbara bustard	<i>Chlamydotis undulata</i>	1
77	House mouse	<i>Mus musculus</i>	1
78	Impala	<i>Aepyceros melampus</i>	4
79	Jackal	<i>Canis mesomelas &amp; C. aureus</i>	1
80	Jaguar	<i>Panthera onca</i>	1
81	Japanese hare	<i>Lepus brachyurus</i>	1
82	Japanese macaque	<i>Macaca fuscata</i>	1
83	Japanese marten	<i>Martes melampus</i>	1
84	Japanese badger	<i>Meles anakuma</i>	1
85	Kaka	<i>Nestor meridionalis</i>	1
86	Kit fox	<i>Vulpes macrotis</i>	1
87	Koala	<i>Phascolarctos cinerus</i>	2
88	Kudu	<i>Tragelaphus strepsiceros</i>	2
89	Lapwing	<i>Vanellus miles &amp; V. vanellus</i>	1
90	Leopard	<i>Panthera pardus</i>	4
91	Leopard frog	<i>Rana sphenoccephala &amp; R. pipiens</i>	1
92	Leopard tortoise	<i>Stigmochelys pardalis</i>	1
93	Lesser prairie chicken	<i>Tympanuchus pallidicinctus</i>	1
94	Lion	<i>Panthera leo</i>	18
95	Lizard	<i>Squamata spp.</i>	2
96	Long-haired rat	<i>Rattus villosissimus</i>	1
97	Long-necked turtle	<i>Chelodina longicollis</i>	1
98	Mallard	<i>Anas platyrhynchos</i>	2
99	Malleefowl	<i>Leipoa ocellata</i>	1
100	Mammal	<i>Mammalia spp.</i>	1
101	Marsh terrapin	<i>Pelomedusa subrufa</i>	1
102	Marten	<i>Martes martes</i>	1
103	Masked palm civet	<i>Paguma larvata</i>	1
104	Mink	<i>Neovison vison</i>	2
105	Mojave desert tortoise	<i>Gopherus agassizii</i>	1
106	Mongolian gazelle	<i>Procapra gutturosa</i>	1
107	Mongoose	<i>Herpestes spp. &amp; Ichnemon spp.</i>	2
108	Moose	<i>Alces alces</i>	5
109	Mouse	<i>Rodentia spp.</i>	3
110	Mule deer	<i>Odocoileus hemionus</i>	6
111	Numbat	<i>Myrmecobius fasciatus</i>	2
112	Opossum	<i>Didelphis virginianus</i>	1
113	Oryx	<i>Oryx dammah</i>	1
114	Otago skink	<i>Oligosoma ottagense</i>	1
115	Painted turtle	<i>Chrysemys picta</i>	1
116	Piping plover	<i>Charadrius melodus</i>	2
117	Porcupine	<i>Hystrix africaeustralis</i>	2
118	Prairie dog	<i>Cynomys ludovicianus</i>	1
119	Pronghorn	<i>Antilocapra americana</i>	3
120	Ptarmigan	<i>Lagopus spp.</i>	2

#	Common name	Scientific name	Count
121	Puma	<i>Puma concolor</i>	1
122	Pygmy owl	<i>Glaucidium brasilianum</i>	1
123	Rabbit	<i>Oryctolagus cuniculus</i>	8
124	Raccoon	<i>Procyon lotor</i>	7
125	Raccoon dog	<i>Nyctereutes procyonoides</i>	1
126	Rat	Rodentia spp.	6
127	Red deer	<i>Cervus elaphus</i>	4
128	Red fox	<i>Vulpes vulpes</i>	16
129	Red grouse	<i>Lagopus lagopus</i>	1
130	Red kangaroo	<i>Osphranter rufus</i>	4
131	Redtailed phascogale	<i>Phascogale calura</i>	1
132	Reptile	Reptilia spp.	1
133	Roe deer	<i>Capreolus capreolus</i>	6
134	Ruppell's fox	<i>Vulpes ruppelli</i>	1
135	Sand cat	<i>Felis margarita</i>	1
136	Scrub hare	<i>Lepus saxatilis</i>	1
137	Sheep	<i>Ovis aries</i>	3
138	Shingleback	<i>Tiliqua rugosa</i>	1
139	Ship rat	<i>Rattus rattus</i>	1
140	Sika deer	<i>Cervus nippon</i>	2
141	Silvereye	<i>Zosterops lateralis</i>	1
142	Skunk	<i>Mephitis</i> spp.	7
143	Snapping turtle	<i>Chelydra serpentina</i>	1
144	Spotted hyena	<i>Crocuta crocuta</i>	1
145	Spotted turtle	<i>Clemmys guttata</i>	1
146	Squirrel	<i>Spermophilus</i> spp.	1
147	Squirrel glider	<i>Petaurus norfolcensis</i>	1
148	Stoat	<i>Mustela erminea</i>	2
149	Sugar glider	<i>Petaurus breviceps</i>	1
150	Swamp wallaby	<i>Wallabia bicolor</i>	1
151	Teal	<i>Anas crecca</i>	1
152	Tern	<i>Sterna albifrons</i>	1
153	Townsend's vole	<i>Microtus townsendii</i>	1
154	Tsessebe	<i>Damaliscus lunatus</i>	1
155	Tui	<i>Prothemadera novaeseelandiae</i>	1
156	Wallaby	Macropodidae spp.	1
157	Warthog	<i>Phacochoerus africanus</i>	4
158	Water buffalo	<i>Bubalus bubalis</i>	1
159	Waterfowl	Anseriformes spp.	1
160	Weasel	<i>Mustela</i> spp.	2
161	Wedge-tailed eagle	<i>Aquila audax</i>	1
162	Wedge-tailed shearwater	<i>Ardenna pacifica</i>	2
163	Western barred bandicoot	<i>Perameles bougainville</i>	3
164	Western gull	<i>Larus occidentalis</i>	1
165	White rhino	<i>Ceratotherium simum</i>	1
166	White-tailed deer	<i>Odocoileus virginianus</i>	9
167	Wild dog	<i>Lycaon pictus</i>	8
168	Wildebeest	<i>Connochaetes taurinus</i>	4
169	Wildlife	Unknown spp.	1
170	Wolf	<i>Canis lupis</i>	2
171	Wombat	Vombatidae spp.	2
172	Yellow-footed rock-wallaby	<i>Petrogale xanthopus</i>	1
173	Zebra	<i>Equus quagga</i> & <i>E. equus</i>	7
<b>Grand total</b>			<b>441</b>



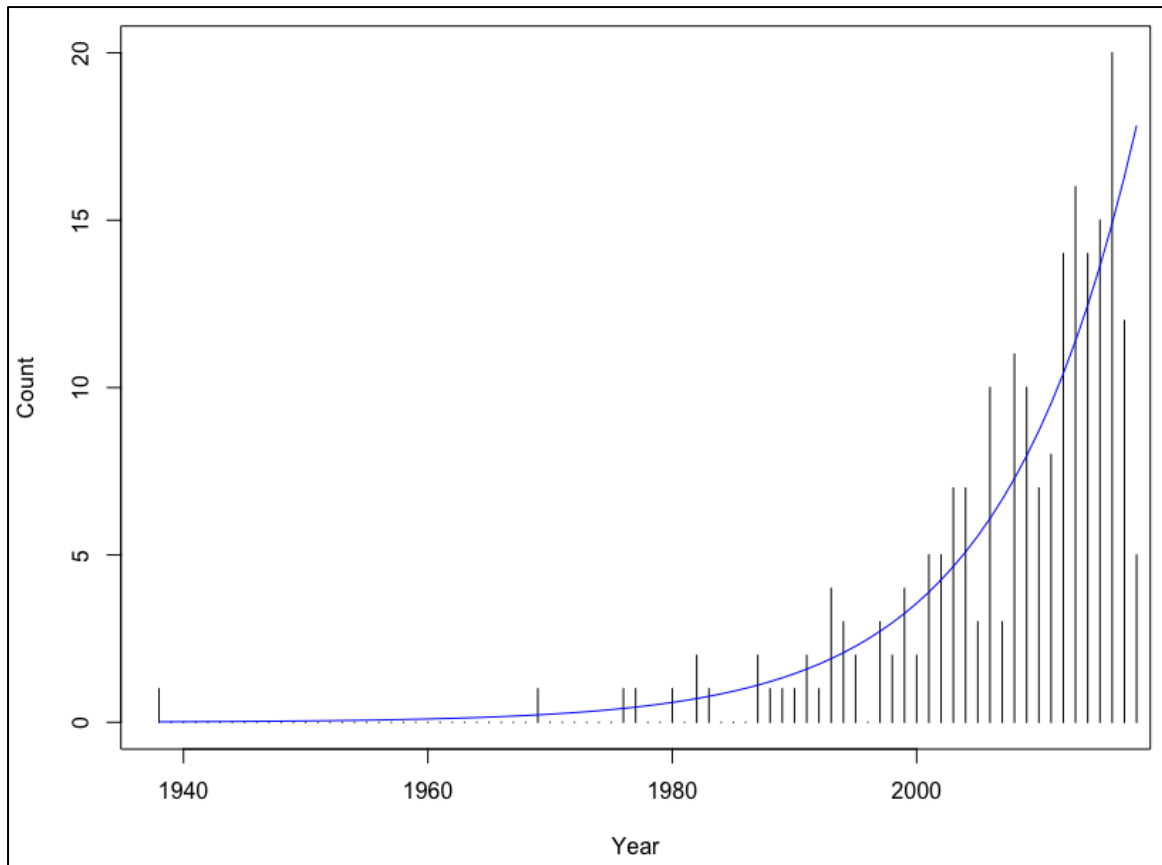
**Figure S1: Percentage of literature per country**

Map of percentage distribution of identified literature across each country, colour ramped by percentage. Created in ArcMap 10.5.1



**Figure S2: Percentage of literature that discusses each fence effect**

Breakdown of discussed fence effects across all literature. Note: Papers may have discussed more than one fence effect.



**Figure S3: Count of exclusion fencing papers over time**

Figure shows the count of papers per year (from 1938-2018). Count is fitted with a GLM (Poisson curve,  $p < 0.001$ )

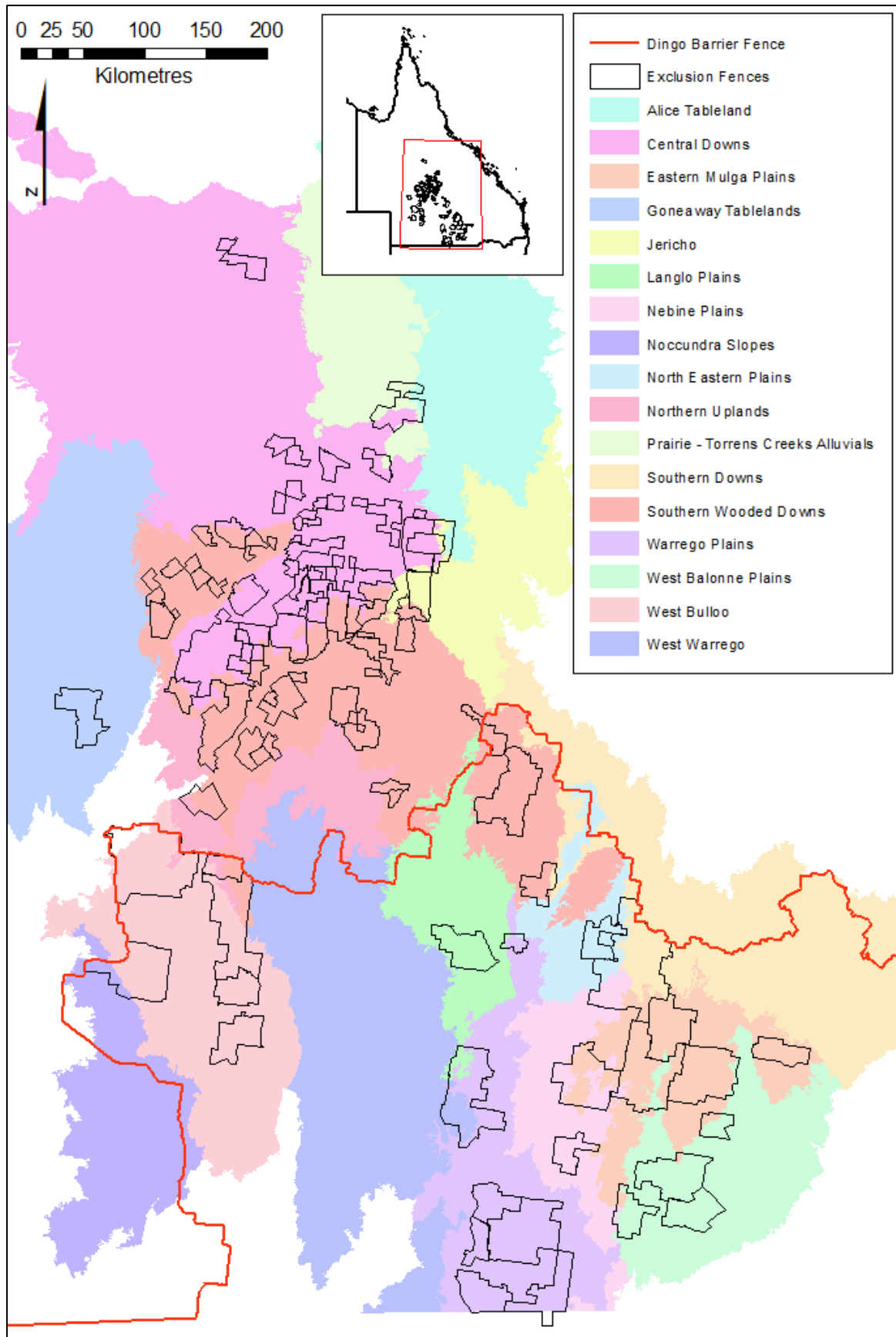
## Appendix II : Supplementary Information (Chapter 3)

**Table S3: Property names, funding source, area, year of completion and centroid location**  
Year completed is based on funding year where land-holders did not report time of completion.

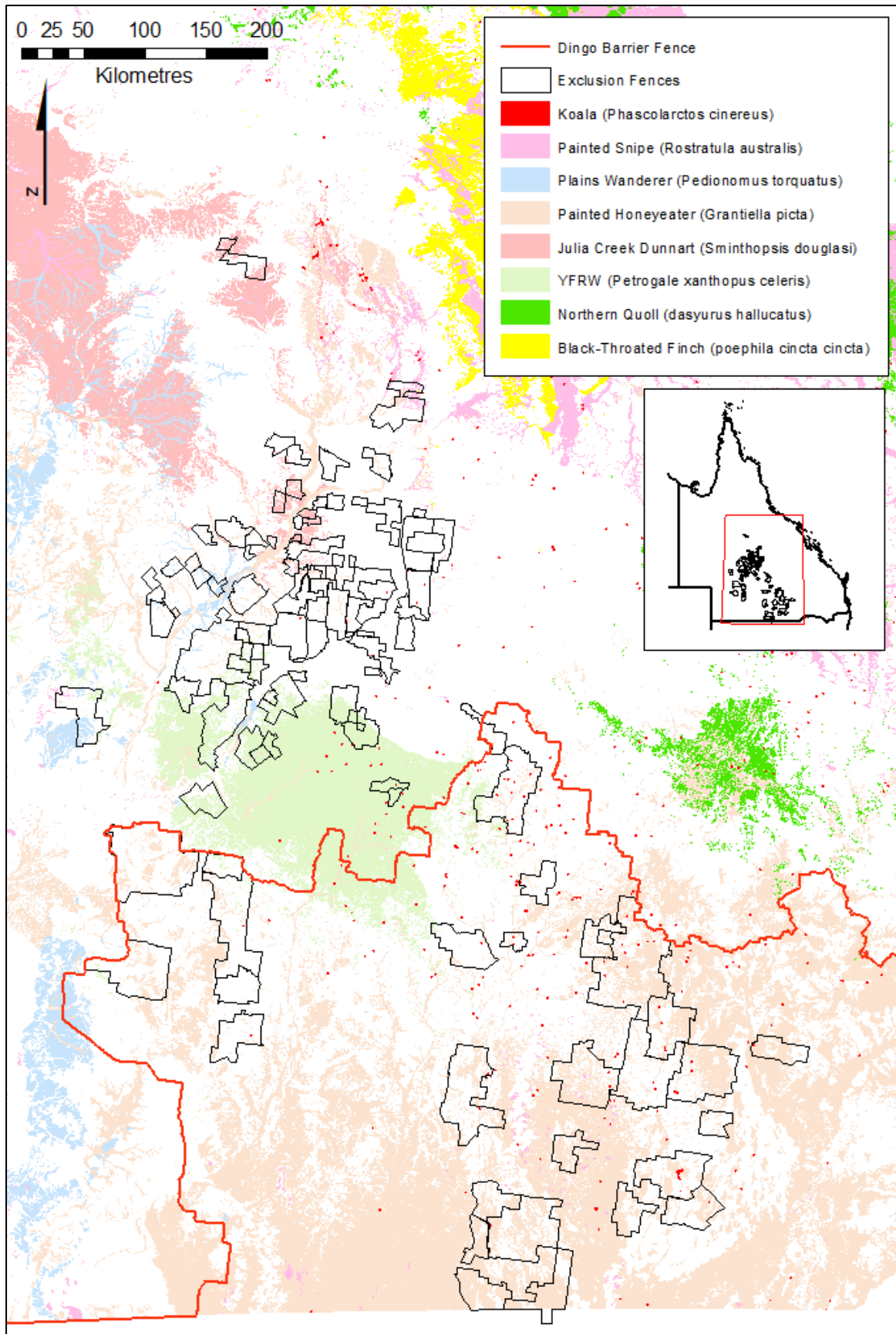
Cluster (Name)	Funding Source	Area (Ha)	Area (Km2)	Year Completed	Centroid	
					Lat	Long
12 Mile	RAPAD_1	48682	487	2018	-23.7543	144.4087
4 Mile	RAPAD_1	31969	320	2018	-23.9945	144.0805
Accord	Private	5086	51	2013	-23.4831	144.8513
Amaroo	Private	14423	144	2014	-25.2113	145.1611
Araleun Trinidad Cluster	CAM2	53875	539	2018	-25.6993	143.7759
Arrilalah	RAPAD_2	58430	584	2018	-23.7109	144.0012
Arrowcreek-Karoola	Private	23004	230	UnKnown	-24.2198	143.9308
Augathella Cluster	CAM2	61892	619	2018	-25.8634	146.3359
Barcaldine Downs	Private	39343	393	UnKnown	-23.8646	144.8689
Barcoo South	RAPAD_2	73797	738	2018	-24.4856	144.2793
Barsdale	LWDEFS	8713	87	2019	-23.5642	144.6794
Beaconsfield	RAPAD_1	41210	412	2018	-23.3673	144.5467
Bellabad	RAPAD_1	39016	390	2018	-23.3569	145.3678
Bellvue	LWDEFS	14618	146	2019	-24.8914	144.0878
Belmore	LWDEFS	3262	33	2019	-23.831	143.435
Bimban	LWDEFS	3971	40	2019	-23.3079	144.4091
Bollon Cluster	CAM2	162460	1625	2018	-28.0024	147.4315
Bollon Sth Cluster	CAM2	107676	1077	2018	-28.2664	147.5891
Boree Downs	Private	12515	125	UnKnown	-24.01	143.9648
Bristol	RAPAD_3	52413	524	2019	-23.1654	145.1467
Camoola Park	LWDEFS	8833	88	2019	-23.0488	144.6043
Clifton Cluster	CAM2	339765	3398	2018	-25.6516	143.1785
Clovelly	LWDEFS	17309	173	2019	-23.6322	144.4209
Clover Hills	RAPAD_1	27226	272	2018	-23.8431	145.2973
Coban Cluster	CAM2	81540	815	2018	-28.1953	145.9295
Cogoon Cluster	CAM2	72866	729	2018	-27.0881	148.2963
Coolagh	Private	9256	93	2012	-24.0189	144.9486
Coombemartin	LWDEFS	8397	84	2019	-23.567	144.5481
Cootabynia Etonvale	RAPAD_3	21480	215	2019	-25.1336	145.1157
Dalmore	RAPAD_3	14877	149	2019	-23.0406	144.2797
Dillulla	LWDEFS	9639	96	2019	-23.1276	144.508
Dundee	LWDEFS	12763	128	2019	-23.5161	144.3716
Dunraven	Private	44568	446	Unknown	-23.6951	145.0516
Eldwick	RAPAD_3	110442	1104	2019	-24.5451	142.6702
Elmesdale	LWDEFS	6574	66	2019	-23.5472	144.6058
Eromanga West Cluster	CAM2	200347	2003	2018	-26.4547	143.049
Eureka	LWDEFS	16705	167	2019	-23.5016	143.4187
Fairfield	LWDEFS	7589	76	2019	-23.1806	144.5374
Fernlee	CAM3	82386	824	2019	-28.2808	147.0647
Fysh Rea	RAPAD_1	33973	340	2018	-22.7676	144.6912
Gillespie	Private	13339	133	Unknown	-24.6062	145.7985
Glass Hut Channel Cluster	CAM2	79833	798	2018	-26.5993	143.8673
Glenferrie	LWDEFS	8341	83	2019	-23.4761	144.7399
Goodberry Hills	LWDEFS	6716	67	2019	-23.1879	144.4262
Janet Downs	Private	14030	140	Unknown	-24.1335	144.0844
Jedburgh Retreat	RAPAD_3	63552	636	2019	-25.2178	143.6287
Kappa-K	Private	14752	148	Unknown	-24.1309	143.9971
Katherine Creek	RAPAD_1	16454	165	2018	-23.574	143.6358
Lagoon Creek	RAPAD_3	39035	390	2016	-23.3363	145.5813
Leander	Private	18441	184	Unknown	-23.3091	144.0795

Cluster (Name)	Funding Source	Area (Ha)	Area (Km2)	Year Completed	Centroid	
					Lat	Long
Linden Nebine	CAM3	64757	648	2019	-27.8292	146.5711
Longway	Private	6797	68	2015	-23.3251	144.2179
Lorne	Private	9611	96	Unknown	-23.0421	144.9201
McGavin	RAPAD_1	28785	288	2018	-23.8468	144.9998
Mellew	Private	52564	526	Unknown	-24.0253	145.2662
Meraree	RAPAD_1	53428	534	2018	-24.524	144.7633
Moble Nerringundah	CAM3	103265	1033	2019	-26.9539	143.8473
Mons	LWDEFS	9236	92	2019	-24.3186	144.4045
Moombria	RAPAD_1	34318	343	2018	-24.1569	145.0234
Moorlands	Private	10226	102	2013	-24.2959	145.0027
Morven	CAM1	376055	3761	2015	-26.4628	147.0859
Mt Harden	Private	67626	676	2010	-24.7329	144.9104
Mt Victoria	LWDEFS	16869	169	2019	-23.2614	143.6772
Mungallala	CAM1	144336	1443	2015	-26.9164	147.3774
Narbethong	RAPAD_3	59068	591	2019	-23.7044	145.3884
Neabul Creek	CAM3	46773	468	2019	-27.2636	147.6753
Neabul Wallara Cluster	CAM2	202265	2023	2018	-27.6293	147.7722
Nebine Cluster	CAM2	190437	1904	2018	-27.2991	146.7023
Newhaven	LWDEFS	16825	168	2019	-24.8283	144.1807
Noorama Cluster	CAM2	123040	1230	2018	-28.8568	146.3914
North Aramac	RAPAD_1	78117	781	2018	-22.3505	145.2489
Northern Barcoo	RAPAD_1	41104	411	2018	-24.4119	143.4869
Parkdale	LWDEFS	12239	122	2019	-23.4806	143.5819
Quilpie	CAM1	194499	1945	2016	-26.1213	143.8347
Road2Road	LWDEFS	20438	204	2019	-23.658	144.6068
Shandon Vale	Private	7153	72	Unknown	-23.1321	145.3927
SLM Exclusion (North)	CAM3	49264	493	2019	-28.4914	145.8042
SLM Exclusion (South)	CAM3	101667	1017	2019	-28.8606	145.8312
South Dangaraga	RAPAD_2	60031	600	2018	-23.8457	144.7255
South Tambo	CAM1	228965	2290	2016	-25.1875	146.1044
Southern Dandaraga Road	LWDEFS	29835	298	2019	-23.831	144.571
Spoilbank	LWDEFS	14407	144	2019	-23.5821	143.246
Spring Plains	LWDEFS	13919	139	2019	-23.7045	143.2841
Stainburn	RAPAD_3	33990	340	2019	-22.762	145.0762
Stamford	RAPAD_2	53542	535	2018	-21.2548	144.0357
Strathdarr	RAPAD_2	52895	529	2018	-23.2697	143.9186
Summer Hill	RAPAD_1	16637	166	2018	-23.0446	144.7216
TamarVentry	LWDEFS	18689	187	2019	-24.2672	144.075
Tocal Creek	RAPAD_3	223560	2236	2019	-24.1573	143.7217
Tomoo Creek	CAM1	231924	2319	2016	-27.2414	147.2029
Toobrack Glouster	LWDEFS	12553	126	2019	-23.6433	143.7688
Ward River Cluster	CAM2	22934	229	2018	-24.4702	144.1857
Waroonga	LWDEFS	9521	95	2019	-26.2862	146.1248
Way Out West	RAPAD_2	47232	472	2018	-23.8816	143.3156
Weewondilla	RAPAD_3	23222	232	2019	-22.6079	144.2903
Well Water Cluster	CAM2	69906	699	2018	-26.2703	146.8156
West Alice	RAPAD_1	90377	904	2018	-23.6241	144.9595
Westbury	LWDEFS	18238	182	2019	-22.943	144.4046
Western Lango Cluster	CAM2	116854	1169	2018	-26.3292	145.7214
Wild Horse	RAPAD_2	89069	891	2018	-24.0094	144.3508
Wild Horse Cluster	LWDEFS	52001	520	2019	-24.0291	144.5365
Woodleigh Creek	Private	25739	257	2015	-22.7013	144.4156
Woodstock	CAM1	241322	2413	2016	-28.4143	146.188
Wyandra	CAM1	226422	2264	2016	-27.408	145.744
Yaraka	RAPAD_1	188200	1882	2018	-24.8271	143.9995





**Figure S4: Biogeographic subregions and cluster fences**  
 Shapefiles of biogeographic subregions, and the location of the Dingo Barrier fence were sourced from Q-Spatial.



**Figure S5: Modelled habitat for some TSP and cluster fence locations**  
 Modelled habitat shapefiles were sourced from Q-Spatial.

**Table S4: List of pest species identified by PMST**

#	Common name	Scientific name	Class
1	House Sparrow	<i>Passer domesticus</i>	Bird
2	Domestic Pigeon	<i>Columba livia</i>	Bird
3	Common Blackbird	<i>Turdus merula</i>	Bird
4	Common Starling	<i>Sturnus vulgaris</i>	Bird
5	Mallard	<i>Anas platyrhynchos</i>	Bird
6	Spotted turtle-dove	<i>Streptopelia chinensis</i>	Bird
7	Common Myna	<i>Acridotheres tristis</i>	Bird
8	Nutmeg Mannikin	<i>Lonchura punctulata</i>	Bird
9	Cane Toad	<i>Rhinella marina</i>	Amphibian
10	Dromedary	<i>Camelus dromedarius</i>	Mammal
11	Wild Dog	<i>Canis familiaris</i>	Mammal
12	Feral Goat	<i>Capra hircus</i>	Mammal
13	Feral Cat	<i>Felis catus</i>	Mammal
14	Red Fox	<i>Vulpes vulpes</i>	Mammal
15	House Mouse	<i>Mus musculus</i>	Mammal
16	Feral Pig	<i>Sus scrofa</i>	Mammal
17	Rabbit	<i>Oryctolagus cuniculus</i>	Mammal
18	Brown Hare	<i>Lepus capensis</i>	Mammal
19	Feral Cattle	<i>Bos taurus</i>	Mammal
20	Feral Deer	(not specified)	Mammal
21	Feral Horse	<i>Equus caballus</i>	Mammal
22	Black Rat	<i>Rattus rattus</i>	Mammal

## Appendix III : Supplementary Information (Chapter 5)

**Table S5: List of species observed at the Quilpie Cluster**

Species observed during the collection of data in the field, or seen on trail cameras.

#	Common Name	Scientific Name
<b>Birds</b>		
1	Apostlebird	<i>Struthidea cinerea</i>
2	Australian Bustard	<i>Ardeotis australis</i>
3	Australian Hobby Falcon	<i>Falco longipennis</i>
4	Australian Raven	<i>Corvus coronoides</i>
5	Australian Ringneck	<i>Barnardius zonarius</i>
6	Australasian Grebe	<i>Tachybaptus novaehollandiae</i>
7	Black Falcon	<i>Falco subniger</i>
8	Black Kite	<i>Milvus migrans</i>
9	Black-eared Cuckoo	<i>Chrysococcyx osculans</i>
10	Black-faced Cuckoo-shrike	<i>Coracina novaehollandiae</i>
11	Black-fronted Dotterel	<i>Elsayornis melanops</i>
12	Black-tailed Native-hen	<i>Gallinula ventralis</i>
13	Black-winged Stilt	<i>Himantopus himantopus</i>
14	Brolga	<i>Grus rubicunda</i>
15	Brown Falcon	<i>Falco berigora</i>
16	Budgerigar	<i>Melopsittacus undulatus</i>
17	(Pied) Butcherbird	<i>Cracticus nigrogularis</i>
18	Cockatiel	<i>Nymphicus hollandicus</i>
19	Common Bronzewing	<i>Phaps chalcoptera</i>
20	Crested bellbird	<i>Oreoica gutturalis</i>
21	Crested Pigeon	<i>Ocyphaps lophotes</i>
22	(Pied) Currawong	<i>Strepera graculina</i>
23	Double-Barred Finch	<i>Taeniopygia bichenovii</i>
24	(Intermediate) Egret	<i>Ardea intermedia</i>
25	Emu	<i>Dromaius novaehollandiae</i>
26	Galah	<i>Cacatua roseicapilla</i>
27	Great Cormorant	<i>Phalacrocorax carbo</i>
28	Grey Fantail	<i>Rhipidura albiscapa</i>
29	Grey Shrike-thrush	<i>Colluricincla harmonica</i>
30	Grey Teal	<i>Anas gracilis</i>
31	Hall's Babbler	<i>Pomatostomus halli</i>
32	Hooded Robin	<i>Melanodryas cucullata</i>
33	Laughing Kookaburra	<i>Dacelo novaeguineae</i>
34	Letterwing Kite	<i>Elanus scriptus</i>
35	Little Black Cormorant	<i>Phalacrocorax sulcirostris</i>
36	Little Corella	<i>Cacatua sanguinea</i>
37	Little Pied Cormorant	<i>Phalacrocorax melanoleucos</i>
38	(Australian) Magpie	<i>Gymnorhina tibicen</i>
39	Magpie-lark	<i>Grallina cyanoleuca</i>
40	Major Mitchell Cockatoo	<i>Cacatua leadbeateri</i>
41	Mulga Parrot	<i>Psephotus varius</i>
42	Pacific Black Duck	<i>Anas superciliosa</i>
43	White-necked Heron	<i>Ardea pacifica</i>
44	Peregrine Falcon	<i>Falco peregrinus</i>
45	Pink-eared Duck	<i>Malacorhynchus membranaceus</i>
46	Plumed Whistling Duck	<i>Dendrocygna eytoni</i>
47	Quail Thrush	<i>Cinclosoma castanotum</i>
48	Red-backed Kingfisher	<i>Todiramphus pyrrhopygia</i>
49	Red-browed Pardalote	<i>Pardalotus rubricatus</i>
50	Red-capped Robin	<i>Petroica goodenovii</i>
51	Red-rumped Parrot	<i>Psephotus haematonotus</i>
5	Savannah Nightjar	<i>Caprimulgus affinis</i>
53	Spinifex Pigeon	<i>Geophaps plumifera</i>

#	Common Name	Scientific Name
54	Sulphur-crested Cockatoo	<i>Cacatua galerita</i>
55	Tree Martin	<i>Petrochelidon nigricans</i>
56	Wedge-tailed Eagle	<i>Aquila audax</i>
57	Whistling Kite	<i>Haliastur sphenurus</i>
58	White-winged Chough	<i>Corcorax melanorhamphos</i>
59	Willy Wagtail	<i>Rhipidura leucophrys</i>
60	(Eastern) Yellow Robin	<i>Eopsaltria australis</i>
61	Yellow-billed Spoonbill	<i>Platalea flavipes</i>
62	Zebra finch	<i>Taeniopygia guttata</i>
<b>Mammals</b>		
1	(European) Cattle	<i>Bos taurus</i>
2	Dingo/Wild Dog/Domestic Dog	<i>Canis familiaris</i>
3	Eastern Grey Kangaroo	<i>Macropus giganteus</i>
4	(Short-beaked) Echidna	<i>Tachyglossus aculeatus</i>
5	Feral Cat	<i>Felis catus</i>
6	Feral Goat	<i>Capra hircus</i>
7	Feral Pig	<i>Sus scrofa</i>
8	Koala	<i>Phascolarctos cinereus</i>
9	Rabbit	<i>Oryctolagus cuniculus</i>
10	Red Fox	<i>Vulpes vulpes</i>
11	Red Kangaroo	<i>Osphranter rufus</i>
12	Sheep	<i>Ovis aries</i>
13	Wallaroo (Euro)	<i>Osphranter robustus</i>
14	Yellow-footed Rock-wallaby	<i>Petrogale xanthopus</i>
<b>Reptiles</b>		
1	Central Bearded Dragon	<i>Pogona vitticeps</i>
2	Gould's Goanna	<i>Varanus gouldii</i>
3	Mulga Snake (King Brown Snake)	<i>Pseudechis australis</i>
4	Nobbi Dragon	<i>Diporiphora nobbi</i>
5	Bynoe's Gecko	<i>Heteronotia binoei</i>

## **SILO (Scientific Information for Land Owners) Data Drill specifications**

- **General information:**

SILO is a database showing climate data from 1889 onwards. SILO is hosted by the Queensland Department of Environment and Science (DES). The central point of the cluster was identified and using SILO's Data Drill, the climate data for the entire study period was interpolated for that position. The location and times specified were:

Latitude: 26 06'S (Degrees Minutes) or -26.10 (as Decimal Degrees)

Longitude: 143 57'E (Degrees Minutes) or 143.95 (as Decimal Degrees)

Elevation: 277m.

Period: 01/04/2017 - 30/04/2019

- **Access information:**

This data was accessed on Wednesday the 5<sup>th</sup> of June, 2019.

Website: <https://legacy.longpaddock.qld.gov.au/silo/datadrill/>

- **References:**

References and scientific reviews of SILO can be found at:

<https://www.longpaddock.qld.gov.au/silo/about/publications-references/>

Primary Reference:

Jeffrey, S.J., Carter, J.O., Moodie, K.B. and Beswick, A.R. (2001). *Using spatial interpolation to construct a comprehensive archive of Australian climate data*, Environmental Modelling and Software, Vol 16/4, pp 309-330. DOI: 10.1016/S1364-8152(01)00008-1

## Appendix IV : Supplementary Information (Chapter 6)

**Table S6: Data collection sheet used for habitat scoring**

Variable	Description
<b>Site No.</b>	<i>Individual Site ID number</i>
<b>GPS Location:</b>	<i>X and Y coordinates</i>
Camera site (if applicable) :	<i>Associated Trail Camera</i>
<b>Occupancy:</b> Scat (+/-)	<i>Presence of YFRW scat</i>
Sighting (+/-)	<i>Sighting of YFRW</i>
<b>Site type:</b> Cliff/Gully/Rockhole/Slope	<i>Type of habitat at primary score site</i>
<b>Lim and Giles (1987):</b> Steep Cliff (+/-)	<i>Vertical rock faces around score site</i>
Water (+/-)	<i>Water around score site</i>
Outcrops (+/-)	<i>Mounds of rock set away from main cliffs/area</i>
Gullies (+/-)	<i>Areas of wash, forming small valleys</i>
Terraces (+/-)	<i>Steps of steep cliff at the score site</i>
Caves (+/-)	<i>Caves, caverns and tunnels at score site</i>
Chimneys (+/-)	<i>Ramps that allow movement between areas</i>
Rock piles (+/-)	<i>Piles of broken away cliff at score site</i>
Boulder size (S/M/L)	<i>Small &lt;1m Medium ~1 or large &gt;1m boulders</i>
Surface Texture (S/I/R)	<i>Smooth, intermediate or Rough rock</i>
<b>Threat Assessment:</b> Goats (+/-)	<i>Presence of goat scat</i>
Domestic Species (+/-)	<i>Presence of cattle dung</i>
Native Species (+/-)	<i>Presence of other native species scat</i>
Clearing (+/-)	<i>Tree/land clearing in area</i>
Mining (+/-)	<i>Resource exploration or mining in area</i>
Other Ag. Use (+/-)	<i>Other notable land use</i>
<b>Short (1982):</b> Height of Cliff (m)	<i>Desktop generated - altitude</i>
Aspect of cliff face (0-360°)	<i>Compass direction</i>
Slope of cliff (0-90°)	<i>Angle of cliff from top to bottom</i>
Distance to water (m-km)	<i>Desktop generated – closest known</i>
<b>EHP Assessment (2017):</b> Canopy - Top (m)	<i>Estimation of average canopy height on cliff</i>
Canopy Height - Ledge (m)	<i>Estimation of average canopy height on ledge</i>
Canopy Height - Flat (m)	<i>Estimation of average canopy height on flat</i>
Canopy Cover - Top (%)	<i>An average of 5 estimations of canopy cover</i>
Canopy Cover - Ledge (%)	<i>An average of 5 estimations of canopy cover</i>
Canopy Cover - Flat (%)	<i>An average of 5 estimations of canopy cover</i>
Shrub Cover - Top (%)	<i>An average of 5 estimations of shrub cover</i>
Shrub Cover - Ledge (%)	<i>An average of 5 estimations of shrub cover</i>
Shrub Cover - Flat (%)	<i>An average of 5 estimations of shrub cover</i>
Ground Vege Cover - Top (%)	<i>An average of 5 estimations of ground cover</i>
Ground Vege Cover - Ledge (%)	<i>An average of 5 estimations of ground cover</i>
Ground Vege Cover - Flat (%)	<i>An average of 5 estimations of ground cover</i>
Ground Vege Height - Top (cm)	<i>An average of 5 measurements of vege height</i>
Ground Vege Height - Ledge (cm)	<i>An average of 5 measurements of vege height</i>
Ground Vege Height - Flat (cm)	<i>An average of 5 measurements of vege height</i>

NOTES:

Appendix V : Supplementary Information (Chapter 7)

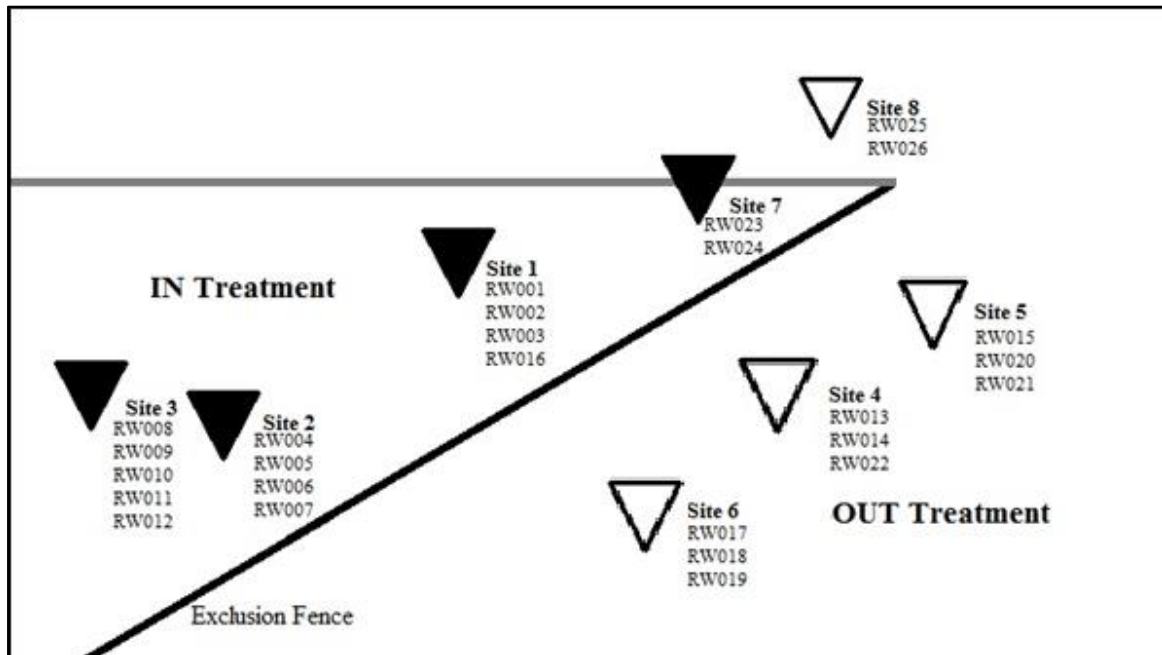


Figure S6: Experimental design and camera trap placement

Table S7: Camera locations, treatments and proximities

Camera ID	Site	Latitude	Longitude	Site	Property	Cluster	Dingo Fence	Closest Camera	Distance (m)	Closest Cam (Dif. Site)	Distance (m)	Site
RW001	1	-26.005834	143.909772	1	Canaway	In	In	RW002	319.91	RW021	18476.94	5
RW002	1	-26.008535	143.908642	1	Canaway	In	In	RW003	293.55	RW021	18233.16	5
RW003	1	-26.010388	143.910738	1	Canaway	In	In	RW002	293.55	RW021	17968.72	5
RW016	1	-26.010188	143.900804	1	Canaway	In	In	RW002	805.75	RW012	18307.19	3
RW004	2	-26.185245	143.917863	2	Ray	In	In	RW005	1269.60	RW010	1529.20	3
RW005	2	-26.196326	143.914626	2	Ray	In	In	RW006	320.47	RW010	760.13	3
RW006	2	-26.199024	143.915782	2	Ray	In	In	RW005	320.47	RW010	967.30	3
RW007	2	-26.202311	143.918066	2	Ray	In	In	RW006	429.80	RW010	1353.91	3
RW008	3	-26.193195	143.901399	3	Ray	In	In	RW009	587.59	RW005	1367.70	2
RW009	3	-26.195558	143.906662	3	Ray	In	In	RW010	71.50	RW005	801.06	2
RW010	3	-26.195087	143.907151	3	Ray	In	In	RW009	71.50	RW005	760.13	2
RW011	3	-26.197392	143.905242	3	Ray	In	In	RW009	247.85	RW005	945.95	2
RW012	3	-26.174812	143.886381	3	Ray	In	In	RW008	2530.15	RW004	3354.78	2
RW013	4	-26.181789	143.991972	4	Aleric	Out	In	RW014	305.63	RW020	2868.12	5
RW014	4	-26.183573	143.98964	4	Aleric	Out	In	RW013	305.63	RW015	2861.72	5
RW022	4	-26.18643	143.99182	4	Aleric	Out	In	RW014	384.29	RW015	3244.96	5
RW015	5	-26.16368	143.971412	5	Aleric	Out	In	RW021	79.56	RW014	2861.72	4
RW020	5	-26.16303	143.97223	5	Aleric	Out	In	RW015	108.97	RW014	2867.19	4
RW021	5	-26.16305	143.97103	5	Aleric	Out	In	RW015	79.56	RW014	2939.94	4
RW017	6	-26.25518	143.94073	6	Fifteen Mile	Out	In	RW018	406.84	RW007	6284.12	2
RW018	6	-26.2517	143.94203	6	Fifteen Mile	Out	In	RW019	206.25	RW007	5976.83	2
RW019	6	-26.25148	143.94408	6	Fifteen Mile	Out	In	RW018	206.25	RW007	6039.90	2
RW023	7	-25.78392	143.97448	7	Canaway	In	In	RW024	813.32	RW026	1838.68	8
RW024	7	-25.77714	143.97137	7	Canaway	In	In	RW023	813.32	RW026	1028.87	8
RW025	8	-25.76723	143.96986	8	Trinidad	Out	Out	RW026	153.19	RW024	1109.05	7
RW026	8	-25.76817	143.96874	8	Trinidad	Out	Out	RW025	153.19	RW024	1028.87	7

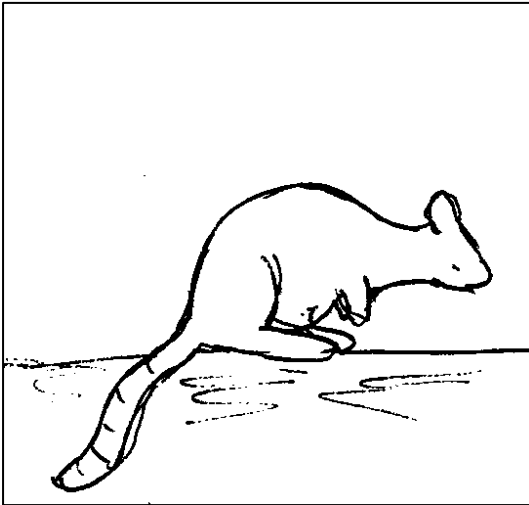


**Table S8: Gantt chart showing when cameras were active**

A timeline showing when cameras were active and functioning correctly during the study period. Blacked out areas indicate active cameras. The 'Camera Days' column shows the number of days each camera was functioning correctly, and totalled by exclusion fence treatment and site.

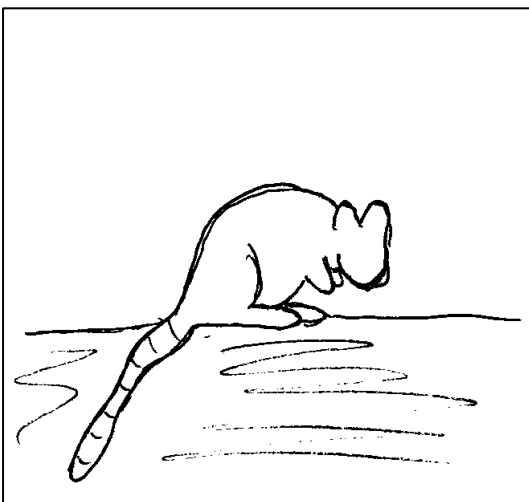
Site & Camera Number	2017								2018												2019				Camera Days
	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	
<b>IN</b>																							<b>9643</b>		
<b>Site 1</b>																							<b>2681</b>		
RW001																							726		
RW002																							691		
RW003																							652		
RW016																							612		
<b>Site 2</b>																							<b>2725</b>		
RW004																							726		
RW005																							674		
RW006																							599		
RW007																							726		
<b>Site 3</b>																							<b>3563</b>		
RW008																							689		
RW009																							696		
RW010																							726		
RW011																							726		
RW012																							726		
<b>Site 7</b>																							<b>674</b>		
RW023																							270		
RW024																							404		
<b>OUT</b>																							<b>5449</b>		
<b>Site 4</b>																							<b>1795</b>		
RW013																							664		
RW014																							726		
RW022																							405		
<b>Site 5</b>																							<b>1435</b>		
RW015																							625		
RW020																							405		
Rw021																							405		
<b>Site 6</b>																							<b>1527</b>		
RW017																							614		
RW018																							407		
RW019																							506		
<b>Site 8</b>																							<b>692</b>		
RW025																							288		
RW026																							404		

Figure S7: Characteristics of scored YFRW behaviours



**Crouching**

- Characterised by rounded back and low head. Front paws may be rested on the ground.
- Differs from 'Foraging' in that the nose is not close to the ground or vegetation.
- Differs from 'Pentapedal Locomotion' in that the tail is relaxed/not ready to bear weight.
- Included in the 'Resting' behavioural group



**Crouching and Looking**

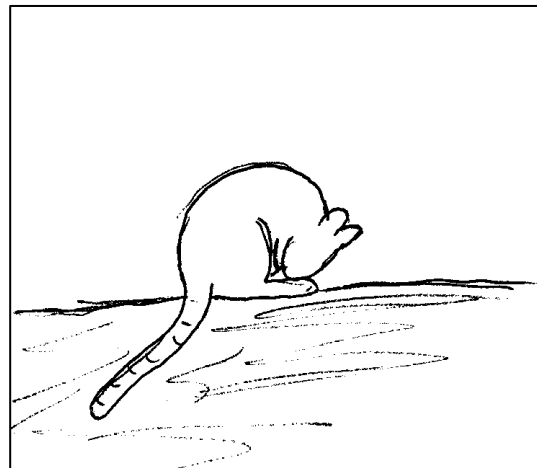
- Characterised by rounded back and low head. Front paws may be rested on the ground.
- Differs from 'Crouching' in that the eyes appear fixated and ears appear alert, pointed forward.

- (cont.) Included in the 'Resting' behavioural group.



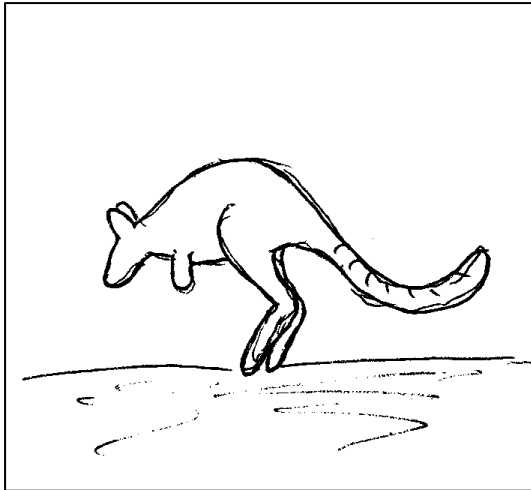
**Foraging**

- Characterised by head/nose close to ground or vegetation.
- May occur in 'Standing', 'Crouching' or 'Pentapedal Locomotion' positions.
- Included in the 'Foraging' behavioural group.



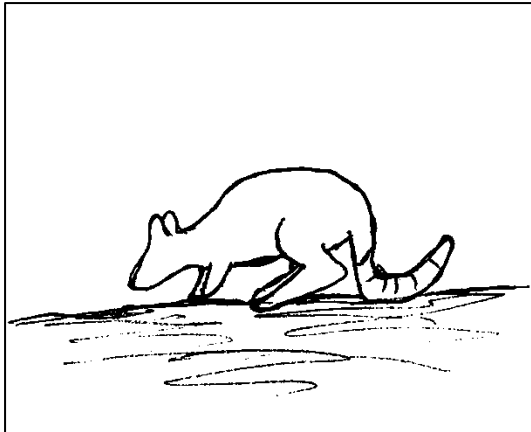
**Grooming**

- Any behaviour in which it appears wallabies are licking/cleaning their fur, pouch or potential small pouch young.
- May occur in the 'Crouching' or 'Standing' position.
- Included in the 'Resting' behavioural group.



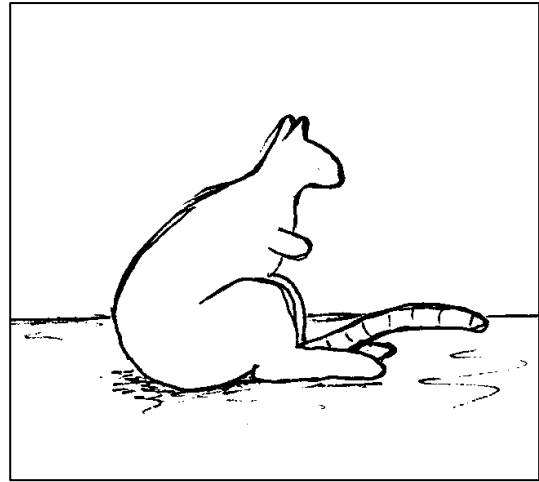
### **Hopping**

- Rapid bipedal movement.
- Distinguished from 'Standing' and 'Crouching' by head and eyes fixated forwards, tail lifted off the ground and image was often blurred.
- Included in the 'Hopping' behavioural group.



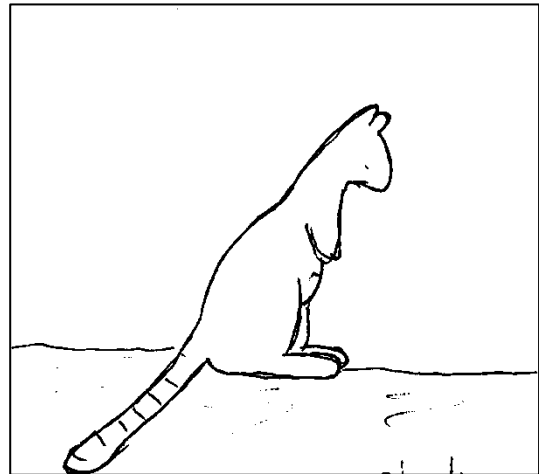
### **Pentapedal Locomotion**

- Characterised by rounded back, all four limbs on the ground and tail bearing/ready to bear weight.
- Differs from 'Foraging' in that the head is not near the ground or vegetation.
- Included in the 'Foraging' behavioural group.



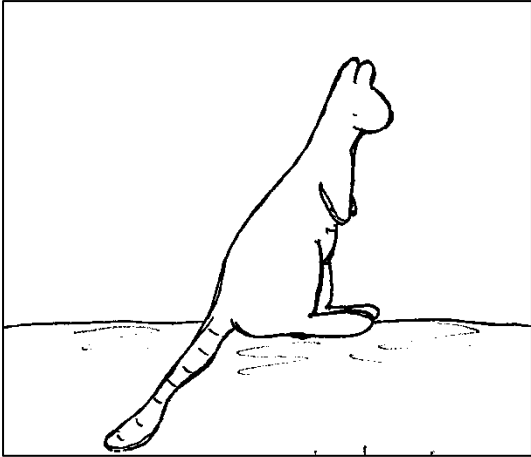
### **Resting**

- Characterised by either laying on their side, or by sitting on rump with tail coming forward, between their legs.
- Included in the 'Resting' behavioural group.



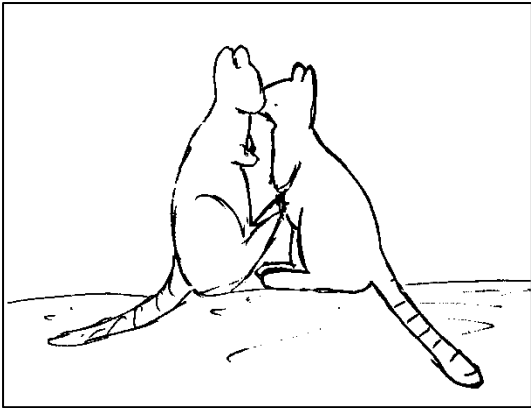
### **Standing**

- Characterised by a vertical straight back and only hind limbs on the ground.
- Differs from 'Resting' and 'Hopping' in that the rump is off the ground and tail is relaxed. Though the tail may still come forward between the legs in this position.
- Included in the 'Resting' behavioural group.



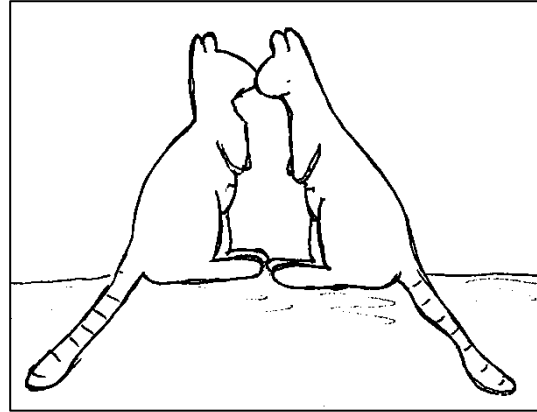
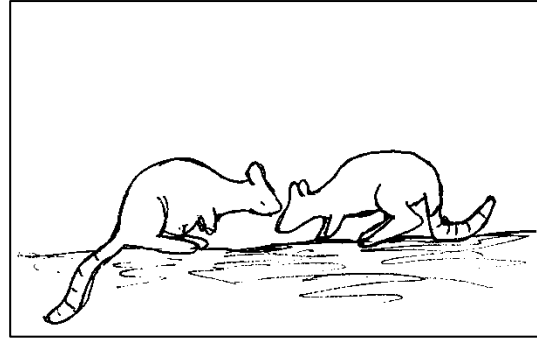
### **Standing and Looking**

- Characterised by a vertical straight back and only hind limbs on the ground.
- Differs from 'Standing' in that the eyes appear fixated and ears appear alert, pointed forward.
- Included in the 'Resting' behavioural group.



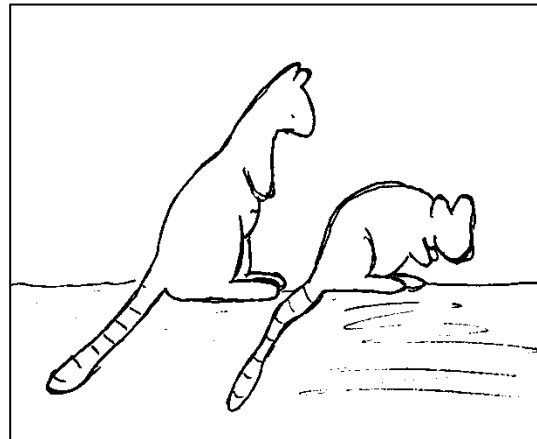
### **Aggressive Interaction**

- Interaction between two or more YFRW.
- Characterised by close proximity between YFRW, often with forelimbs wrapped around the other individual or hind limbs raised to kick other individual. The image is also often blurred.
- Included in the 'Interacting' behavioural group.



### **Affiliative Interaction**

- Characterised by very close proximity between two or more YFRW, often with heads/faces meeting to interact.
- Included in the 'Interacting' behavioural group.



### **Other Interaction**

- Any interaction between two or more YFRW that was not characterised as 'Aggressive' or 'Affiliative'.
- Included in the 'Interacting' behavioural group.

**Table S9: Table of species counts by treatment, site and camera**

Table showing the raw photo count for each camera, and the sum of the ecologically relevant species counted across each camera. The table is distributed by treatment and site, with totals for each calculated at top of each treatment or site section. YFRW = yellow-footed rock-wallaby, EGK = Eastern Grey Kangaroo, Euro = Wallaroo, RK = Red Kangaroo.

Treatment Site Camera	Raw Photo Count	Sum of Animals	YFRW	Dingos	Goats	EGK	Euro	RK	Rab bit	Cat
<b>IN</b>	<b>137352</b>	<b>118808</b>	<b>9533</b>	<b>109</b>	<b>95806</b>	<b>3808</b>	<b>2862</b>	<b>923</b>	<b>263</b>	<b>39</b>
<b>Site1</b>	<b>31268</b>	<b>47756</b>	<b>2046</b>	<b>15</b>	<b>40200</b>	<b>2248</b>	<b>1183</b>	<b>378</b>	<b>180</b>	<b>38</b>
RW001	3813	8020	226	0	7497	0	28	0	14	29
RW002	10717	15292	228	0	14842	68	1	5	45	0
RW003	8853	13686	422	15	9235	1702	827	328	85	0
RW016	7885	10758	1170	0	8626	478	327	45	36	9
<b>Site2</b>	<b>29210</b>	<b>47178</b>	<b>1839</b>	<b>50</b>	<b>41402</b>	<b>687</b>	<b>543</b>	<b>154</b>	<b>0</b>	<b>0</b>
RW004	7058	8158	48	26	4860	556	185	114	0	0
RW005	1763	1812	1591	4	136	33	30	5	0	0
RW006	17298	36132	119	13	35775	25	107	20	0	0
RW007	3091	1076	81	7	631	73	221	15	0	0
<b>Site3</b>	<b>47369</b>	<b>22067</b>	<b>4364</b>	<b>43</b>	<b>14184</b>	<b>589</b>	<b>1100</b>	<b>314</b>	<b>83</b>	<b>0</b>
RW008	1105	1030	70	21	405	96	338	14	0	0
RW009	41139	15639	3402	2	10851	10	435	125	83	0
RW010	749	408	32	20	78	0	109	120	0	0
RW011	1939	3242	129	0	2850	57	131	39	0	0
RW012	2437	1748	731	0	0	426	87	16	0	0
<b>Site7</b>	<b>29505</b>	<b>1807</b>	<b>1284</b>	<b>1</b>	<b>20</b>	<b>284</b>	<b>36</b>	<b>77</b>	<b>0</b>	<b>1</b>
RW023	28199	1624	1178	1	20	268	36	74	0	1
RW024	1306	183	106	0	0	16	0	3	0	0
<b>OUT</b>	<b>197916</b>	<b>25988</b>	<b>6559</b>	<b>297</b>	<b>13488</b>	<b>1212</b>	<b>368</b>	<b>80</b>	<b>31</b>	<b>9</b>
<b>Site4</b>	<b>6510</b>	<b>4227</b>	<b>2477</b>	<b>20</b>	<b>1016</b>	<b>406</b>	<b>155</b>	<b>25</b>	<b>0</b>	<b>0</b>
RW013	621	525	145	0	240	30	35	15	0	0
RW014	3675	3161	1871	12	775	310	120	5	0	0
RW022	2214	541	461	8	1	66	0	5	0	0
<b>Site5</b>	<b>8591</b>	<b>3470</b>	<b>958</b>	<b>45</b>	<b>1957</b>	<b>394</b>	<b>0</b>	<b>0</b>	<b>31</b>	<b>0</b>
RW015	5869	1110	297	0	802	0	0	0	0	0
RW020	333	273	104	0	129	40	0	0	0	0
RW021	2389	2087	557	45	1026	354	0	0	31	0
<b>Site6</b>	<b>180635</b>	<b>17090</b>	<b>2292</b>	<b>232</b>	<b>10515</b>	<b>228</b>	<b>115</b>	<b>32</b>	<b>0</b>	<b>4</b>
RW017	6346	5654	1814	55	3505	77	67	32	0	0
RW018	110201	4026	259	74	57	141	48	0	0	0
RW019	64088	7410	219	103	6953	10	0	0	0	4
<b>Site8</b>	<b>2180</b>	<b>1201</b>	<b>832</b>	<b>0</b>	<b>0</b>	<b>184</b>	<b>98</b>	<b>23</b>	<b>0</b>	<b>5</b>
RW025	687	586	404	0	0	128	38	15	0	0
RW026	1493	615	428	0	0	56	60	8	0	5
<b>Total</b>	<b>335268</b>	<b>144796</b>	<b>16092</b>	<b>406</b>	<b>109294</b>	<b>5020</b>	<b>3230</b>	<b>1003</b>	<b>294</b>	<b>48</b>

**Table S10: List of recorded species from camera traps**

#	Common Name	Scientific Name
1	Bird species.	Aves spp.
2	Cattle	<i>Bos taurus</i>
3	Common Bronzewing	<i>Phaps chalcoptera</i>
4	Crested Bellbird	<i>Oreoica gutturalis</i>
5	Crow	<i>Corvus bennetti</i>
6	Domestic Dog	<i>Canis familiaris</i>
7	Eastern Grey Kangaroo	<i>Macropus giganteus</i>
8	Emu	<i>Dromaius novaehollandiae</i>
9	Euro/Wallaroo	<i>Osphranter robustus</i>
10	European Rabbit	<i>Oryctolagus cuniculus</i>
11	Feral Cat	<i>Felis catus</i>
12	Feral Goat	<i>Capra hircus</i>
13	Grey Shrike Thrush	<i>Colluricincla harmonica</i>
14	Hall's Babbler	<i>Pomatostomus halli</i>
15	Humans	<i>Homo sapiens</i>
16	Sacred Kingfisher	<i>Todiramphus sanctus</i>
17	Lizard spp.	Squamata spp.
18	Magpie	<i>Gymnorhina tibicen</i>
19	Chestnut Quail Thrush	<i>Cinlosoma castanotum</i>
20	Red Kangaroo	<i>Osphranter rufus</i>
21	Domestic Sheep	<i>Ovis aries</i>
22	Short-billed Echidna	<i>Tachyglossus aculeatus</i>
23	Spotted Nightjar	<i>Eurostopodus argus</i>
24	Wedge Tailed Eagle	<i>Aquila audax</i>
25	Wild dog/dingo	<i>Canis familiaris</i>
26	Willy Wagtail	<i>Rhipidura leucophrys</i>
27	Yellow-footed Rock-wallaby	<i>Petrogale xanthopus</i>

**Table S11: Table of animals and ecologically relevant species per camera-day**

This table displays the sum of species per camera-day (sum of scored species/camera-day) for all ecologically relevant species. Macropods/camera-day is equal to the sum of Euro, EGK, RK and Unknown Macropod scores per camera/camera-day.

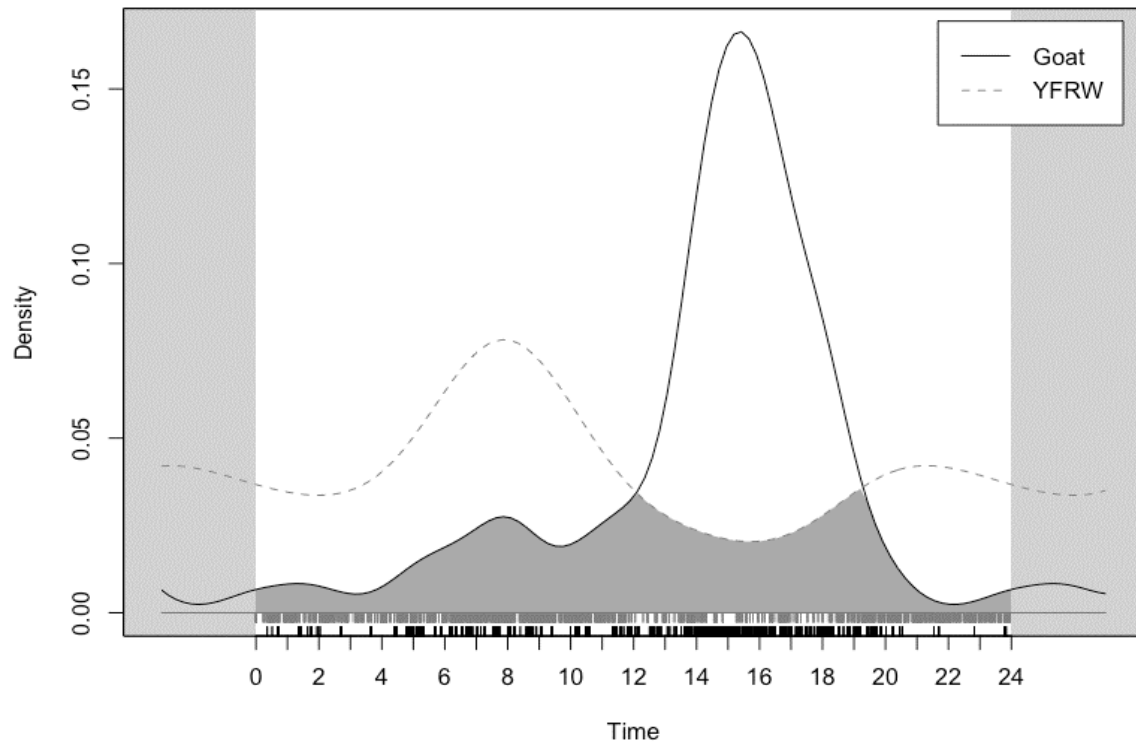
Camera	Camera days (c-d)	Animals/c-d	YFRW/c-d	Wild Dogs/c-d	Goats/c-d	EGK/c-d	Euro/c-d	RK/c-d	Macropods (exc. YFRW)/c-d	Rabbit/c-d	Cat/c-d
RW001	<b>726</b>	11.05	0.31	0.00	10.33	0.00	0.04	0.00	0.22	0.02	0.04
RW002	<b>691</b>	22.13	0.33	0.00	21.48	0.10	0.00	0.01	0.17	0.07	0.00
RW003	<b>652</b>	20.99	0.65	0.02	14.16	2.61	1.27	0.50	5.93	0.13	0.00
RW004	<b>726</b>	11.24	0.07	0.04	6.69	0.77	0.25	0.16	4.02	0.00	0.00
RW005	<b>674</b>	2.69	2.36	0.01	0.20	0.05	0.04	0.01	0.10	0.00	0.00
RW006	<b>599</b>	60.32	0.20	0.02	59.72	0.04	0.18	0.03	0.37	0.00	0.00
RW007	<b>726</b>	1.48	0.11	0.01	0.87	0.10	0.30	0.02	0.47	0.00	0.00
RW008	<b>689</b>	1.49	0.10	0.03	0.59	0.14	0.49	0.02	0.77	0.00	0.00
RW009	<b>696</b>	22.47	4.89	0.00	15.59	0.01	0.63	0.18	0.92	0.12	0.00
RW010	<b>726</b>	0.56	0.04	0.03	0.11	0.00	0.15	0.17	0.33	0.00	0.00
RW011	<b>726</b>	4.47	0.18	0.00	3.93	0.08	0.18	0.05	0.33	0.00	0.00
RW012	<b>726</b>	2.41	1.01	0.00	0.00	0.59	0.12	0.02	1.10	0.00	0.00
RW013	<b>664</b>	0.79	0.22	0.00	0.36	0.05	0.05	0.02	0.12	0.00	0.00
RW014	<b>726</b>	4.35	2.58	0.02	1.07	0.43	0.17	0.01	0.66	0.00	0.00
RW015	<b>625</b>	1.78	0.48	0.00	1.28	0.00	0.00	0.00	0.00	0.00	0.00
RW016	<b>612</b>	17.58	1.91	0.00	14.09	0.78	0.53	0.07	1.42	0.06	0.01
RW017	<b>614</b>	9.21	2.95	0.09	5.71	0.13	0.11	0.05	0.35	0.00	0.00
RW018	<b>407</b>	9.89	0.64	0.18	0.14	0.35	0.12	0.00	0.46	0.00	0.00
RW019	<b>506</b>	14.64	0.43	0.20	13.74	0.02	0.00	0.00	0.02	0.00	0.01
RW020	<b>405</b>	0.67	0.26	0.00	0.32	0.10	0.00	0.00	0.10	0.00	0.00
RW021	<b>405</b>	5.15	1.38	0.11	2.53	0.87	0.00	0.00	0.89	0.08	0.00
RW022	<b>405</b>	1.34	1.14	0.02	0.00	0.16	0.00	0.01	0.18	0.00	0.00
RW023	<b>270</b>	6.01	4.36	0.00	0.07	0.99	0.13	0.27	1.42	0.00	0.00
RW024	<b>404</b>	0.45	0.26	0.00	0.00	0.04	0.00	0.01	0.05	0.00	0.00
RW025	<b>288</b>	2.03	1.40	0.00	0.00	0.44	0.13	0.05	0.63	0.00	0.00
RW026	<b>404</b>	1.52	1.06	0.00	0.00	0.14	0.15	0.02	0.31	0.00	0.01

**Table S12: Chi-square test of equal proportions for YFRW behaviours, and age for YFRW and goats**

	Proportion IN	Proportion OUT	X-squared	df	p-value
YFRW Pouch Young	0.012	0.007	9.73	1	0.002
YFRW Juveniles	0.053	0.018	122.12	1	2.18e-28
YFRW Juv.+PY	0.065	0.025	128.64	1	8.14e-30
YFRW Resting	0.474	0.472	0.04	1	0.848
YFRW Hopping	0.103	0.217	398.09	1	1.44e-88
YFRW Foraging	0.255	0.141	301.54	1	1.52e-67
YFRW Interactions	0.021	0.042	59.98	1	9.60e-15
YFRW Other behav.	0.148	0.128	13.33	1	0.0002
Feral Goat Kids	0.037	0.012	233.09	1	1.26e-52

**Table S13: T-tests of significance in difference of activity across exclusion fencing**

	Value/camera-day	t-stat	df	p-value	u of IN	u of OUT
<b>YFRW Age class</b>	Adult	-0.05	23.72	0.96	0.75	0.77
	Juvenile	1.42	15.62	0.18	0.04	0.01
	Pouch Young (PY)	0.53	23.85	0.60	0.01	0.01
	Juvenile+PY	0.98	21.22	0.34	0.06	0.03
<b>Pooled Behaviour</b>	Pooled Resting	0.14	22.58	0.89	0.41	0.39
	Pooled Foraging	0.72	15.26	0.48	0.22	0.12
	Pooled Hopping	-0.65	10.72	0.53	0.09	0.18
	Pooled Interaction	-0.64	12.67	0.53	0.02	0.03
	Pooled Other	0.28	23.92	0.78	0.13	0.11
<b>Goat Age Class</b>	Adult Goats	1.83	16.72	0.09	9.91	2.24
	Kid Goats	2.53	14.42	0.02	0.37	0.03
<b>ANOVA</b>	<b>Value/camera-day</b>	<b>F-stat</b>	<b>df</b>	<b>p-value</b>		
<b>Wildlife</b>	Animals	2.72	(1,6)	0.15		
	YFRW	0.003	(1,6)	0.96		
	Dogs	1.78	(1,6)	0.23		
	Goats	2.94	(1,6)	0.14		
	Kids	4.23	(1,6)	0.09		
<b>YFRW Age class</b>	Female YFRW	3.20	(1,6)	0.12		
	Male YFRW	1.54	(1,6)	0.26		
	Pouch Young	0.37	(1,6)	0.57		
	Adult YFRW	0.008	(1,6)	0.93		
	Juvenile YFRW	5.72	(1,6)	0.053		
<b>Pooled Behaviour</b>	Other	0.13	(1,6)	0.73		
	interact	0.72	(1,6)	0.43		
	Hopping	0.63	(1,6)	0.46		
	Forage	0.90	(1,6)	0.38		
	Rest	0.06	(1,6)	0.82		

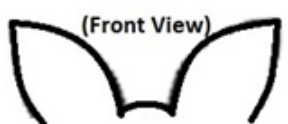


**Figure S8: Comparison of YFRW activity to feral goats in the OUT treatment ( $D_{hat4} = 0.37$ )**



**YFRW trapping data collection sheet**

- **MC:** Microchip ID number.
- **Date:** Date of capture event.
- **H/R(start):** Heart rate in beats per 15 seconds at first capture.
- **H/R(end):** Heart rate in beats per 15 seconds immediately prior to release.
- **Time:** Stopwatch reading, started at first approach to trap.
- **Weight:** Scale reading in kilograms (minus weight of trapping bag).
- **Sex:** Male or female animal.
- **R/foot:** Length of right foot from toe to heel in millimetres.
- **L/foot:** Length of left foot from toe to heel in millimetres.
- **Tail:** Length of tail from tip to base in millimetres.
- **Locale:** Trap site name.
- **Name:** Informal name of individual wallaby.
- **Comments:** Any usual/noteworthy observations about the trapping event/YFRW
- **Diagram of Rock-wallaby ears:** Indication of where the ear biopsy and ear tag were placed. Aids in identification of YFRW in photos and in the event of microchip failure.
- **Fecundity:** Data recorded on pouch young. Tick boxes indicate if pouch young was present and whether an ear biopsy was taken from the pouch young. Data also recorded on Sex, tail length (millimetres) and microchip number (if applicable).

<b>MC:</b>	
<b>Date:</b> /    /	<b>Trap:</b> .....
<b>H/R(start):</b> .....b/15sec	<b>Time:</b> .....
<b>H/R(end):</b> .....b/15sec	<b>Time:</b> .....
<b>Weight:</b> .....kg	<b>Sex:</b> M / F
<b>R/Foot:</b> .....mm	<b>L/Foot:</b> .....mm
<b>Tail:</b> .....mm	<b>Locale:</b> .....
<b>Name:</b> .....	
Comments.....	
.....	
.....	
	
<b>PY:</b> <input type="checkbox"/>	<b>Fecundity</b>
<b>DNA</b> <input type="checkbox"/>	<b>Sex:</b> ..... <b>Tail:</b> .....mm
	<b>M.Chip</b> .....

## Design of Soft-walled treadle traps

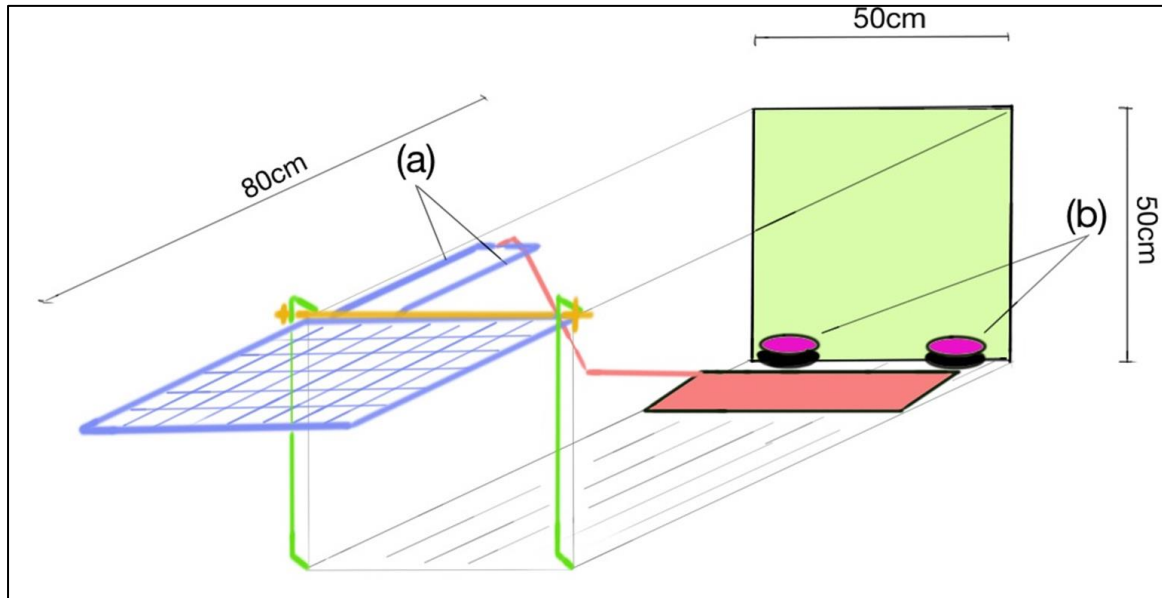


Figure S9: Diagram of soft-walled treadle trap

- **Function:**

The trap mechanism is shown in red. When an animal enters the trap it moves to the back to access the bait and water containers (b). When the treadle is depressed, the front gate (shown here in blue) is released by the treadle arm (red line). When the front gate drops and closes, the bar-lock (orange) slides down the rails (green), locking the animal in the trap. The animal is then accessed through the back gate (light green), which slides upwards and can be fully removed. During free baiting periods the front gate is locked open at (a) with two padlocks.

- **Design:**

The back gate is a sheet of thin metal. The frame of the trap (black lines) is comprised of metal rods. The floor and front gate are made from solid meshed wire. The roof and side walls are made from softer chicken-wire meshing, and shade cloth material. The dimensions of the trap (as displayed) are 50cm by 50cm by 80cm.

## **Tissue Dissection (aseptic protocol)**

Aseptic protocol was employed for the dissection of tissue samples into smaller tissue samples for extraction. This preserves half (or more) of the samples for later extraction if required. This process is best performed under a fume hood to allow extraction of gases from the flame process, and limit cross contamination, but it is not STRICTLY necessary.

1. First, all instruments and required solutions are prepared. The instruments required for this protocol are: scalpel and handle, 2 sets of fine point tweezers, open flame e.g. Bunsen burner, 100% ethanol wash bottle, beaker of 100% ethanol, lab wipes/lab tissues, disposable dishes (one for each sample, lab weigh boats are good for this purpose), gloves, lab coat and glasses.
2. All samples require a new labelled tube. If the newly dissected samples are to be extracted immediately after dissection, each new tube should contain 240 $\mu$ L of Digsol, as required for the first step of the extraction protocol. If they are to be stored, the tube should contain ~1ml of Longmire's storage buffer.
3. All instruments are placed into the beaker of ethanol, then removed and excess ethanol burnt off over the Bunsen burner. Hold the instrument in the flame briefly, and remove. Allow the ethanol on the instrument to burn out. And lay each instrument out on a new lab wipe, be careful that the flame has completely expired, the flame can be hard to see.
4. After this process, pick up each instrument and using the wash bottle of ethanol over the beaker, rinse each instrument and place back down on the wipe, do not flame the instruments this time.
5. Prepare a single dish (weigh boat) and using the tweezers remove the original sample from its tube and place it in the dish. Using the scalpel and tweezers, halve the sample. If one 'half' is bigger, use the tweezers to place this part back in the original sample tube, and then the smaller half to the new labelled tube.
6. Place the instruments back in the beaker of ethanol and discard the lab wipe and the dish.
7. Repeat steps 3 to 6 for all samples.

## **Tissue DNA Extraction (Ethanol precipitation)**

1. Move 3-4mm<sup>3</sup> of samples into a labelled 1.5ml tube, (unnecessary if proceeding straight from dissection protocol)
2. Add 240µL of Digsol
3. Add 10µL of 20mg/ml Proteinase K
4. Incubate at 50°C for a few hours or overnight
5. Spin down tubes, add 190µL of 7.5M AmAc
6. Vortex/shake, let sit for 15 minutes
7. Centrifuge/spin on maximum speed for 20 min
8. Transfer clear supernatant to new labelled tube, discard pellet (repeat if any pellet transfers to the new tube)
9. Add 1ml of 100% ethanol to supernatant tubes
10. Mix tubes gently, inverting them 5-10 times
11. Inspect and note DNA quantities visible
12. Spin on maximum speed for 15 minutes
13. Gently tip out/remove ethanol from tube, invert tube onto absorbent paper to dry
14. Add 1ml of 70% ethanol to supernatant tubes
15. Spin on maximum speed for 10 minutes
16. Gently tip out/remove ethanol from tube, invert tube onto absorbent paper to completely dry
17. Add TE buffer to pelleted DNA, volume based on DNA observed in Step 11. From 50-200µL for small to large.
18. Resuspend DNA by vortexing tube
19. Incubate at 37°C for a few hours or overnight
20. Use straight away or spin down and freeze

## **Qubit™ Fluorometric Quantification– High Sensitivity Kit - Invitrogen**

1. Prepare working solution by diluting 1:200 Qubit™ reagent with Qubit™ Buffer, 200µL per sample and per standard, and an extra volume for pipetting error (n+3)
2. Aliquot 190µL of working solution into two tubes and add 10µL of Standard 1 to the first, and 10µL of Standard 2 to the second.
3. Aliquot 198µL of working solution to n tubes
4. Add 2µL of a sample DNA to each tube
5. Incubate for 2 minutes
6. Calibrate Qubit™ using Standard 1 and Standard 2 tubes,
7. Insert Sample tubes and record DNA quantity in ng/mL

## ThermoFisher Scientific NanoDrop™ 8000 - DNA Quality Control

1. All tissue samples were quality controlled on a NanoDrop™ 8000
2. The NanoDrop™ is first cleaned with ddH<sub>2</sub>O or MilliQ water, and then blanked with elution buffer (TE buffer). To do this, 2ul of the water is loaded onto each measurement pedestal, the lid closed, and a run completed. The process is repeated for TE buffer to blank the readings.
3. Sample IDs were entered into the appropriate dialog box for all samples before processing.
4. 2ul of 8 samples are then loaded onto each of the measurement pedestals, the lid closed and a run completed. Ensure that all pedestals that have a sample loaded are activated on the desktop screen before running. If an error occurs with any sample, reload, clean the pedestals with a lab tissue and reload just that pedestal with a volume from the same sample. Activate just that pedestal on the desktop and run again.
5. After all 8 samples have been read, and the run is complete, the lights for the corresponding column will go green on both the NanoDrop™ and the desktop display. After this occurs you can lift the lid/arm and clean the pedestals by placing a folded lab tissue between the pedestals and closing the lid.
6. Repeat steps 3 and 4 until all samples have been completed.
7. If the NanoDrop™ asks for re-blanking, clear whatever samples are present from the pedestals and load 2ul of TE buffer, click 'Blank' on the desktop.
8. Save the table of results from NanoDrop™, and if required save the image from each column of data (this is only required if you desire the spectral image graph). The table of results should be saved in a .txt format for easy transfer to Microsoft Excel.
9. Results should be carefully assessed to ensure all samples are of an appropriate quality. A 260/280 ratio of ~1.8 is desirable. A spectral graph showing a smooth absorbance trough at ~230nm and a single peak at ~260nm before decreasing to ~0 absorbance by ~300nm is desired.

For more information of NanoDrop™ readouts and use, see: [www.thermofisher.com](http://www.thermofisher.com) and navigate to the NanoDrop™ 8000 user manual.

## Unfiltered data – Reports and data exploration

**Table S14: Base frequencies**

Base	Frequency (%)
A	27.95
G	23.62
T	27.35
C	21.08

**Table S15: Proportion of SNP types**

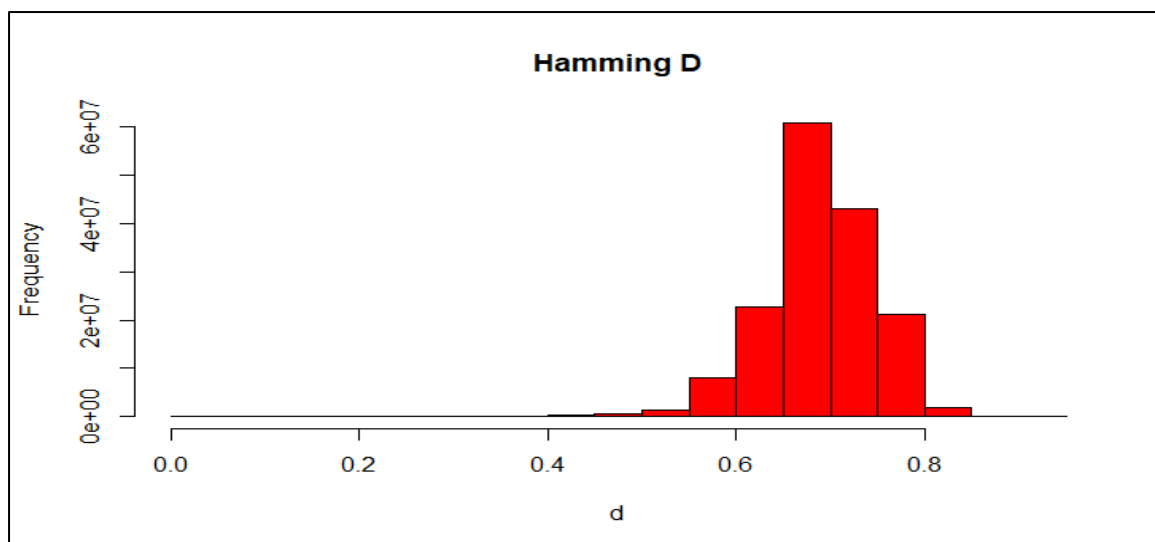
SNP type	Percentage
Transitions (ts)	68.23%
Transversions (tv)	31.77%
tv/ts ratio	2.1478

**Table S16: Proportion of SNPs with missing genotypes**

Percentage of missing values	SNP count	Percentage of total SNPs
Loci with no missing values	9988	55.9%
< 5% missing values	13659	76.4%
< 10% missing values	15241	85.3%
< 15% missing values	15852	88.7%
< 20% missing values	16294	91.2%
< 25% missing values	16559	92.6%
< 30% missing values	16776	93.9%
< 35% missing values	16975	95%
< 40% missing values	17117	95.8%
< 45% missing values	17264	96.6%
< 50% missing values	17365	97.2%
< 55% missing values	17452	97.6%
< 60% missing values	17565	98.3%
< 65% missing values	17626	98.6%
< 70% missing values	17733	99.2%
< 75% missing values	17824	99.7%

**Table S17: Proportion of individuals with missing genotypes**

Percentage of missing values	No. included	% of tot.	No. Removed
Individuals no missing values	0	0	92
with less than or equal to 5%	37	40.2	55
with less than or equal to 10%	88	95.7	4
with less than or equal to 15%	91	98.9	1
with less than or equal to 20%	92	100	0

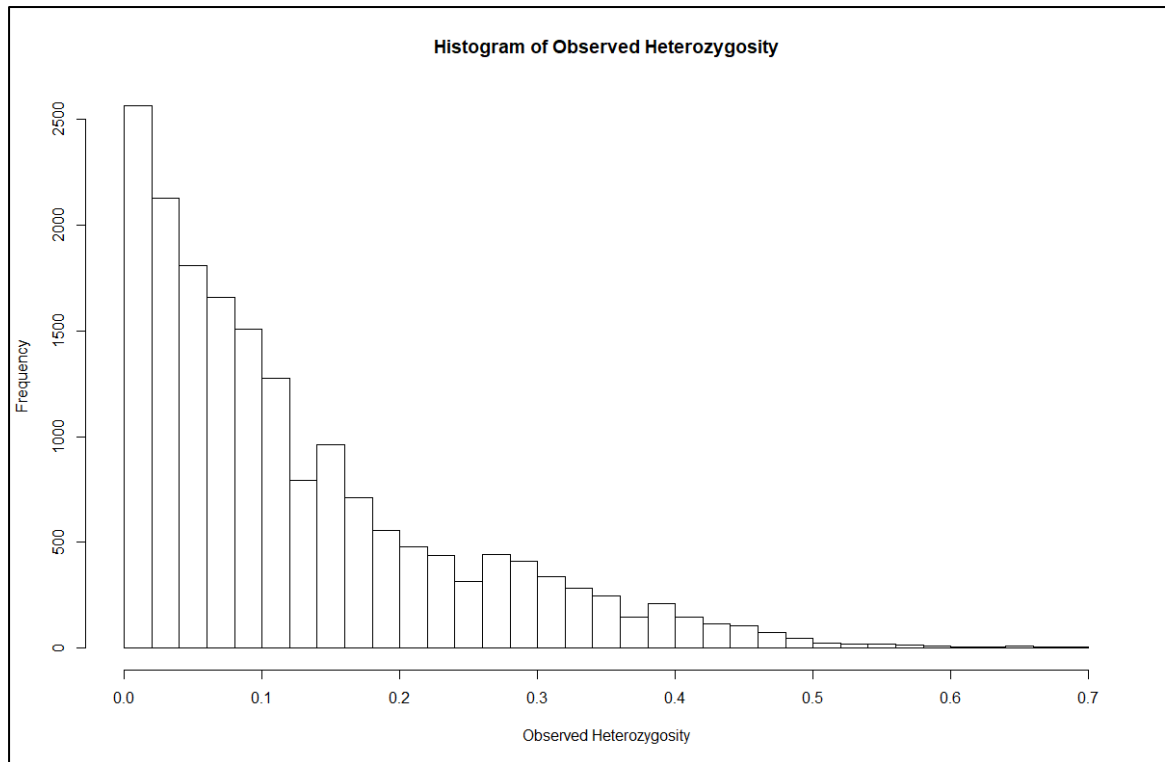


**Figure S10: Frequency plot of pairwise hamming distances**  
 Mean Hamming Distance 0.69+/-0.06SD (0 - 0.93)

**Table S18: Average repeatability of SNPs**

Proportion = Count [as % of total]
1.0 = 15296 [85.6%]
0.995 = 15296 [85.6%]
0.99 = 15296 [85.6%]
0.985 = 15296 [85.6%]
0.98 = 15296 [85.6%]
0.975 = 16749 [93.7%]
0.97 = 16833 [94.2%]
0.965 = 16850 [94.3%]
0.96 = 16867 [94.4%]
0.955 = 16871 [94.4%]
0.95 = 17390 [97.3%]
0.945 = 17410 [97.4%]
0.94 = 17428 [97.5%]
0.935 = 17436 [97.6%]
0.93 = 17632 [98.7%]
0.925 = 17711 [99.1%]
0.92 = 17716 [99.1%]
0.915 = 17723 [99.2%]
0.91 = 17726 [99.2%]
0.905 = 17797 [99.6%]
0.9 = 17824 [99.7%]
0.895 = 17824 [99.7%]
0.89 = 17828 [99.7%]
0.885 = 17863 [99.9%]
0.88 = 17864 [99.9%]
0.875 = 17866 [100%]





**Figure S11: Report of Observed Heterozygosity by loci**

## Dataset 1 – Post-filter (All samples)

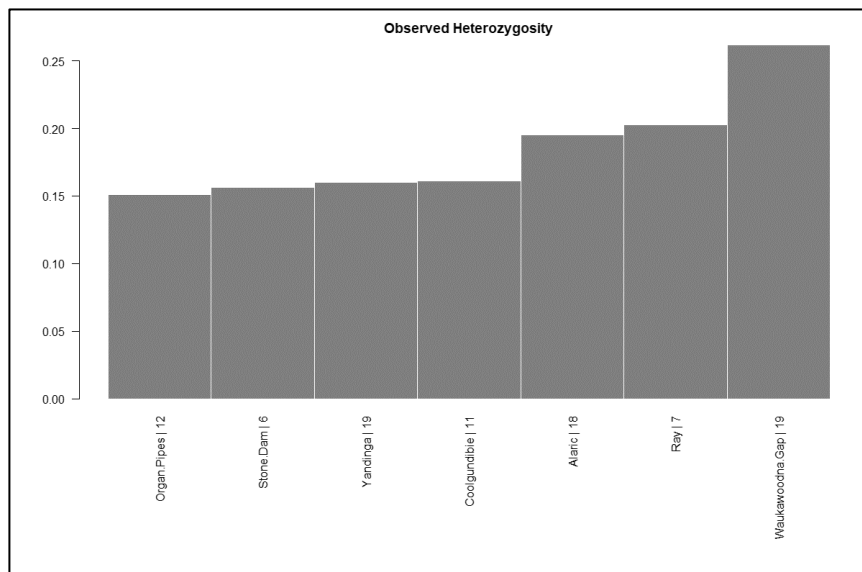


Figure S12: Comparison of observed heterozygosity among all colonies

Table S19: Examination of pairwise population private and fixed alleles (all colonies)

p1	p2	pop1	pop2	N1	N2	fixed	priv1	priv2	totalpriv	mdf
1	2	Alaric	Coolgundibie	18	11	877	4333	3068	7401	0.444
1	3	Alaric	Organ Pipes	18	12	823	4141	3120	7261	0.441
1	4	Alaric	Ray	18	7	0	774	142	916	0.071
1	5	Alaric	Stone Dam	18	6	918	4436	3011	7447	0.443
1	6	Alaric	waukawoodna Gap	18	19	196	1983	3687	5670	0.377
1	7	Alaric	Yandinga	18	19	732	3916	3215	7131	0.439
2	3	Coolgundibie	Organ Pipes	11	12	0	227	471	698	0.061
2	4	Coolgundibie	Ray	11	7	1076	3525	4158	7683	0.446
2	5	Coolgundibie	Stone Dam	11	6	0	462	302	764	0.074
2	6	Coolgundibie	waukawoodna Gap	11	19	48	569	3538	4107	0.268
2	7	Coolgundibie	Yandinga	11	19	0	102	666	768	0.057
3	4	Organ Pipes	Ray	12	7	1012	3597	3986	7583	0.443
3	5	Organ Pipes	Stone Dam	12	6	0	630	226	856	0.066
3	6	Organ Pipes	waukawoodna Gap	12	19	37	589	3314	3903	0.262
3	7	Organ Pipes	Yandinga	12	19	0	188	508	696	0.047
4	5	Ray	Stone Dam	7	6	1112	4263	3470	7733	0.446
4	6	Ray	waukawoodna Gap	7	19	273	1943	4279	6222	0.382
4	7	Ray	Yandinga	7	19	913	3775	3706	7481	0.441
5	6	Stone Dam	waukawoodna Gap	6	19	49	542	3671	4213	0.267
5	7	Stone Dam	Yandinga	6	19	0	100	824	924	0.062
6	7	waukawoodna Gap	Yandinga	19	19	29	3046	641	3687	0.257

Table S20: Private allele distance matrix (all colonies)

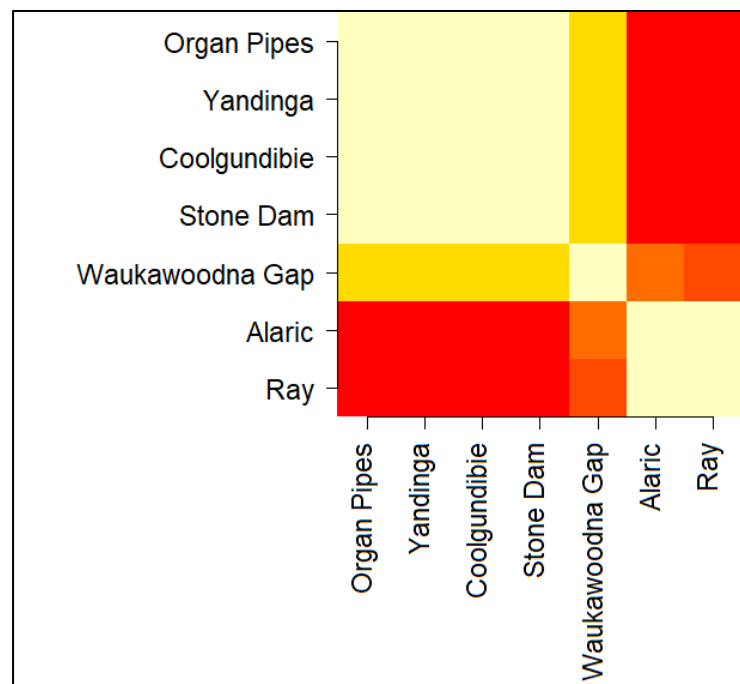
	Alaric	Cool	Organ	Ray	Stone	waukawoodna	Yandinga
Alaric	0	10	9	0	10	2	8
Cool	10	0	0	12	0	1	0
Organ	9	0	0	11	0	0	0
Ray	0	12	11	0	12	3	10
Stone	10	0	0	12	0	1	0
W.Gap	2	1	0	3	1	0	0
Yandi	8	0	0	10	0	0	0

**Table S21: Fixed allele distance matrix (all colonies)**

	Alaric	Cool	Organ	Ray	Stone	Waukawoodna	Yandinga
Alaric	NA	7527	7387	930	7571	5781	7255
Cool	7527	NA	724	7809	786	4128	806
Organ	7387	724	NA	7709	876	3932	716
Ray	930	7809	7709	NA	7861	6329	7605
Stone	7571	786	876	7861	NA	4242	946
W.Gap	5781	4128	3932	6329	4242	NA	3708
Yandi	7255	806	716	7605	946	3708	NA

**Table S22: Euclidean genetic distance matrix (all colonies)**

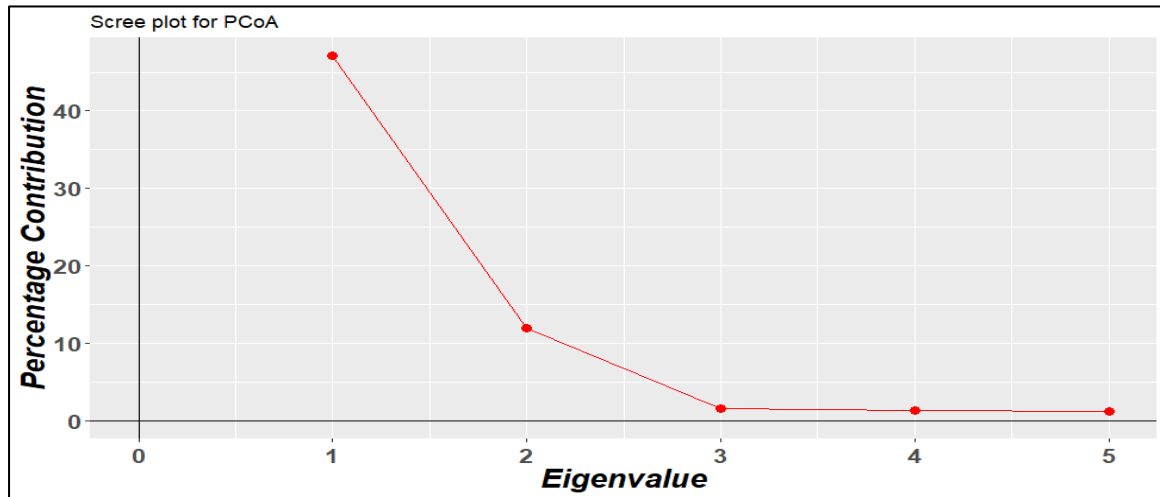
	Alaric	Cool	Organ	Ray	Stone	Waukawoodna	Yandinga
Alaric	0.0000	52.1183	51.5703	11.6284	52.1688	43.5311	51.1539
Cool	52.1183	0.0000	11.0398	52.8330	13.7532	34.5658	10.3056
Organ	51.5703	11.0397	0.0000	52.2987	11.7473	33.8224	8.22993
Ray	11.6284	52.8330	52.2987	0.0000	52.8793	44.3607	51.8589
Stone	52.1688	13.7533	11.7473	52.8793	0.0000	34.5658	10.9469
W.Gap	43.5312	34.5658	33.8224	44.3607	34.5658	0.0000	33.1720
Yandi	51.1540	10.3056	8.2299	51.8588	10.9469	33.1720	0.0000



**Figure S13: Heat-map of Nei's genetic distance matrix (all colonies)**

**Table S23: Nei's genetic distance matrix (all colonies)**

	Ray	Organ	W.Gap	Alaric	Yandinga	Stone	Cool
Ray	0.00000	0.446816	0.321843	0.018374	0.440295	0.454929	0.454854
Organ	0.44681	0.000000	0.170050	0.436437	0.008871	0.017927	0.015847
W.Gap	0.32184	0.170050	0.000000	0.312025	0.164194	0.176354	0.176784
Alaric	0.01837	0.436437	0.312025	0.000000	0.430541	0.444758	0.444608
Yandi	0.44030	0.008871	0.164194	0.430541	0.000000	0.015573	0.013820
Stone	0.45493	0.017927	0.176354	0.444758	0.015573	0.000000	0.024544
Cool	0.45485	0.015847	0.176784	0.444608	0.013820	0.024544	0.000000



**Figure S14: Scree plot of Eigen values (all colonies)**

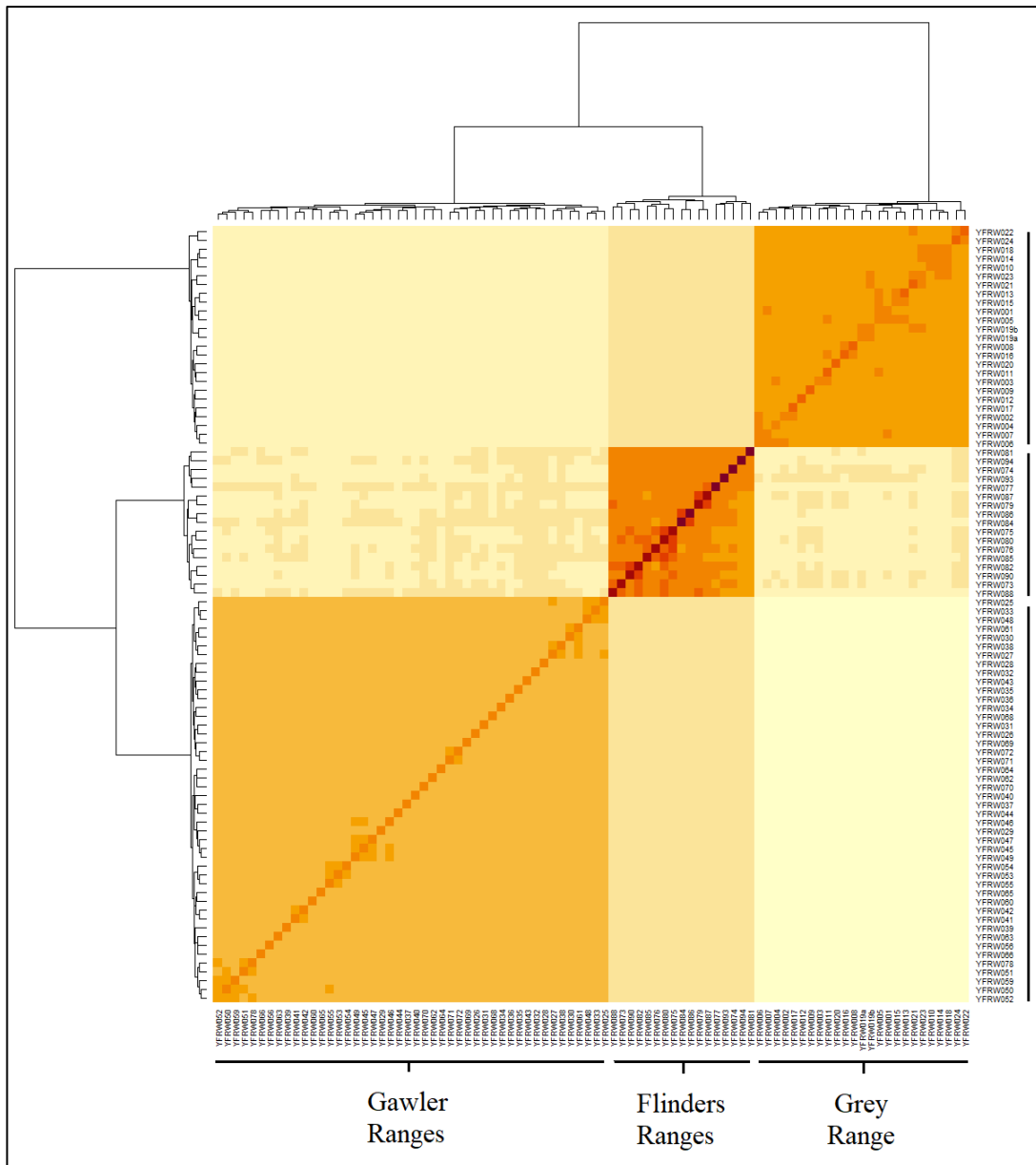


Figure S15: Heat-map and dendrogram of all samples from GRM (all colonies)

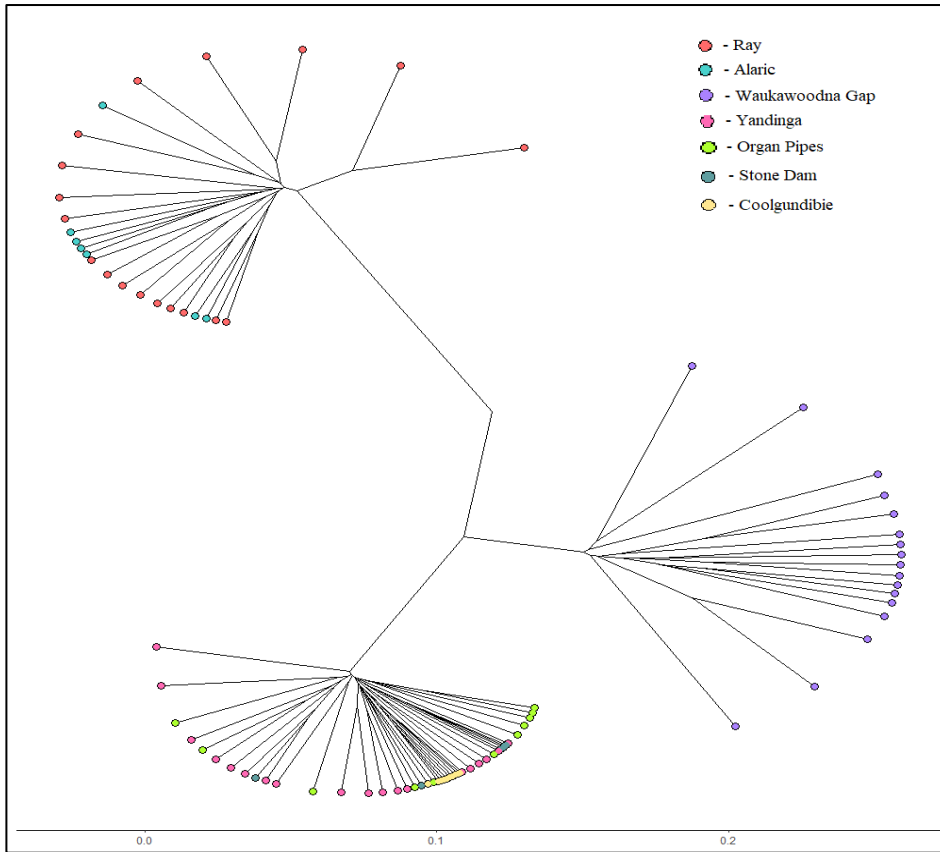


Figure S16: Unrooted dendrogram, calculated by IBS (all colonies)

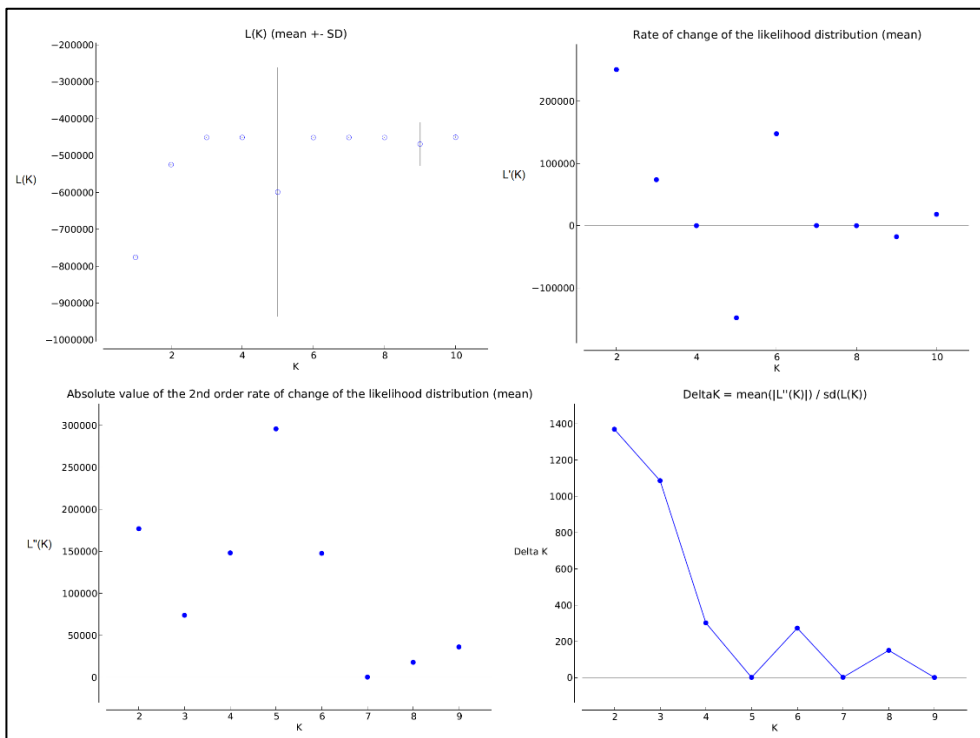


Figure S17: Output graphs of Structure Harvester (all colonies)

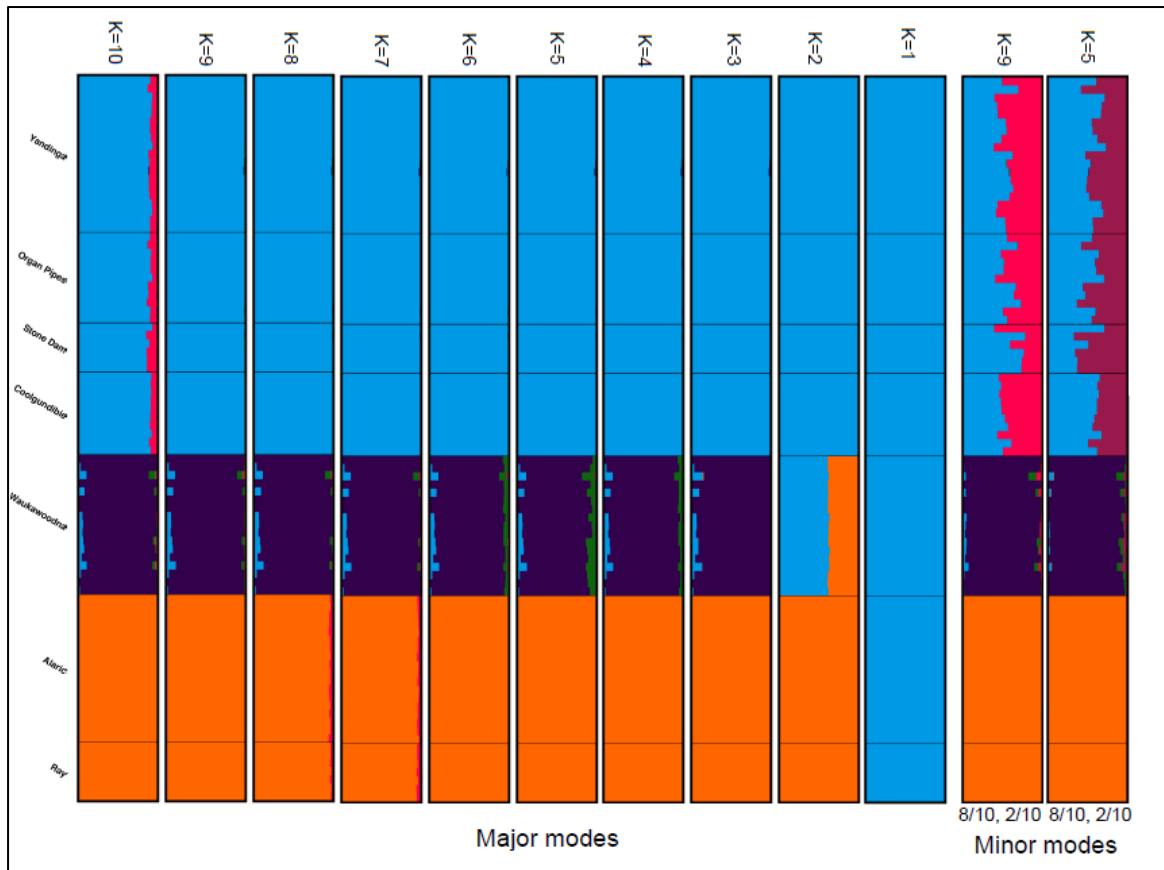


Figure S18: Bar plots for all values of K (CLUMPAK – all colonies)

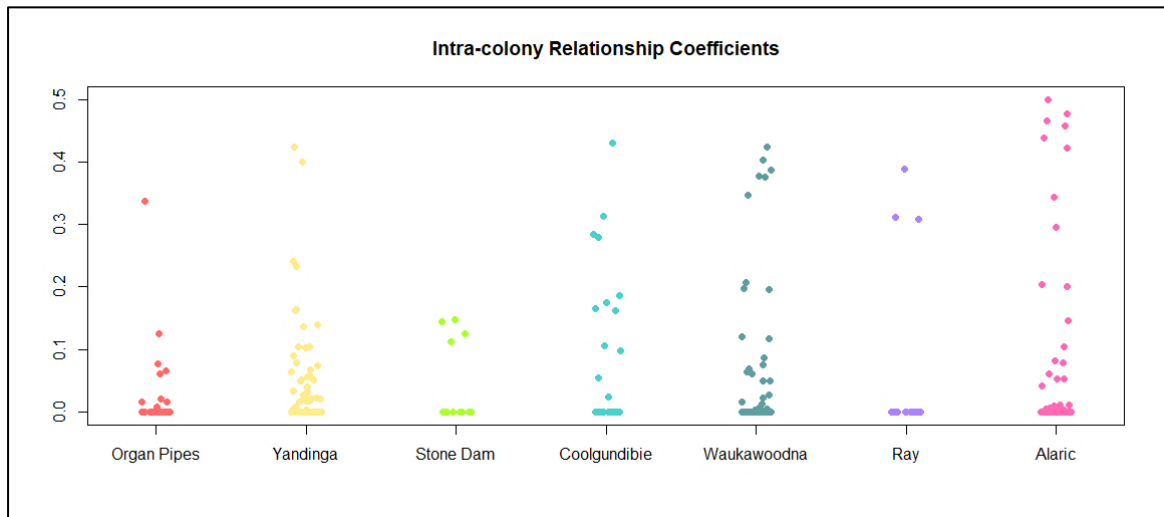


Figure S19: Intra-colony relationship coefficients (all colonies)

**Table S24: Table of intra-colony relationships greater than 0 (all colonies)**

Population	Colony	<i>n</i>	Average intra-colony <i>r</i>	% <i>r</i> > 0	% <i>r</i> > 0.0625
Gawler	Organ Pipes	11	0.013	9/55 = 16.4%	10/55 = 7.27%
Gawler	Yandinga	19	0.018	38/171 = 22.2%	16/171 = 9.36%
Gawler	Stone Dam	6	0.035	4/15 = 26.7%	4/15 = 26.7%
Gawler	Coolgundibie	10	0.051	12/45 = 26.7%	10/45 = 22.2%
Flinders	Waukawoodna	17	0.027	25/136 = 18.4%	15/136 = 11.0%
Grey	Ray	7	0.048	3/21 = 14.3%	3/21 = 14.3%
Grey	Alaric	18	0.029	26/153 = 17.0%	14/153 = 9.15%



## Dataset 2 – Post-filter (Gawler Ranges)

**Table S25: Examination of pairwise population private and fixed alleles (Gawler colonies)**

p1	p2	pop1	pop2	N1	N2	fixed	priv1	priv2	totalpriv	mdf
1	2	Coolgundibie	Organ Pipes	10	11	0	118	252	370	0.145
1	3	Coolgundibie	Stone Dam	10	6	0	345	190	535	0.177
1	4	Coolgundibie	Yandinga	10	19	0	10	293	303	0.136
2	3	Organ Pipes	Stone Dam	11	6	0	400	111	511	0.153
2	4	Organ Pipes	Yandinga	11	19	0	10	159	169	0.107
3	4	Stone Dam	Yandinga	6	19	0	5	443	448	0.144

**Table S26: Nei's genetic distance matrix (Gawler colonies)**

	[,1]	[,2]	[,3]	[,4]
<b>Organ Pipes</b>	0.000000	0.028230	0.056408	0.050983
<b>Yandinga</b>	0.028230	0.000000	0.048947	0.044437
<b>Stone Dam</b>	0.056408	0.048947	0.000000	0.076661
<b>Coolgundibie</b>	0.050983	0.044437	0.076661	0.000000

**Table S27: NeEstimator output of Gawler Ranges (for all lowest allele frequencies – Gawler colonies)**

Number of Loci = 3578  
Mating Model: Random  
Lowest allele frequencies used, ordered in each population:  
0.0500 0.0200 0.0100  
Up to 17 righthmost characters can be shown for population names.

Population # then :Name	Samp Size	Crit. Value	Weighted H. Mean	#Indep. Alleles	r^2	Exp(r^2) Sample	Ne^	CIs for Ne^				
								Parametric	Jackknife	Samp	(Eff.df)	
1:YFRW050	10	0.0500	10.0	5389380	0.172280	0.149870	8.4	11.6	11.9	5.5	35.9	990
		0.0200	10.0	5389380	0.172280	0.149870	8.4	11.6	11.9	5.5	35.9	990
		0.0100	10.0	5389380	0.172280	0.149870	8.4	11.6	11.9	5.5	35.9	990
2:YFRW061	11	0.0500	10.7	5194505	0.141802	0.125583	26.3	16.9	17.3	11.2	30.6	3193
		0.0200	10.7	5828894	0.131780	0.128481	31.1	87.6	96.1	28.6	Infinite	3091
		0.0100	10.7	5828894	0.131780	0.128481	31.1	87.6	96.1	28.6	Infinite	3091
3:YFRW044	6	0.0500	6.0	4892852	0.318820	0.267178	4.5	3.0	3.0	2.0	8.8	1269
		0.0200	6.0	4892852	0.318820	0.267178	4.5	3.0	3.0	2.0	8.8	1269
		0.0100	6.0	4892852	0.318820	0.267178	4.5	3.0	3.0	2.0	8.8	1269
4:YFRW025	19	0.0500	18.8	6211676	0.070939	0.062814	35.1	35.8	36.5	22.9	72.6	2238
		0.0200	18.8	6359513	0.069192	0.062659	36.6	44.9	45.9	27.2	108.6	2418
		0.0100	18.8	6359513	0.069192	0.062659	36.6	44.9	45.9	27.2	108.6	2418

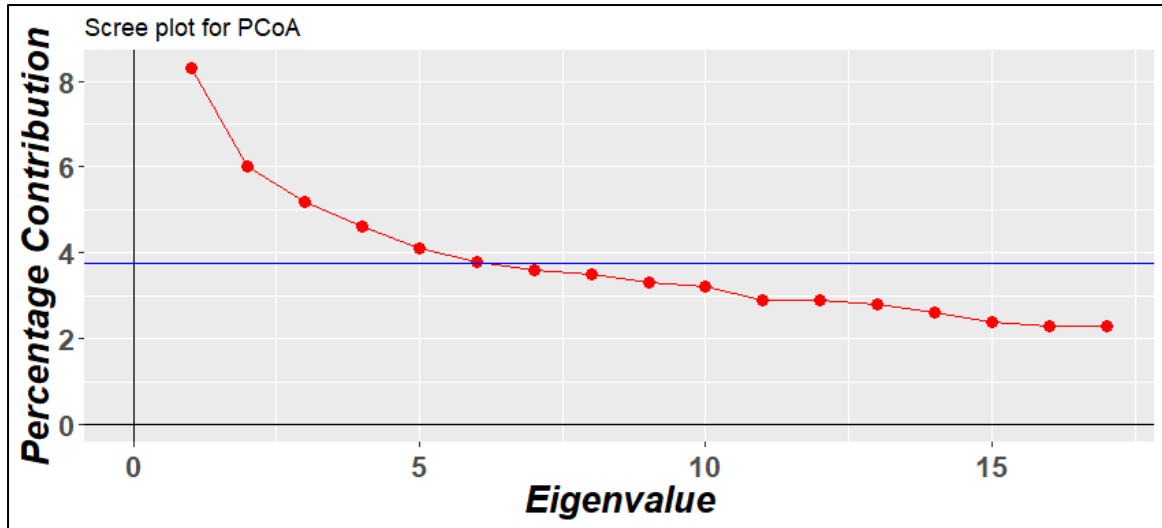


Figure S20: Scree plot of Eigen values (Gawler colonies)

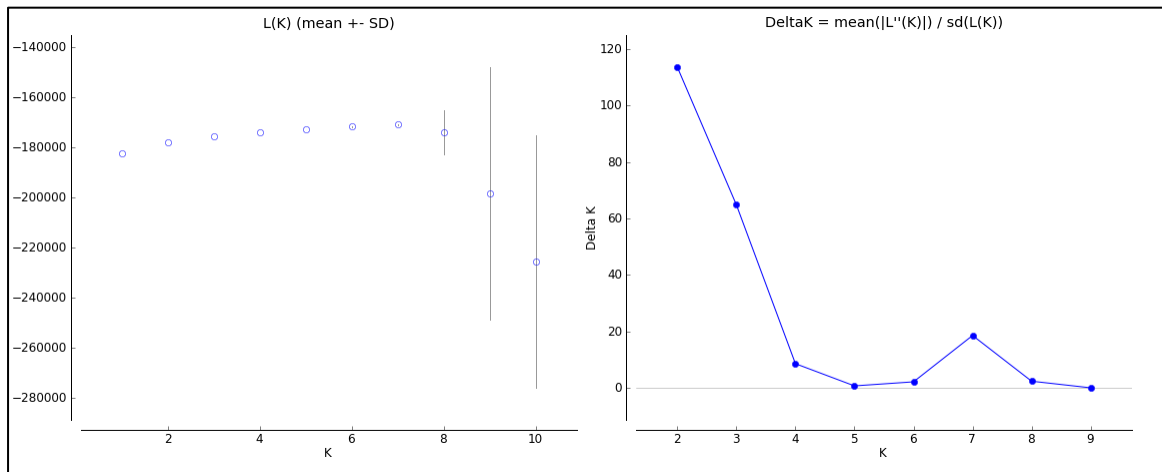


Figure S21: Output graphs of Structure Harvester (Gawler colonies)

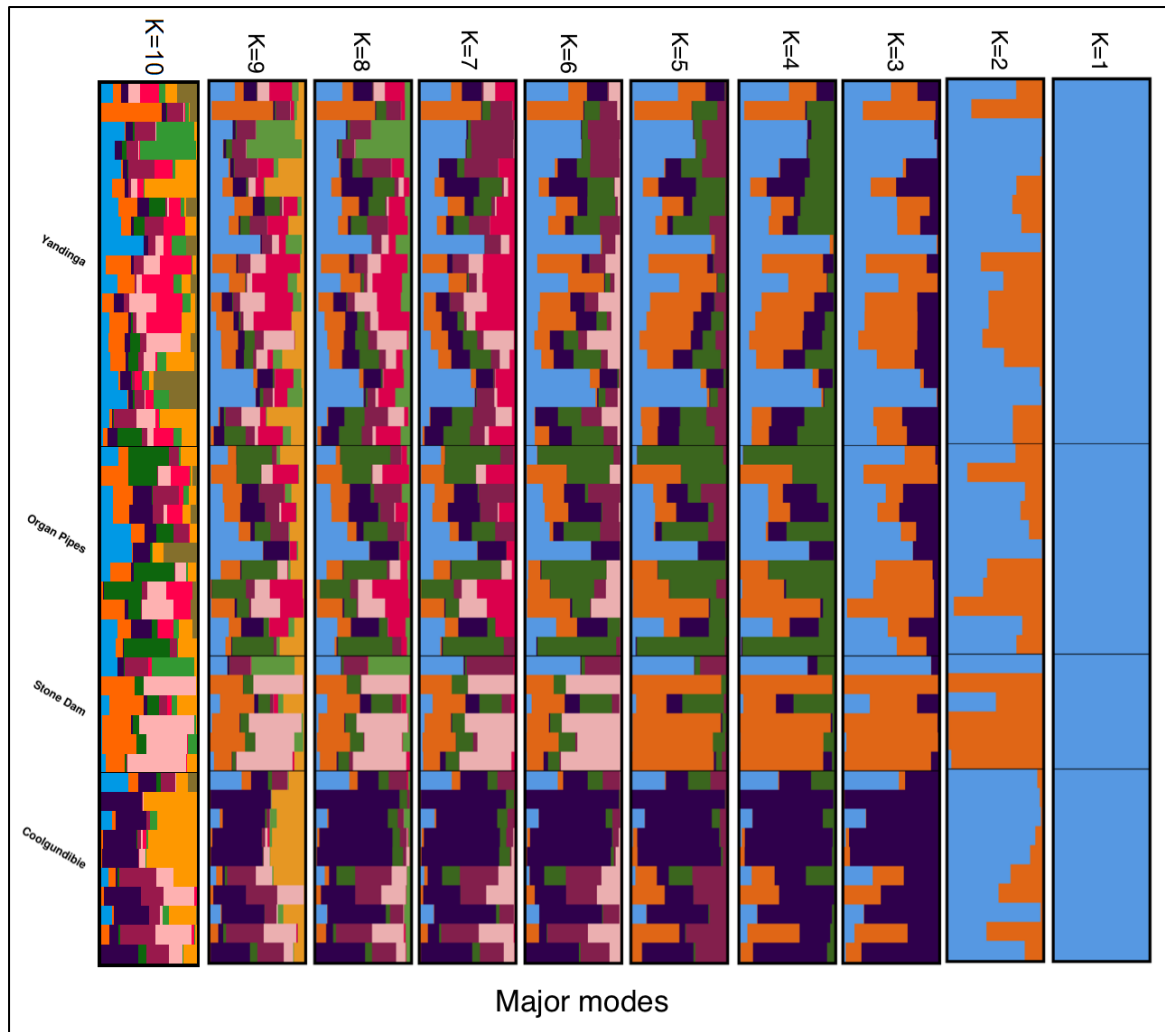


Figure S22: Bar plots of all values of K (major modes – Gawler colonies)

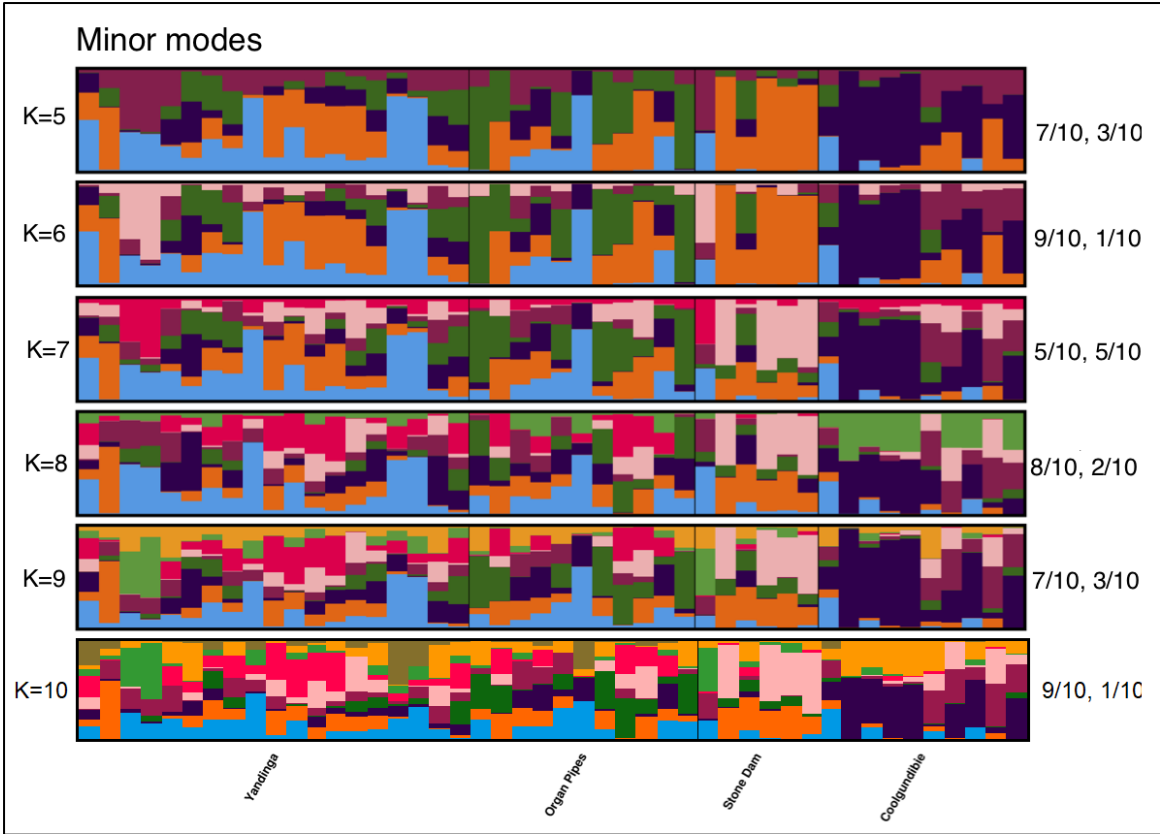


Figure S23: Bar plots of  $\alpha$  values of  $K$  (minor modes – Gawler colonies)

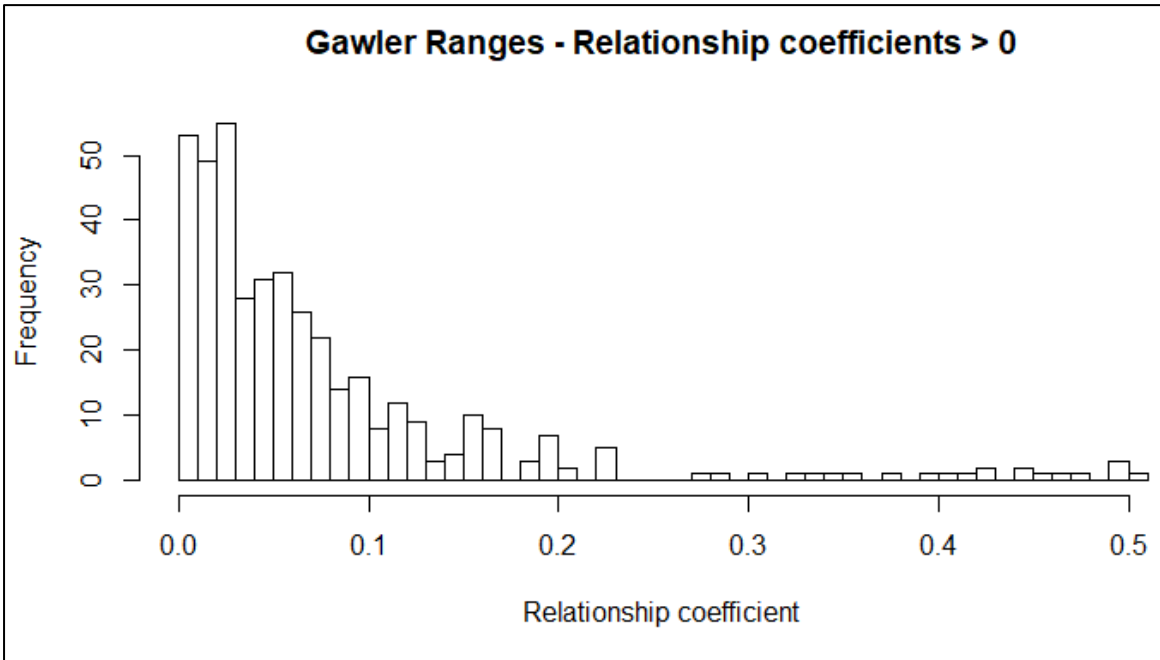


Figure S24: Histogram of  $r > 0$  from IBD analysis (Gawler colonies)

**Table S28: Output of GeneClass2 (Gawler colonies)**

Population 1) Coolgundibie, 2) Organ pipes, 3) Stone dam and 4) Yandinga as sampling colony. Samples in red are putative migrants. Green column indicates assignment colony.

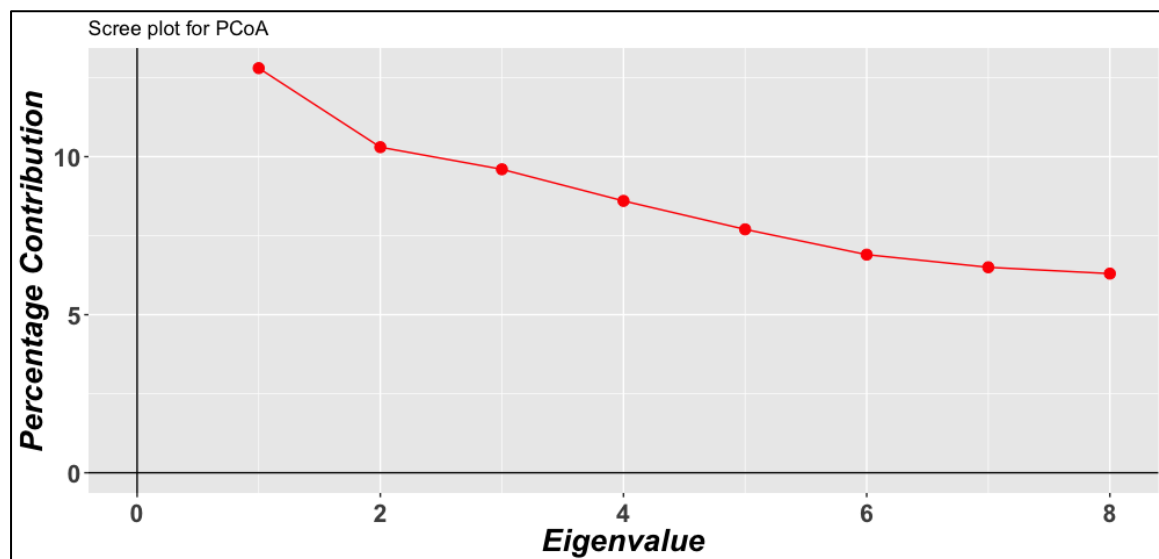
Assigned sample	home	$\log(L_{\text{home}} / L_{\text{max}})$	probability	1 $\log(L)$	2 $\log(L)$	3 $\log(L)$	4 $\log(L)$	Nb. of loci
1/	1	0.000	0.500	1184.983	1432.711	1601.235	1406.292	3575
1/	1	0.000	0.500	1243.592	1399.057	1556.532	1371.103	3577
1/	1	0.000	0.500	1149.700	1379.752	1529.694	1337.813	3576
1/	1	0.000	0.500	1180.174	1383.133	1396.524	1335.984	3577
1/	1	1.113	0.000	1327.425	1393.570	1386.898	1326.312	3573
1/	1	0.000	0.500	1147.603	1354.441	1479.071	1342.176	3575
1/	1	127.519	0.000	1452.305	1324.786	1576.578	1331.417	3574
1/	1	0.000	0.500	1244.618	1445.273	1560.615	1376.275	3575
1/	1	59.840	0.000	1432.143	1433.829	1425.729	1372.302	3572
1/	1	0.000	0.500	1192.533	1386.842	1550.771	1365.897	3573
2/	2	157.855	0.000	1503.452	1534.478	1668.864	1376.623	3568
2/	2	0.000	0.500	1604.721	1379.010	1570.444	1451.795	3567
2/	2	62.546	0.000	1473.589	1418.163	1577.848	1355.617	3569
2/	2	0.000	0.500	1611.007	1330.702	1422.069	1398.983	3569
2/	2	99.705	0.000	1350.824	1410.821	1480.665	1311.116	3572
2/	2	56.835	0.000	1365.810	1396.545	1541.550	1339.711	3552
2/	2	25.571	0.000	1388.657	1324.851	1450.104	1299.279	3309
2/	2	0.000	0.500	1561.126	1347.317	1408.979	1426.904	3508
2/	2	84.131	0.000	1615.722	1471.493	1387.362	1460.442	3550
2/	2	0.000	0.500	1532.228	1335.628	1534.536	1419.707	3531
2/	2	0.000	0.500	1580.146	1344.743	1557.899	1403.205	3573
3/	3	176.771	0.000	1477.408	1316.545	1493.316	1343.864	3578
3/	3	0.000	0.500	1507.283	1415.136	1174.072	1405.398	3575
3/	3	0.000	0.500	1596.642	1442.343	1217.040	1395.064	3573
3/	3	0.000	0.500	1504.213	1446.101	1308.384	1396.323	3573
3/	3	505.720	0.000	1500.412	1471.234	1818.494	1312.774	3578
3/	3	0.000	0.500	1499.346	1358.820	1110.213	1347.213	3574
4/	4	0.000	0.500	1497.038	1460.734	1563.354	1347.729	3550
4/	4	0.000	0.500	1446.916	1410.554	1538.296	1408.332	3569
4/	4	0.000	0.500	1648.254	1511.448	1640.795	1399.032	3523
4/	4	0.000	0.500	1562.234	1472.730	1478.020	1456.136	3574
4/	4	27.720	0.000	1400.505	1397.132	1342.886	1370.605	3574
4/	4	0.000	0.500	1640.649	1489.564	1677.745	1446.895	3570
4/	4	0.000	0.500	1462.071	1501.341	1573.079	1446.209	3575
4/	4	0.000	0.500	1636.730	1596.290	1643.314	1437.113	3568
4/	4	0.000	0.500	1477.141	1420.031	1431.643	1328.026	3494
4/	4	29.924	0.000	1588.047	1379.347	1573.680	1409.272	3535
4/	4	0.000	0.500	1561.016	1465.474	1503.658	1351.712	3568
4/	4	0.000	0.500	1535.831	1391.287	1453.249	1372.339	3577
4/	4	0.500	0.000	1440.822	1370.481	1415.706	1370.981	3577
4/	4	0.000	0.500	1666.864	1506.210	1723.445	1434.730	3576
4/	4	0.000	0.500	1518.239	1449.210	1586.708	1443.678	3573
4/	4	72.667	0.000	1631.039	1472.679	1457.919	1530.586	3571
4/	4	0.000	0.500	1399.948	1397.579	1501.792	1305.138	3571
4/	4	0.000	0.500	1530.949	1392.645	1590.169	1354.648	3574
4/	4	0.000	0.500	1690.338	1494.491	1593.976	1367.915	3572

### Dataset 3 – Post-filter (Flinders Ranges)

**Table S29: NeEstimator output for Waukawoodna Gap (for all lowest allele frequencies – Flinders Colony)**

```

Number of Loci = 7979
LD mating model: Random
(Symbol "No S*" in Frequency means that NO Singleton Alleles are accepted.)
Population      1 [YFRW073]  (Number of Individuals = 17)
*****
-----
Lowest Allele Frequency Used    0.050      0.020      0.010      No S*      0+
-----
LINKAGE DISEQUILIBRIUM METHOD
Harmonic Mean Sample Size =      17.0      17.0      17.0      17.0      17.0
Independent Comparisons = 31828231  31828231  31828231  31828231  31828231
OverAll r^2 =                    0.083757  0.083757  0.083757  0.083757  0.083757
Expected r^2 Sample =            0.070516  0.070516  0.070516  0.070516  0.070516
Estimated Ne^ =                   21.4      21.4      21.4      21.4      21.4
95% CIs for Ne^
* Parametric                      21.4      21.4      21.4      21.4      21.4
                                   21.5      21.5      21.5      21.5      21.5
* JackKnife on Samples             13.5      13.7      13.7      13.5      13.7
                                   41.0      39.5      39.5      41.0      39.5
    
```



**Figure S25: Scree plot of Eigen values (Flinders Colony)**

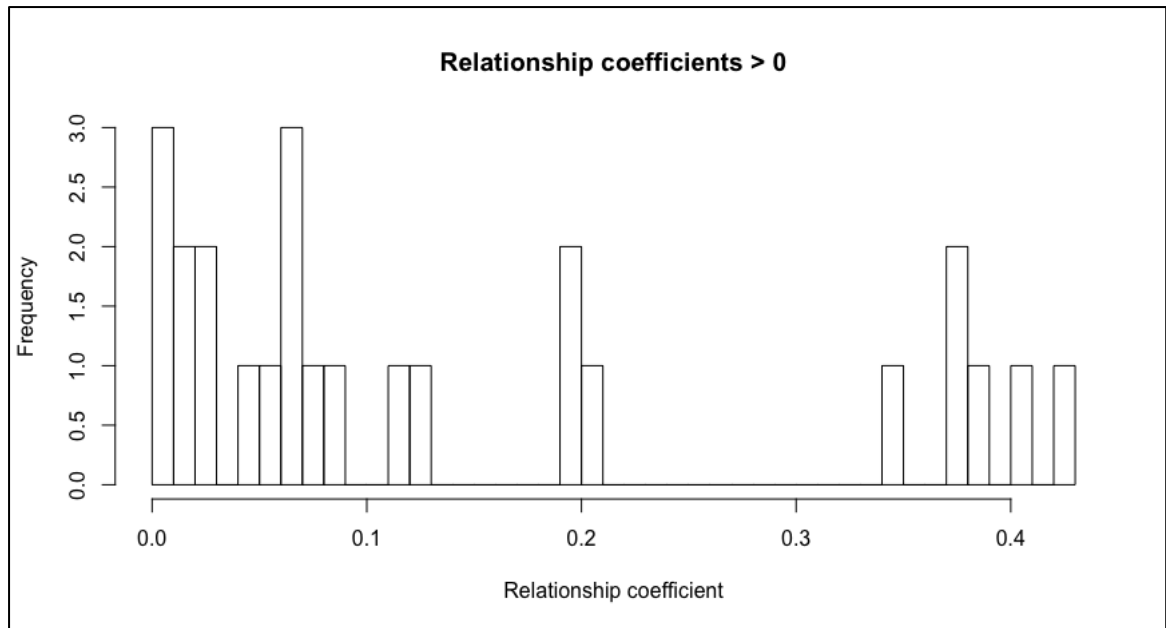


Figure S26: Histogram of  $r > 0$  from IBD analysis (Flinders Colony)

## Dataset 4 – Post-filter (Grey Range)

**Table S30: Examination of private and fixed alleles (Grey Range colonies)**

p1	p2	pop1	pop2	N1	N2	fixed	priv1	priv2	totalpriv	mdf
1	2	Alaric	Ray	18	7	0	726	35	761	0.136

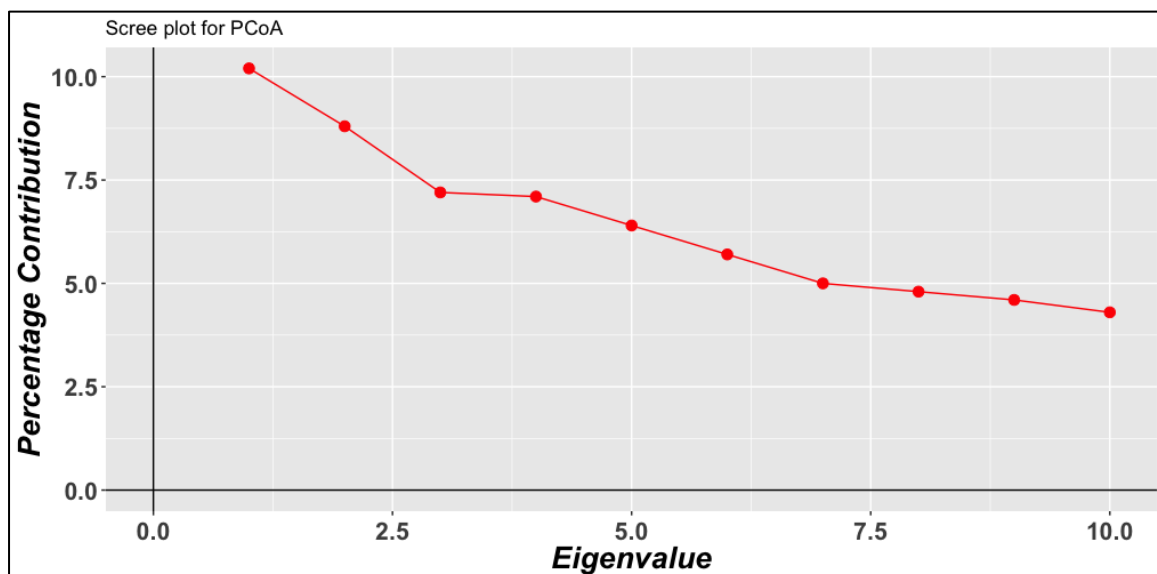
**Table S31: Nei's genetic distance matrix (Grey Range colonies)**

	[,1]	[,2]
Ray	0.000000	0.043289
Alaric	0.043289	0.000000

**Table S32: NeEstimator output for the Grey Range (for all lowest allele frequencies – Grey Range colonies)**

Number of Loci = 5481  
Mating Model: Random  
Lowest allele frequencies used, ordered in each population:  
0.0500 0.0200 0.0100  
Up to 17 righthmost characters can be shown for population names.

Population # then :Name	Samp Size	Crit. Value	Weighted H. Mean	#Indep. Alleles	r^2	Exp(r^2) Sample	Ne^	CIs for Ne^				
								Parametric	Jackknife	Samp (Eff.df)		
1:YFRW008	18	0.0500	18.0	14271988	0.073981	0.063423	20.9	27.2	27.5	14.6	83.7	750
		0.0200	17.9	14825889	0.073452	0.066039	21.3	39.5	40.1	18.5	395.2	816
		0.0100	17.9	14825889	0.073452	0.066039	21.3	39.5	40.1	18.5	395.2	816
2:YFRW001	7	0.0500	7.0	11298383	0.283415	0.234956	5.1	3.2	3.2	1.9	12.8	694
		0.0200	7.0	11298383	0.283415	0.234956	5.1	3.2	3.2	1.9	12.8	694
		0.0100	7.0	11298383	0.283415	0.234956	5.1	3.2	3.2	1.9	12.8	694



**Figure S27: Scree plot of Eigen values (Grey Range colonies)**



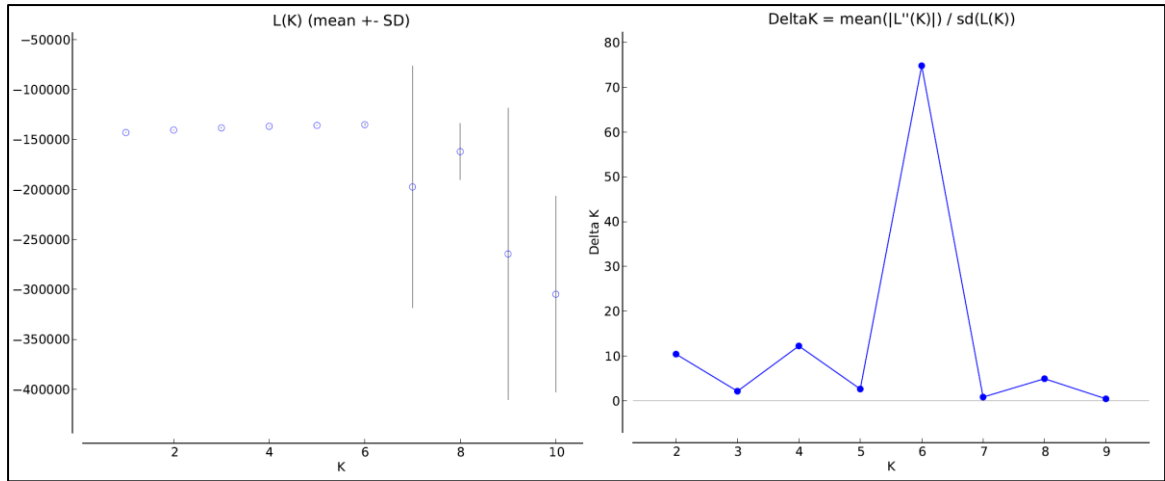


Figure S28: Output graphs of Structure Harvester (Grey Range colonies)

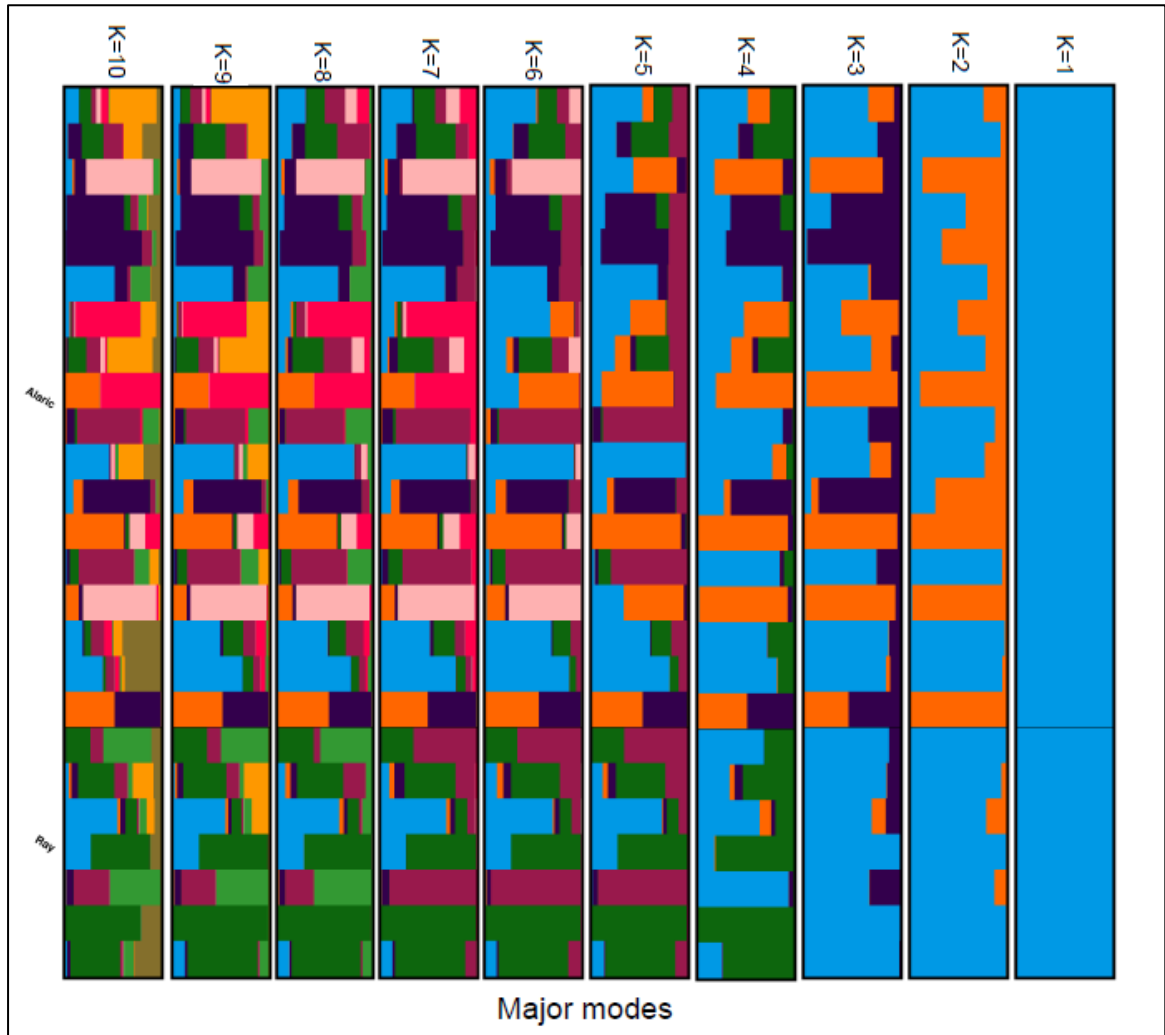


Figure S29: Bar plots for all values of K (major modes – Grey Range colonies)

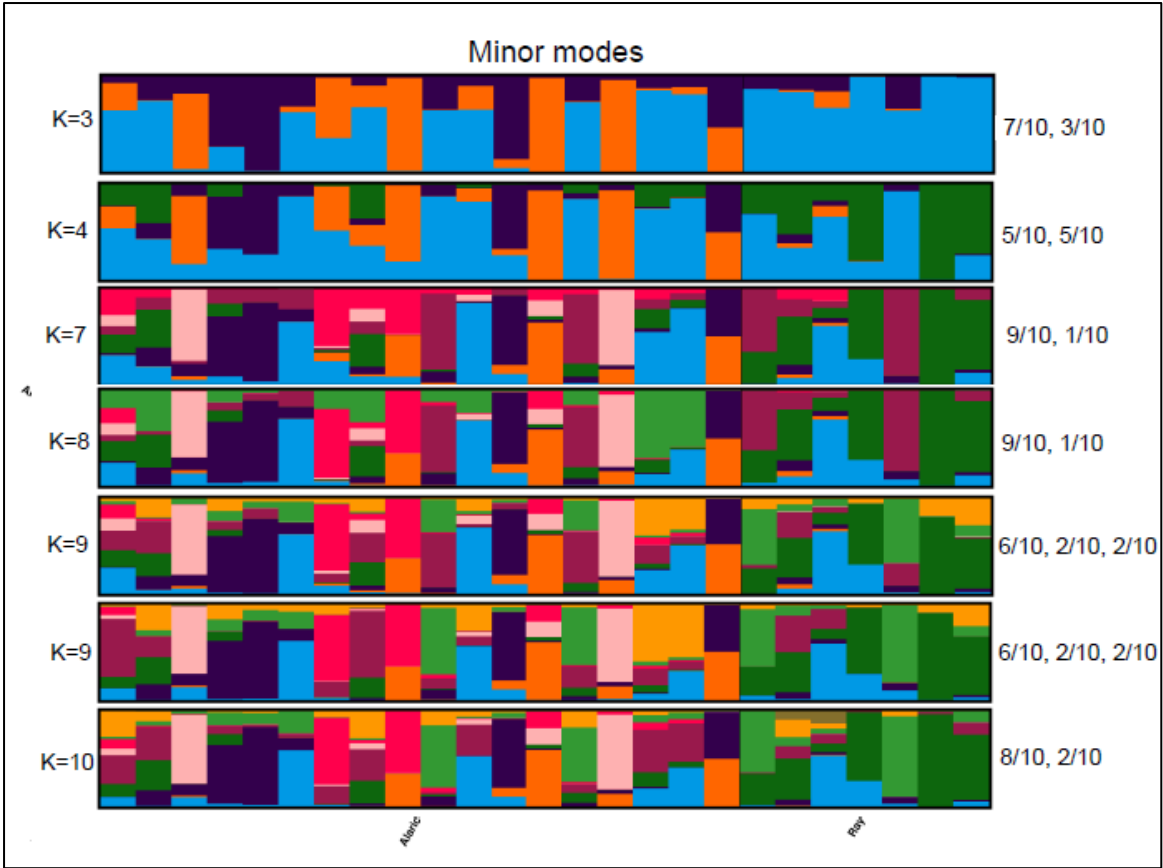


Figure S30: Bar plots for all values of K (minor modes – Grey Range colonies)

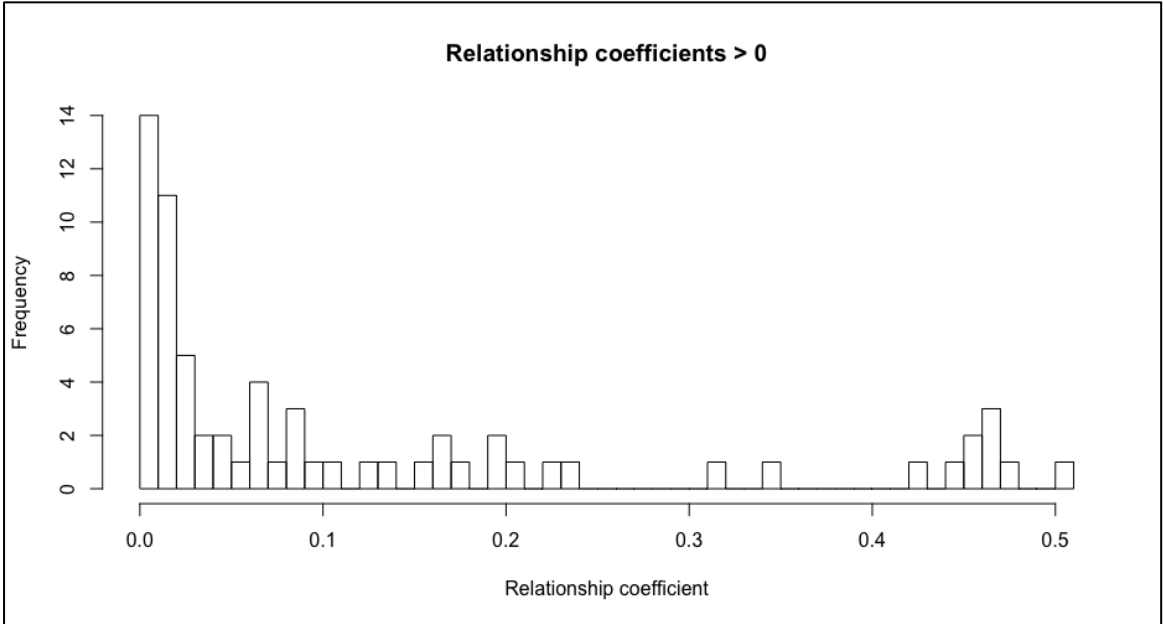


Figure S31: Histogram of  $r > 0$  from IBD (Grey Range colonies)

**Table S33: Output of GeneClass2 (Grey Range colonies)**

Population 1) Alaric, and 2) Ray as sampling colony. Samples in red are putative migrants. Green column indicates assignment colony.

				1	2	Nb. of loci
Assigned sample	home	Hog(L_home / L_max)	probability	Hog(L)	Hog(L)	
1/	1	0.000	0.500	2005.986	2113.064	5473
1/	1	0.000	0.500	2108.084	2221.254	5471
1/	1	0.000	0.500	1910.633	2135.776	5475
1/	1	0.000	0.500	2009.196	2055.857	5477
1/	1	0.000	0.500	2144.445	2217.122	5473
1/	1	0.000	0.500	1993.610	2108.686	5478
1/	1	0.000	0.500	1786.042	2170.233	5475
1/	1	0.000	0.500	1989.452	2057.942	5480
1/	1	0.000	0.500	2080.843	2207.539	5471
1/	1	0.000	0.500	2007.411	2071.298	5474
1/	1	0.000	0.500	1793.237	2190.009	5480
1/	1	0.000	0.500	2023.784	2306.884	5477
1/	1	0.000	0.500	1890.189	2322.908	5474
1/	1	0.000	0.500	2160.345	2316.735	5471
1/	1	0.000	0.500	2019.808	2375.267	5473
1/	1	0.000	0.500	2187.793	2552.761	5469
1/	1	0.000	0.500	1858.703	2296.047	5479
1/	1	0.000	0.500	2125.751	2447.588	5457
2/	2	0.000	0.500	2021.044	1768.332	5476
2/	2	0.000	0.500	2043.076	2017.717	5472
2/	2	149.602	0.000	2046.598	2196.200	5473
2/	2	0.000	0.500	2127.162	1862.065	5476
2/	2	192.074	0.000	1938.001	2130.075	5472
2/	2	0.000	0.500	2104.890	1731.238	5475
2/	2	0.000	0.500	2001.887	1877.607	5478

## Appendix VII : Ancillary Research Note – Microsatellite analysis of DNA from yellow-footed rock-wallaby scat

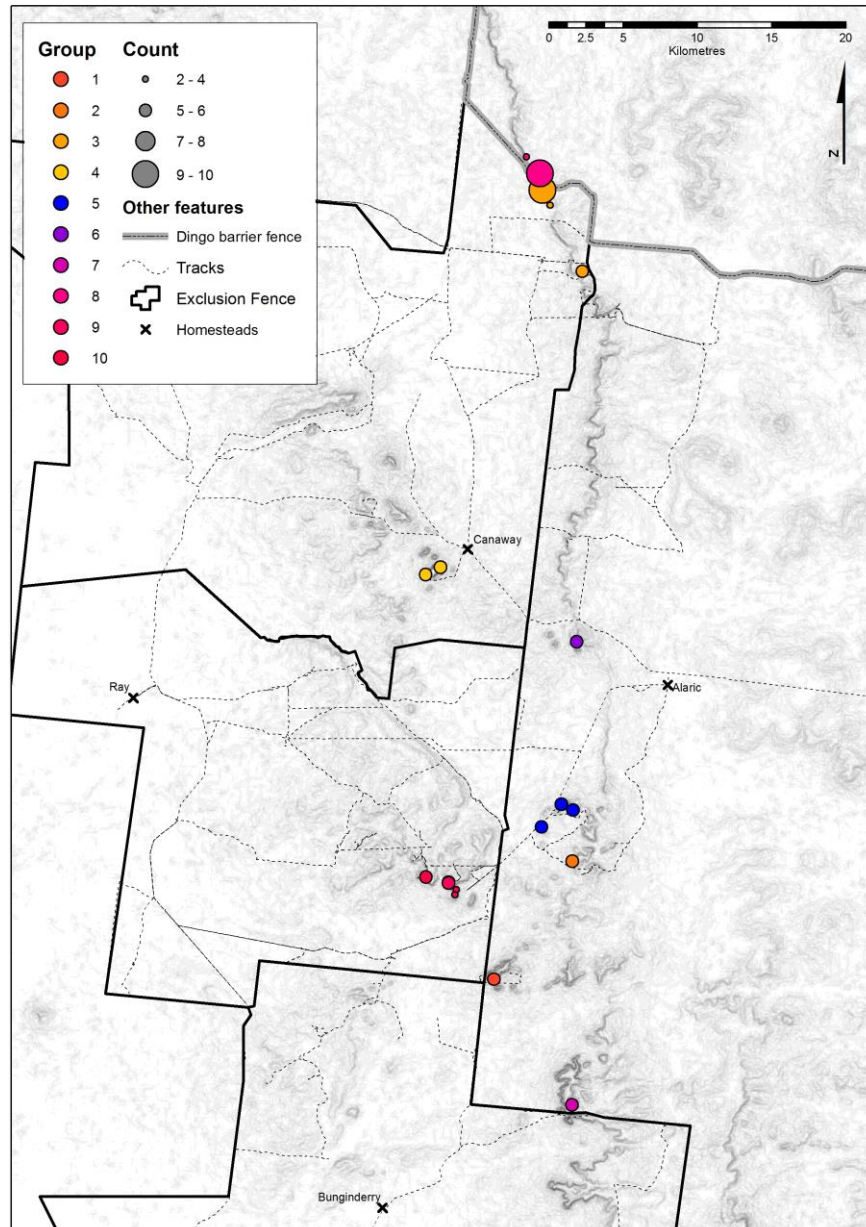
**Reference:** Smith, D. (Unpublished). Microsatellite analysis of DNA from yellow-footed rock-wallaby scat. In “*The effects of exclusion fencing on the yellow-footed rock-wallaby (Petrogale xanthopus celeris)*”. (Doctoral Thesis), University of Southern Queensland, Australia.

### Introduction & Methods

This thesis attempted to assess the genetic structure of populations at the study site in Western Queensland (Chapter 5) through a study of polymorphic microsatellite loci using DNA extracted from yellow-footed rock-wallaby (YFRW) scat. Colonies of YFRW were located using the methods described in Chapter 6. YFRW scat can be difficult to find, particularly in areas where YFRW may be in low densities. However, it has a distinct shape compared with other species that occupy the same areas. The scat of other *Macropodidae* spp. that move into the cliff areas (Red kangaroos, Eastern Grey Kangaroo and Euro) are large and spherical in appearance, goat scat (present in large quantities) is more similar but often smaller and nearer round (Triggs, 2004). YFRW scat is elongated, similar to the shape of a bullet, and tapered at one end. YFRW scat was often found on top of large rocks that sat out from the main area of cliff. As YFRW scat was common in these topographic features, and this scat was not mixed with goat scat, we often examined these locations first. When fresh (dark, wet) scat was found, pellets were bagged in zip-lock bags and labelled with the location and date of collection. In some cases, multiple scat collections occurred from the same colony.

Scats were kept in a dark and cool place whilst in the field (a small esky kept in the vehicle), and promptly transferred to the freezer (-4°C) at the end of each field trip. Scats were divided into theoretical colonies based on collection location, and a number of DNA extractions from each of these populations was completed (Figure S32). Scat extractions were completed using the QIAamp Fast DNA Stool Mini Kit and protocols. Following extraction, potential DNA presence was quantified using an Invitrogen Qubit™ Fluorometre with a high-sensitivity dsDNA assay protocol. When present, DNA was amplified using 14 microsatellite marker pairs (Table S34) using Polymerase Chain

Reaction (PCR). Initial PCRs were successful without fluorescent M13. After the addition of \*M13 primers further PCR trials were completed to optimise results. Final PCRs were performed using a 20µl reaction containing 1µl of extracted template DNA, 0.2 mM dNTPs, 1× PCR buffer (Bioline), 2µl of reverse primer (2µM), 1µl of 6-FAM, VIC, PET or NED M13(-21) primers, 0.5µl pmol of \*M13 forward primer (2µM) and 0.1U of Taq DNA Polymerase (Bioline).



**Figure S32: YFRW scat collection sites**

Locations of YFRW scat collections. Coloured by group, based on location. Proportional marker size based on quantity of scats used in analysis. Where possible and excepting high value sites, five scats were used per site. A broader scope of this location can be seen in Figure 4.1.

PCR conditions for all loci were as follows: 95°C (5 min), a touch down sequence of 10 cycles at 94°C (30 s), 60–50°C (-1°C per cycle - 45 seconds), 72°C (45 s), followed by 30 cycles at 94°C (30 s), 50°C (45 s), 72°C (45 s) and a final extension 72°C (3 min) followed by an infinite hold at 12°C. Successful amplifications were observed using agarose gel electrophoresis with 2% agarose and photographed using a Bio Rad Gel Doc™ EZ Imager and Image Lab™ software. These samples were then purified using ethanol precipitation before they were transferred to the Australian Genome Research Facility (AGRF) for capillary electrophoresis. The results of capillary electrophoresis were examined in STRand (v2.4.110 – Toonen & Hughes, 2001).

**Table S34: Microsatellite loci amplified in scat DNA assessments**

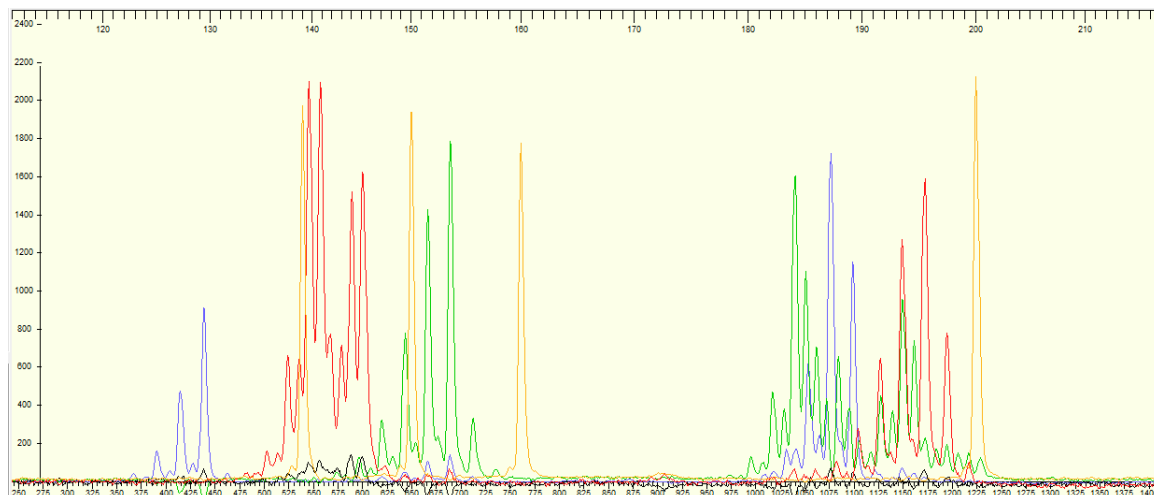
The locus, size range (in base pairs), and \*M13 fluorescent dyes used. Source shows the original research that presented the loci.

Locus	Size (bp)		*M13 dye	Source
<i>Macropus eugenii</i>				
Me14	160	210	VIC	Taylor & Cooper (1998)
Me15	225	270	6-FAM	
Me16	240	280	VIC	
Me17	110	135	PET	
Me2	230	280	NED	
<i>Petrogale assimilis</i>				
Pa297	120	155	6-FAM	Spencer et al. (1995)
Pa385	160	190	6-FAM	
Pa593	130	160	NED	
Pa595	210	285	PET	
Pa597	125	170	VIC	
<i>Petrogale xanthopus</i>				
Y148	185	200	6-FAM	Pope et al. (1996)
Y151	175	210	PET	
Y170	135	170	PET	
Y76	190	215	VIC	

## Results & Discussion

In total, scats were collected from 19 YFRW colonies. Several of these colonies were close to others, and as such were congregated into groups (Figure S32). Where possible, DNA was extracted from five scat pellets from each site. A focus was also placed on four sites that occur on either side of the DBF, as such 13 scat samples were used from each side. DNA was successfully extracted from 81 scat samples, though DNA quantity was often low (mean = 7.5 ng/μL). Trials of amplification of DNA were successful for all loci without the addition of \*M13 fluorescent dyes. After adding \*M13 dyes were added to the reaction

mix PCR trials were mostly successful, though not with consistency. Ultimately, a trial of seven samples with high concentration of DNA (>10 ng/μL - and 1 negative control) was successfully amplified and sent to AGRF for capillary electrophoresis.

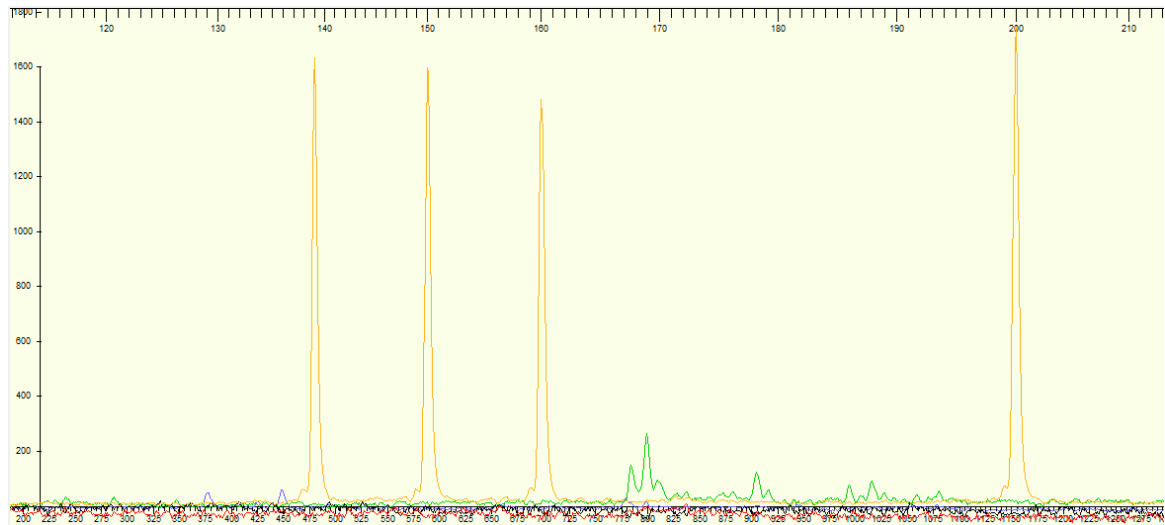


**Figure S33: Example chromatogram of successful trial sample**

The resulting chromatographs produced by capillary electrophoresis were viewed in STRand (Figure S33). Several loci had peaks an order of magnitude greater than others and dilution (by AGRF) was necessary to create viewable chromatographs. These differences in peak size were consistent across samples, indicating that certain primer pairs performed better under the PCR conditions, rather than pipetting error. This can be easily accounted for prior to fragment analysis, by adjusting the ratio of each PCR product in the final multiplexed sample. The chromatographs also showed that some locus peaks were missing from samples, despite observable and correctly sized bands at gel electrophoresis for that sample and primer pair. This tends to indicate that the \*M13 fluorescence was not being incorporated during PCR in that reaction mix; however, these missing peaks were not consistent across samples, and may also indicate degraded or poor quality DNA. Despite the need for multiplex ratio adjustments and missing peaks the trial was successful, with acceptable levels of missing data.

Further PCRs of samples with lower quantities of DNA (<10ng/μL) resulted in low yields or no appropriate sized bands observable after gel electrophoresis. Due to funding limitations and time constraints, samples that showed signs of partial success (faint bands observed after gel electrophoresis), were multiplexed at the appropriate ratios and sent to AGRF (n=46). Somewhat expectantly, the resultant chromatographs had high frequencies

of missing data, and were unable to be analysed (Figure S33). As a result of these poor results this aspect of the project was abandoned.



**Figure S34: Example chromatogram from final capillary electrophoresis**

I suspect the failure to amplify DNA consistently was due to both to the poor quality and low quantity DNA that was extracted from scats. While attempts were made to source fresh scat, even obviously fresh scat (collected immediately after an observed excretion) was often quite dry, likely due to a combination of the YFRW's own hydro-regulation and due to the high temperature, low humidity and drought at the study site during the study period that were not factors in the research this study draws on (see Piggott et al., 2006; Piggott, Banks, & Taylor, 2006). Scats therefore often absorbed large quantities of the buffer solutions used in DNA extraction and resulted in inconsistent and low DNA yields. For future studies, I recommend avoiding scat that does not already have high water content, and more robust techniques that optimise amplification of low quality DNA be explored.



## References

- PIGGOTT, M., BANKS, S., STONE, N., BANFFY, C., & TAYLOR, A. (2006). Estimating population size of endangered brush - tailed rock - wallaby (*Petrogale penicillata*) colonies using faecal DNA. *Molecular Ecology*, 15(1), 81-91.
- PIGGOTT, M., BANKS, S., & TAYLOR, A. (2006). Population structure of brush - tailed rock - wallaby (*Petrogale penicillata*) colonies inferred from analysis of faecal DNA. *Molecular Ecology*, 15(1), 93-105.
- TOONEN, R., & HUGHES, S. (2001). Increased throughput for fragment analysis on an ABI Prism® 377 automated sequencer using a membrane comb and STRand software. *Biotechniques*, 31(6), 1320-1325.
- TRIGGS, B. (2004). *Tracks, Scats and Other Traces: A field guide to Australian mammals (rev. ed.)*. Australia: Oxford University Press.