Aspects of Yellow-footed Rock-wallaby (*Petrogale xanthopus xanthopus*) Ecology in Connected and Isolated Habitat to Inform Future Climate Change Management



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I would like to dedicate this thesis to:

Manfred "Manny" Lee-Gunkel,
Jacqueline Hindmarch
and
Gladys "Gangan" Lee-Gunkel

My constant source of inspiration and strength. If it wasn't for them, I wouldn't be in the position I am today. I owe them more than I could ever give.

Table of Contents

Thesis Declaration……1
Acknowledgements……2
Acknowledgement of Country ……4
Thesis Abstract……5

General Introduction: *"Declining biodiversity, and the status of the semi-arid adapted yellow-footed rock-wallaby (Petrogale xanthopus xanthopus)"*……7

Declining Biodiversity……8
Climate Change in Australia……9
Rock-wallaby (*Petrogale*) Species in Australia……10
The Yellow-footed Rock-wallaby……11
Habitat and Ecology……11
Water and Nutrition……16
Threats to the Yellow-footed Rock-wallaby……18
Historic Population Declines……18
Population Recovery ('Operation Bounceback')……18
Current and Future Threats……19
Thesis Aims……20

Chapter One: "*Population demographics, health and breeding of yellow-footed rock-wallabies (Petrogale xanthopus xanthopus) in connected and isolated habitat in semi-arid South Australia"*……21

Statement of Authorship……22
Abstract……23
Introduction……23
Methods……25
Ethical Statement……25
Study Sites……25
Trapping and Sampling……29
Blood Biochemical Analysis……30
Statistical Analysis……31
Results……32
Trapping……32
Breeding……33
Sexual Maturity……36
Females……36
Males……36
Physical Condition……37
Italowie Gap……37
Bimba Hill……38
Blood Biochemistry……40
Discussion……42
Conclusions……46

Chapter Two: "*Population genetics of two yellow-footed rock-wallaby (Petrogale xanthopus xanthopus) colonies in connected and isolated habitat in semi-arid South Australia"*……48

Statement of Authorship……49
Abstract……51
Introduction……52
Methods……53
Ethical Statement……53

Study Sites……53
Sample Acquisition……56
DNA Extraction and Quantification…..……56
Analysis and Statistics……...56
Results……..57
Sample Collection and SNP Data……..57
Genetic Diversity……...58
Kinship Analysis……59
Discussion……62
Conclusions & Further Research……65

Chapter Three: *"The diet of the yellow-footed rock-wallaby (Petrogale xanthopus xanthopus) and dietary overlap with sympatric herbivores in semi-arid South Australia"*……67

Statement of Authorship……...68
Abstract……70
Introduction……..71
Methods……72
Ethical Statement……...72
Study Sites……72
Sample Acquistion……76
Sample Preparation and DNA Extraction..……77
Library Preparation and Hybridisation Capture with myBaits®……77
Sequencing Data Processing……..78
Informative Analysis……..79
Results……..80
Yellow-footed Rock-wallaby Diet at Italowie Gap……...80
Dietary Overlap with Sympatric Herbivores at Italowie Gap……...83
Yellow-footed Rock-wallaby Diet at Bimba Hill……..85
Dietary Overlap with Sympatric Herbivores at Bimba Hill……..88
Discussion……90
Further Research……93
Conclusions……..93

Chapter Four: "*The influence of season, habitat and diet on the faecal microbiome of the yellow-footed rock-wallaby (Petrogale xanthopus xanthopus)"*……...95

Statement of Authorship……...96
Abstract……98
Introduction……..98
Methods……..100
Ethical Statement……100
Study Sites……100
Sample Acquisition……...104
Laboratory Processing……104
Faecal Microbiome……....104
Diet……105
Sequence Preparation and Informative Analysis……106
Faecal Microbiome……106
Diet……106
Results……107
Faecal Microbiome……..107
Site and Seasonal Differences……108
Intra-individual Variation……112
Dietary Influences……115
Italowie Gap……..115
Bimba Hill……117

Discussion……..119
Conclusions……120
Data Availability……121

Chapter Five: "*Habitat characteristics and the future distribution of the yellow-footed rock-wallaby (Petrogale xanthopus xanthopus)"*…….122

Statement of Authorship……123
Abstract……..124
Introduction……125
Methods……..126
Survey Locations and Methodology……126
Preparation of Informative Environmental Layers for Input into Models……...128
Preparation of Fine-scale Scat Survey Data……128
Development of Statistical Models Using Regional Scat Survey Data……...131
Preparation of Broad-scale Presence-only Data……..131
Species Distribution Models with Broad-scale Presence-only Data……...132
Future Climate Change Analysis……132
Results……133
Statistical Models……133
Species Distribution Models……....137
Discussion……..……143
Conclusions……146

General Discussion: *"Key Findings and Conservation Implications for the Yellow-footed Rock-wallaby (Petrogale xanthopus xanthopus)"*……...148

Introduction……149
Key Findings……..149
Current Population Status, Future Research and Management Directions……154
Connected Population Management Strategies……...154
Isolated Population Management Strategies……156
The Species' Future Distribution and Management Actions……...157
The Final Word……..159

Supplementary Chapter: "*A guide to trapping, handling, and processing small to medium sized macropodids: with examples from the iconic, semi-arid adapted yellow-footed rock-wallaby (Petrogale xanthopus)"*……...160

Statement of Authorship……161
Abstract……..162
Introduction……162
Methods……..163
Trap Design and Setup…….....163
Active Trapping…….……..165
Processing……167
Order of Observations and Morphometric Sampling……..169
Measurements……...169
Hind-end Measurements……..170
Pes……170
Tail……170
Pouch Assessment and Measurement of Pouch Young……171
Scrotal Measurements……...173
Anterior Measurements……...173
Ear Length……174
Head Width/Length……...175

Sample Acquisition……...177
Microbiome……177
Faecal……..177
Pouch……..177
Oral……177
Storage……177
Tissue……178
Blood……180
Identification and Marking…….....181
Microchips……181
Ear-tags……..182
Return of Furred Pouch Young……183
Vitamin E Injection……..185
Releasing……186
Main Points and Conclusions……187

References……...188

Appendices……..211
Appendix 1……212
Appendix 2……216
Appendix 3……217
Appendix 4……227
Appendix 5……230
Appendix 6……233

Thesis Declaration:

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree. The author acknowledges that copyright of published works contained within the thesis resides with the copyright holder(s) of those works. I give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time. I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

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To my family, I wouldn't have gotten to this position in my life without you all. Mum, part of why I started on this journey was for you, so you could see my chasing my passion and bettering myself as a person. I have seen you show strength through some of the worst times of your life and that inspires me every day. Manny, you are probably the biggest reason why I did this degree. You've always reinforced to me how important education is, and seeing the look in your eyes when I achieve something is one of my biggest driving forces. I hope that what I have achieved here makes you proud. Gangan, you and Manny have always supported me throughout my life, teaching me so many important skills and lessons. I like to think that our times spent together in the garden, or the kitchen, have given me the tools to achieve anything in life that I put my mind to. I rely on you both more than you'll ever know. Sarah, thank you for always being there for me, even when I didn't deserve it. We finally got to the end of this, no more of me stressing out, sitting for hours on end looking at my laptop, and getting super frustrated and cranky. Well, at least not for my PhD anyway! To all my family, I love you all and I hope this makes you proud.

To Shannon Kleemann and Stacey Dix, I couldn't have got through the last couple of years without you guys. You've always been there helping me in whatever way you can and have become real, true friends. Shannon, you've been there throughout, and I hope you know how much I appreciate having you there, sharing all these moments with me, and the effort you've put into making this thesis happen. And, of course, helping me cope with Tags' idiosyncrasies! I hope we all can continue to conquer the world together in the future, one numbat at a time. May the laughs, quote list, flipping echidnas and pushing wombats continue for a very long time.

Now, enough of the soppy stuff. On a professional level there are many people who have helped me through this journey which I'd like to acknowledge. My supervisors and advisors, Adam Croxford, Raphael Eisenhofer, Jeremy Austin, and Bertram Ostendorf, thank you for your time and for always guiding me. Other people which have helped me throughout this project I'd like to acknowledge are Michelle Waycott, Kor Van Dijk, Ed Biffin, Graeme Finlayson, Tessa Bradford, Geoff Axford, Rob Brandle, Lindel Andrews, Jodi Buchecker, Mark Eldridge, Sally South, Brian Rich, Peter McCartney, Wayne Rohrig, Steve McKechnie, Wayne Boardman, Lucy Woolford, Anne-lise Chaber, Ryan O'Handley and Zach Staude. I'd also like to make a special mention to Sian Johnson and Peter Watkins with National Parks and Wildlife Service SA, for who I would never have been able to do this. You both welcomed us into your worlds, spent your own time free-feeding traps, and allowed us into the great insights and knowledge you have of yellow-foots and your parks. I am extremely grateful for all you've both done to make this project a success.

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Acknowledgement of Country:

I would like to acknowledge that this project was conducted on the Traditional Lands of the Kaurna, Adnyamathanha and Wilyakali people. I pay my respect to their Elders past, present, and emerging.

In the Adnyamathanha culture, the yellow-footed rock-wallaby, or 'andu' in Yura ngawarla (Aboriginal language), is a significant animal and represents a connection to their ancestral spirits.

During this project I have found that the Adnyamathanha's connection with their country is deeply engrained and their knowledge of this environment is irreplaceable. What troubles me most is that this culture and knowledge is at risk of disappearing. Already details around andu Dreaming stories have been lost. The in-depth cultural knowledge that does still persist is highly classified and only passed onto initiated Yura men. But with no Yura Elders now present in the community to perform initiation ceremonies, this knowledge will one day be lost to time.

However, the scarcely available information that has been shared with me throughout this project always reiterates the importance of keeping the andu on country for the continuation of Adnyamathanha culture. I believe that this project has greatly benefited from the Adnyamathanha's knowledge of country which has been passed onto me, and I am immensely privileged and grateful to have received it. My hope is that projects involving the andu will continue, and will aid in retaining cultural knowledge. However, the most important focus of andu research should be centred around keeping this extremely sacred animal on country for many years to come.

I hope to continue to learn from and work with the Adnyamathanha people, the true custodians of andu county in the Flinders and Gammon Ranges, to achieve this goal.

Thesis Abstract:

Advancing the ecological knowledge of Australia's unique marsupial species is vital to inform efficient and effective conservation management strategies as climate change continues to have an impact. This thesis examined the effect of habitat connectivity on yellow-footed rock-wallaby (YFRW; *Petrogale xanthopus xanthopus*) populations to identify areas in their distribution at greatest risk from climate change, and to identify the ecological factors which may influence their future viability. The bulk of this thesis was informed by data obtained from Italowie Gap in the Vulkathunha-Gammon Ranges National Park (highly connected) and at Bimba Hill in the Bimbowrie Conservation Park (isolated outcrops), where trapping was conducted in 2021/22 to examine the impacts of habitat connectivity and rainfall. Additionally, scat surveys were conducted to identify significant YFRW habitat characteristics, and the outcomes used to develop a species distribution model (SDM) to identify areas in the YFRWs distribution at greatest risk from climate change.

Some key findings associated with habitat connectivity involved population demographics and genetic diversity. Results indicated that sub-adult YFRWs were less prevalent in the highly connected Italowie Gap, compared to the isolated Bimba Hill colony, suggesting habitat connectivity influences population demographics through potential migration pathways. Genetic diversity was higher at Italowie Gap than at Bimba Hill, likely also influenced by migration rates and gene flow. Concerningly, Bimba Hill showed similar levels of genetic diversity to that of a captive YFRW population, indicating that as climate change progresses and populations become more fragmented, genetic management will likely be required to maintain isolated YFRW colonies.

Other key findings from this project concerning diet, blood biochemistry, and faecal microbiome, were associated with rainfall and local vegetation communities. The detection of herb species in diet samples was common throughout. However, dietary diversity increased during dry periods with more shrub species being detected. Blood biochemistry and diet were found to be tightly linked. Urea, creatinine and some antioxidants were influenced by high rainfall which triggered a pulse of resource abundance, and altered vegetation nutrient content. Faecal microbiome was also influenced by diet, where microbial composition varied in response to the dominant plant species detected in diet samples. Faecal microbiome was significantly different between study sites, with Italowie Gap having higher microbial richness throughout. This is, however, also likely influenced by population genetic diversity which is known to effect faecal microbial diversity and richness.

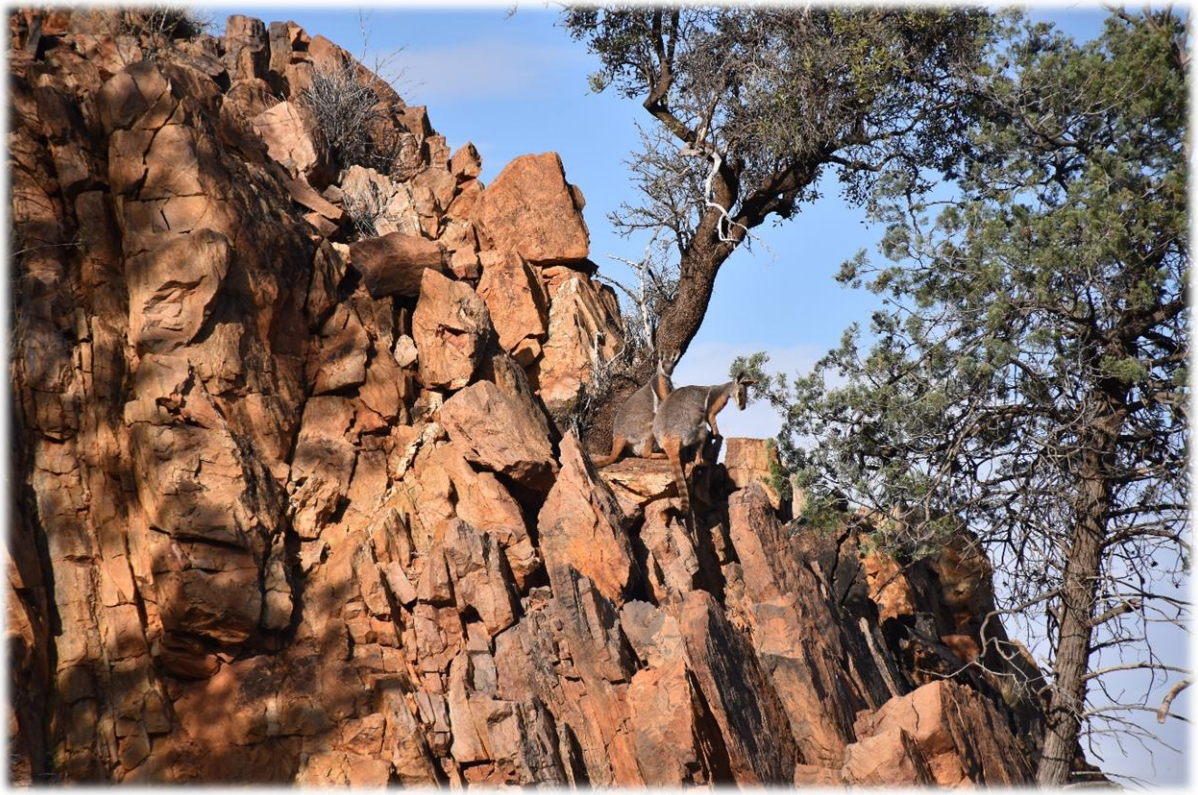
Scat survey data suggested that terrain ruggedness is most indicative of YFRW habitat, with mean annual temperature also having an influence. Through the use of an SDM, the distribution of the YFRW is predicted to contract towards high quality, connected habitat as climate change progresses. Some connected habitat in the southern Flinders Ranges is predicted to become more suitable for habitation, with large contractions of poor-quality habitat occurring in the isolated populations in the

Gawler and Olary Ranges, and western New South Wales. As issues with genetic and faecal microbial diversity were identified as limiting factors in isolated populations, climate change is expected to pose a significant further risk to the viability of these YFRW populations.

The implementation of management practices now in these high-risk populations to alleviate the impacts of climate change is recommended, such as the implementation of genetic management practices. Although this thesis has provided a detailed understanding of two colonies in connected and isolated habitat, on-going monitoring into the future will be needed to determine the ecological changes that occur in these colonies due to climate change. Using these colonies as models will enable the information gathered to be used across the wider YFRW distribution.

General Introduction

"Declining biodiversity, and the status of the semi-arid adapted yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*)"



"*The yellow-footed rock-wallaby is still fairly abundant in certain parts of South Australia, but from many of its old haunts it has completely disappeared*"

– Prof Wood Jones, 1924

General Introduction: Declining biodiversity, and the status of the semi-arid adapted yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*)

Werner L.E

Declining Biodiversity:

Across the globe, human activities affect approximately 83% of the earth's land surface (Sanderson *et al*. 2002) and approximately 59% of its oceans (Halpern *et al*. 2019). These activities have impacted wildlife species globally, leading to population declines and a significant rise in the number of species threatened with extinction. This is due to processes such as habitat loss and fragmentation, predation and competition with introduced pest species, increased disease prevalence, and over-hunting or poaching (Turner 1996; Andersen *et al.* 2004; Somerville 2016; Schlaepfer *et al*. 2018; Ghoddousi *et al*. 2019; Moore *et al*. 2021). As a result, the abundance of wildlife populations has declined by 69% since 1970, with the most severe losses occurring in tropical areas (WWF 2022).

However, Australia is not exempt, with the continent experiencing the worst mass extinction event in recorded history with more than 10% of endemic mammal species becoming extinct in the past 200 years, and a further 21% of species classified as threatened (Woinarski *et al*. 2015). The same factors identified elsewhere around the globe, and in particular the establishment of feral predator and competitive herbivore species, are also responsible for the decline of Australia's wildlife species diversity and abundance (Woinarski *et al*. 2015). In western New South Wales for example, it was estimated that between 1841-1901 the establishment of the sheep (*Ovis aries*) industry contributed to the local extinction of 24 local mammal species (Lunney 2001). The top four threats identified as causing population declines or extinctions of Australian mammals are: predation by feral cats (*Felis catus*), predation by red foxes (*Vulpes vulpes*), competition with livestock and feral herbivores, and habitat loss and fragmentation (Woinarski *et al*. 2019). To date, 28 mammal species have gone extinct in Australia since colonisation (Figure 1), with each of these extinction events attributed to one or more of the threats listed previously.

Feral cats in Australia have been associated with the extinction of at least 16 mammal species (critical weight range, 35-5500g; Johnson & Isaac 2009), and are thought to be responsible for a further 97 vertebrate and invertebrate species becoming threatened (Johnson 2006; Doherty *et al*. 2015; Woinarski *et al*. 2015; Tuft *et al*. 2021). The red fox has also had catastrophic impacts on Australian wildlife, and has been implicated in the loss or decline of 58 species (Woinarski *et al*. 2015).

As well as predators, exotic herbivores, such as the feral goat (*Capra hircus*), feral pig (*Sus scrofa*), camel (*Camelus dromedarius*) and European rabbit (*Oryctolagus cuniculus*), were introduced into the

Australian landscape and are now widespread throughout the continent, competing with native herbivores for food and water resources (Dawson & Ellis 1979; Lawrence 2010; Creese *et al* 2019). All of the invasive pest species in Australia pose a significant threat to our native wildlife, and as such, wildlife conservation programs often involve the control of feral species to minimise their impacts.

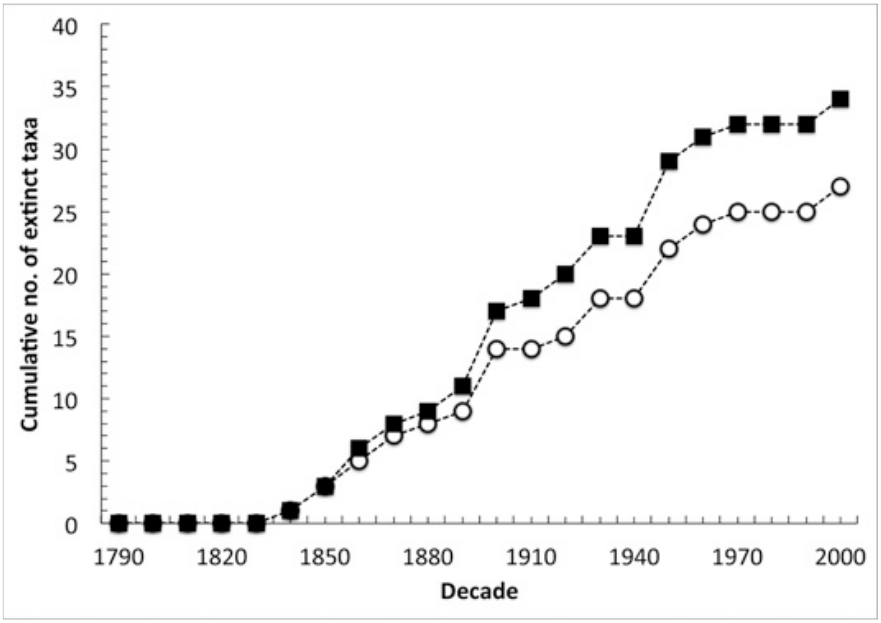


Figure 1: The cumulative number of extinct mammal taxa in Australia since European colonisation. Light circles denote extinct species, dark squares include extinct subspecies. Replicated from Woinarski *et al*. (2014).

Climate Change in Australia:

More recently, the traditional anthropogenic impacts on biodiversity are being exacerbated by an emerging threat; climate change (Beever & Belant 2012; Pant *et al*. 2021; Beebee 2022; Abedin *et al*. 2024). Predictions suggest that as global climate change progresses there will be an increase in temperature extremes, drought frequency, severity and duration, and a higher frequency of severe weather events (Weiskopf *et al.* 2020; Department for Environment and Water 2022). These climate related changes have all been identified previously to be associated with a decline in global biodiversity (Habibullah *et al*. 2022). As climatic factors become more unpredictable and extreme as climate change progresses, greater losses of biodiversity are anticipated. This will be particularly evident in regions already experiencing other threats and habitat destructive factors, such as Latin America and the Caribbean, where species abundance has decreased by ~94% since 1970 (WWF 2022).

Australia's weather is determined by two main weather drivers; the El Nino-Southern Oscillation and the Indian Ocean Dipole, but these drivers are becoming less predictable and more severe with the progression of climate change, resulting in more variable weather patterns across the continent (Head *et al*. 2014). This shift in weather patterns suggests that Australia will also experience climate-related losses to species biodiversity, and will require innovative management strategies to protect environmental heterogeneity.

Since climate records began in 1910, Australia has warmed by an average of 1.47ºC and this is predicted to worsen as climate change progresses (State of the Climate 2022, www.bom.gov.au/state-of-the-climate/). Some of the most climatically variable areas of Australia are found in the arid and semi-arid regions. The semi-arid regions of Australia are host to many unique fauna and flora species, adapted to survive in these harsh conditions (Tyndale-Biscoe 2005). However, these regions are predicted to experience an increase in the frequency, severity, and duration of drought events, an increase in mean annual temperature, and a decrease in annual rainfall, with more erratic and unreliable rainfall events occurring as climate change worsens (Head *et al*. 2014; Department for Environment and Water 2022). As the arid and semi-arid regions of Australia typically rely on summer rainfall and their iconic boom-and-bust cycles to maintain the region's biodiversity, climate change threatens to impact the balance of these ecosystem with local species forced to adapt or decline. The effect that regional climatic changes will have on Australia's wildlife is largely unknown, but when investigations have been undertaken, habitat specialist species such as the mountain pygmy possum (*Burramys parvas*) and the platypus (*Ornithorhynchus anatinus*) have been predicted to be severely impacted by climate change (Klamt *et al*. 2011; Broome *et al*. 2013). Sadly, in many cases the impacts of climate change may not be understood until it is too late for conservation actions to be implemented successfully.

Rock-wallaby (*Petrogale*) Species in Australia:

Habitat specialist wildlife in the arid and semi-arid regions are predicted to be most susceptible to local climatic changes, threatening their future viability (Crompton *et al*. 2008; Shi *et al*. 2015; Kutschera *et al*. 2016). One group that has been faring particularly poorly since European settlement are the rock-wallabies, or *Petrogale* genus. These habitat specialist macropodids, many of whom live in semi-arid Australia, are threatened by exotic pests and habitat loss and/or fragmentation, and as a consequence, many of the *Petrogale* taxa are now threatened (Lavery *et al*. 2021). There are currently 25 taxa within the *Petrogale* genus, ranging in weight from 1-12kg (Lavery *et al*. 2021; Baker & Gynther 2023). However, the common factor across all *Petrogale* species is their reliance on rocky structures within their habitat, such as outcrops, gorges, and cliffs, for water conservation and protection from weather extremes and predation (Wilson *et al.* 1976; Short 1982; Ruykys 2017; Baker

& Gynther 2023; Mallam *et al*. 2023). Reliance on these rock structures, and the protection and microclimate they provide naturally, places restrictions on population expansion due to the limited carrying capacity in this preferred habitat. This exposes *Petrogale* populations to the negative impacts of dry environmental conditions and drought, which are predicted to become more prevalent with future climate change.

The Yellow-footed Rock-wallaby:

The yellow-footed rock-wallaby (YFRW; *Petrogale xanthopus*) is endemic to Australia and is classified as Vulnerable (EPBC classification; Baker & Gynther 2023). The species is separated into two distinct subspecies, *P. x. xanthopus* in South Australia and New South Wales, and *P. x. celeris* in Queensland (Copley 1981; 1983; Gordon *et al*. 1993). *P. x. xanthopus* is classified as Vulnerable in South Australia (National Parks and Wildlife Act 1972) and Endangered in New South Wales (Threatened Species Conservation Act 1995) (Baker & Gynther 2023). Whilst *P. x. celeris* is classified as Vulnerable in Queensland (Nature Conservation Act 1992) (Baker & Gynther 2023). The colouration of the YFRW provides great camouflage amongst their habitat, while the large hindfeet, rubbery foot pad, short toe-nail, and long striped tail provide balance and traction when jumping across rocks. Their distinctive facial markings, yellow feet, and striped tail are their most identifiable features (Figure 2), making the YFRW an iconic Australian marsupial species, a tourism drawcard, and a flagship species for conservation agencies (e.g. Nature Foundation, Zoos SA).

Habitat and Ecology:

The YFRW is found in semi-arid South Australia throughout the Flinders, Gammon, Olary, and Gawler Ranges (Figure 3; Copley 1981; Copley 1983; Lim *et al*. 1987), with some small populations in Mutawintji National Park and Coturaundee Range in New South Wales (Figure 3; Dovey *et al*. 1997). YFRWs, like all rock-wallabies, live in rocky gorges, ranges, and outcrops often with steep inclines, small crevices, and complex rock structures (Wilson *et al.* 1976; Copley 1981; Short 1982; Lim & Giles 1987; Lim *et al*. 1987; Ruykys 2017; Michael & Lindenmayer 2018). The habitat of the YFRW can be highly variable across their distribution, ranging from steep gorges with fairly simple rock fissures and crevices in the Flinders and Gammon Ranges, to low areas of isolated, rocky outcrops with complex rock jumbles in the Olary Ranges (Figure 4). The variability of YFRW habitat makes tailoring conservation actions difficult as local habitat factors may be influencing animal behaviour, habitat usage and survival due to differences in resource availability.

Few studies have been conducted on YFRW habitat preferences, but the data that is available has found unique characteristics associated with YFRW habitat in New South Wales (Lim & Giles 1987)

and south-western Queensland (Smith & Allen 2021). However, due to high environmental heterogeneity across the species' distribution, broad-scale and accurate extrapolation of preferential habitat characteristics is not possible based on the existing limited on-ground habitat surveys.

Areas suitable for YFRW habitation are often limited in size and isolated, restricting the carrying capacity of the habitat and increasing the need for migration to sustain genetic diversity. Genetic investigations have been conducted on many of Australia's rock-wallaby species to monitor for inbreeding effects and losses of genetic diversity (Browning *et al*. 2001; Eldridge *et al*. 2004; Ruykys & Lancaster 2015; West *et al*. 2018). Typically, it has been found that genetic diversity is maintained in mainland colonies, whereas island populations appear highly inbred (Eldridge *et al*. 1999; Jones Lennon *et al.* 2011) reflecting the impact of isolation. This suggests that migration must be occurring within mainland rock-wallaby populations at a reasonable rate to maintain genetic diversity. Although YFRW migration rates have been described previously as being low and sporadic when using radio-tracking and ear tagging methods (Sharp 1997a), more recent genetic studies have found the migration occurs more frequently than previously suggested (Potter *et al*. 2020; Smith *et al*. 2023a).

Contemporary YFRW genetic investigations are few, with only two published studies in the last 20 years (Potter *et al*. 2020; Smith *et al*. 2023a). Both of these studies reported that YFRW colonies had high to moderate levels of genetic diversity with low levels of inbreeding. However, how this level of genetic diversity is maintained, and the environmental or intrinsic factors which influence migration dynamics, are poorly understood. One mechanism which has been proposed for maintaining their genetic diversity is the utilisation of breeding groups which are typically composed of a dominant male with multiple adult females, and several sub-adults and juveniles (Hazlitt *et al*. 2006; Sharp 1997a). Additionally, YFRWs can breed continuously throughout the year with embryonic diapause utilised to boost reproductive rates (Figure 5; Poole *et al*. 1985; Tyndale-Biscoe 2005; Armati *et al*. 2006).

Most of our understanding of YFRW reproduction has been obtained from captive animals (Poole *et al*. 1985). However, studies on wild YFRWs found that births can occur across the year, with a notable increase during autumn and spring (Lim *et al*. 1987; Sharp & McCallum 2015), but if these characteristics are representative of other wild YFRWs colonies is currently unknown. As noted in most other semi-arid fauna, a high rate of breeding and successful recruitment is reflective of environmental conditions (Kelly *et al*. 2013; Cavalcanti *et al*. 2016; Barros *et al*. 2018), and as such, YFRW breeding is likely no exception.



Figure 2: *Top:* A sub-adult male yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*), Olary Ranges, South Australia (Photo: S. Dix). *Bottom left:* A yellow-footed rock-wallaby, Gammon Ranges, South Australia (Photo: S. Kleemann). *Bottom right:* The hindfoot of a yellow-footed rock-wallaby showing the ridges and rippled appearance which give them grip on rocky surfaces (Photo: S. Kleemann).

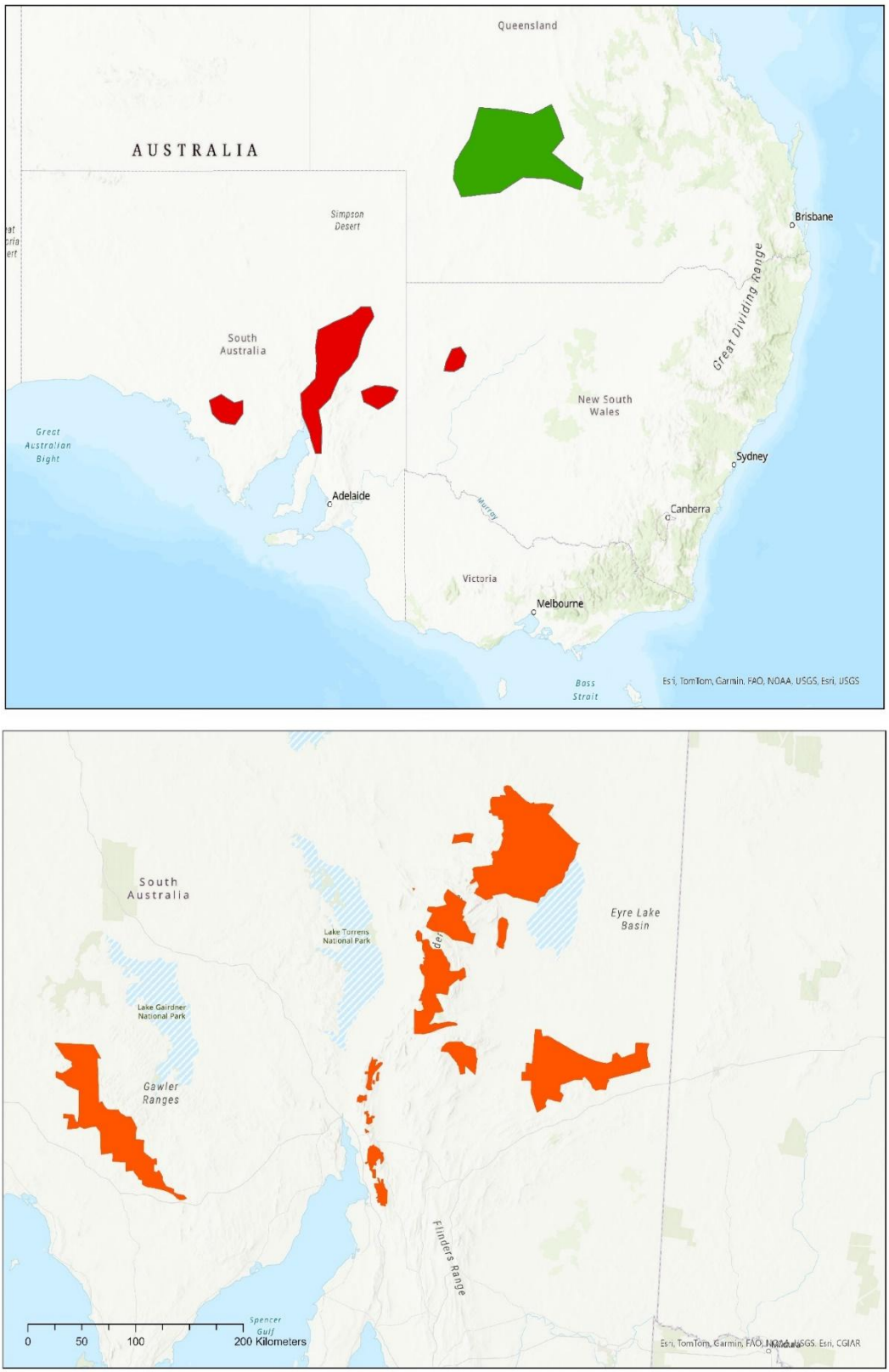


Figure 3: *Top*: A map showing the current distribution of the yellow-footed rock-wallaby subspecies, *Petrogale xanthopus xanthopus* (red) in South Australia and New South Wales, and *Petrogale xanthopus celeris* (green) in Queensland, Australia. *Bottom*: A map of the current footprint of Operation Bounceback in South Australia.



Figure 4: The variability of yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) habitat across the northern Flinders/Gammon Ranges (*top*; Photo: D. Taggart), central and lower Flinders Ranges (*middle*; Photo: S. Kleemann), and Olary Ranges (*bottom*; Photo: L. Werner) of South Australia.

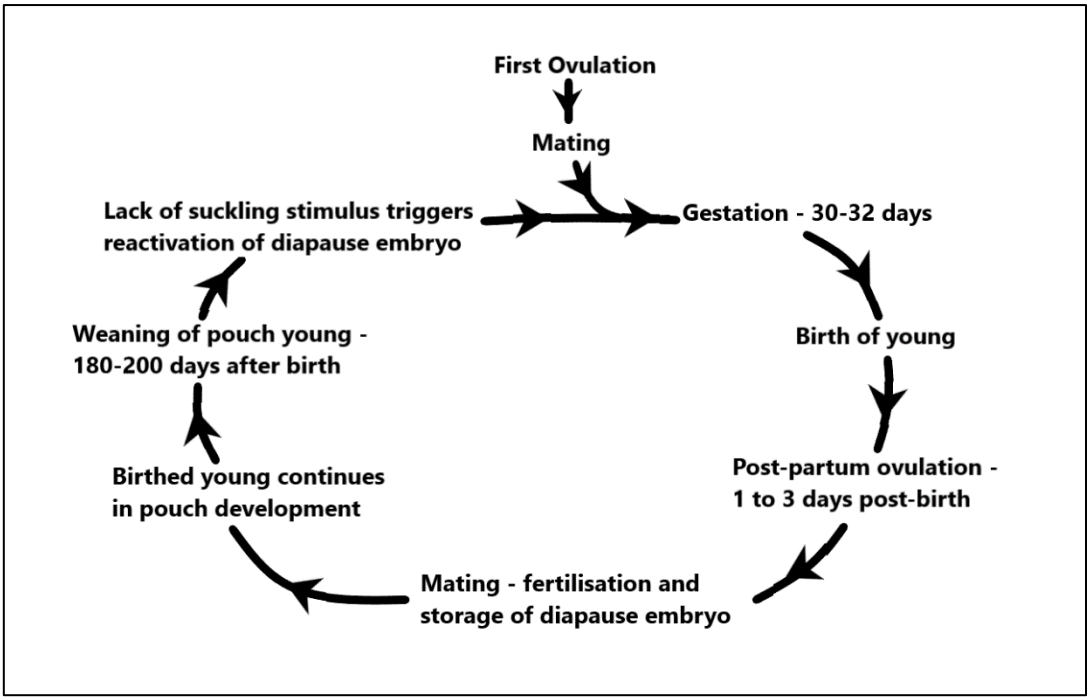


Figure 5: A schematic figure showing the cycle of embryonic diapause, utilised by macropodid species, such as the yellow-footed rock-wallaby (*Petrogale xanthopus*).

Water and Nutrition:

Adult YFRWs are capable of surviving without access to free water in cooler months. However, they do require free water when vegetation water content drops below 20% (Lim *et al*. 1987; Lapidge & Munn 2011; Sharp 2011), which occurs commonly in the semi-arid zone. Access to free water is often limited in the Australian semi-arid zone, and as such, YFRWs utilise a variety of permanent and ephemeral water holes, springs, and creeks across their habitat to meet their requirements, and additionally obtain moisture from the vegetation they ingest (Copley 1981; Lim *et al*. 1987; Lapidge & Munn 2011). During times of low free water availability or vegetation moisture, YFRWs can also acquire moisture from dew accumulated on rocks (Lim 1987). Juvenile and sub-adult YFRWs on the other hand, are likely to be more susceptible to drought conditions, as has been observed in black-footed rock-wallabies (*Petrogale lateralis lateralis*; Ruykys 2011; West *et al*. 2017). This suggests that if water availability and vegetation moisture is low for a prolonged period of time, population abundance will likely decrease due to a lack of recruitment.

Previous studies on YFRW dietary preferences examined seasonal changes in diet using micro-histological examination of faecal plant fragments, showing that YFRWs are opportunistic feeders, grazing and browsing, depending upon resource availability, showing a preference towards grasses and forbs when available (Dawson & Ellis 1979; Copley & Robinson 1983; Lim *et al*. 1987; Lapidge 2000; 2001). This technique however has limitations, with many plant fragments unable to be

identified. This is further complicated as plant communities are highly variable across the YFRW distribution, and as such, the dietary information currently available is not representative of the species more broadly. Further YFRW diet studies are required from across their distribution and in a variety of habitats during different conditions (i.e. drought or following high rainfall) to properly characterise the dietary preferences of this species in target regions, and track changes in dietary intake associated with changes in local vegetation communities and rainfall.

YFRWs use foregut microbial fermentation evolved to access the nutrients from their diet (Hume 1999; Tyndale-Biscoe 2005; Armati *et al*. 2006). Microbial fermentation is a key factor in enabling YFRWs to survive in their harsh, unpredictable habitat, allowing them to utilise dry, low-quality feed for energy. This type of digestion also allows access to microbial proteins, B-vitamins, and the recycling of nitrogen/urea, aiding in retaining substances vital for the animal's health (Armati *et al*. 2006). The adaptation of recycling nitrogen limits the amount of urea and ammonia produced by the body that is needed to be excreted by the kidneys, thus limiting the volume of water excreted as urine; an adaption to conserve water (Hume 1999; Tyndale-Biscoe 2005). The gut microbiome of some Australian marsupials has been characterised previously (Chhour *et al*. 2010; Cheng *et al*. 2015; Brice *et al*. 2019; Eisenhofer *et al*. 2021), but the composition of the YFRWs microbiome is currently unknown. The gut microbiome has previously been found to influence health parameters (Haller 2018; Amato *et al*. 2021; Huang *et al*. 2022; Pannoni *et al*. 2022; Ribas *et al*. 2023), and has recently been identified as a critical component to be incorporated into wildlife conservation programs (Banerjee *et al*. 2020). As such, the characterisation of the YFRW microbiome is needed as this may inform important future conservation actions.

The lack of contemporary YFRW studies means there may be population characteristics which are allowing colonies to adapt to their changing environment, but have not yet been recognised. One way of monitoring for these changes is by examining blood biochemistry, which can provide useful insights into the hydration and nutritional status of the animal, as well as information about environmental conditions (i.e. plant water content). Blood biochemical analyses have only previously been conducted on reintroduced YFRWs (Lapidge 2001), but prolonged, long-term examination has not been conducted. Tracking blood biochemical changes through chemical analyses and how it changes with rainfall may provide insights into seasonal stressors on YFRW colonies and inform management practices in times of low rainfall.

Threats to the Yellow-footed Rock-wallaby:

Historic Population Declines:

At the time of European settlement, YFRWs were abundant in the Flinders Ranges, described as being the most abundant mammal species in the area (Copley 1981; Lim *et al*. 1987).

> *"The rock-wallaby is by far the most abundant of animals, and yet it is a much-persecuted creature."* (Fountain 1907).

Following European settlement in South Australia, hunting of YFRWs became a common past time. They were often considered to be a pest, feeding on farmer's gardens, and drinking from stock water troughs (Babbage 1972). Hunting was fuelled by the fur-trade in London where a YFRW pelt could fetch "*a shilling and fourpence*" (Lydekker 1894). European hunting of this species continued until 1919, when a significant decline of the species was recognised and the YFRW was declared a protected species in South Australia under the Animal Protection Act (1912). This Act made it illegal to take or kill rock-wallabies, or to sell their pelts (Lim *et al*. 1987).

Although protected from human persecution, YFRW numbers continued to decline into the mid-1900's, mostly attributed to the establishment of the red fox, feral goat and rabbit in the landscape (Hume *et al.* 2004; Tyndale-Biscoe 2005; Hayward *et al.* 2011; Abbott *et al*. 2014; Sharp *et al.* 2014; Michael & Lindenmayer 2018). In the 1990's it was reported that at least 15% of YFRW colonies had become extinct since European settlement (Copley and Alexander 1997).

Population Recovery ('Operation Bounceback'):

After legislative protection of YFRWs was introduced, foxes became the major limiting factor of population viability and recovery. In 1992, 'Operation Bounceback', a South Australian government feral predator and herbivore control program was initiated (Bounceback Celebrating 20 Years, Report, 2012). Today this program is still ongoing, and has seen many biodiversity successes, in partnership with local Government departments, the South Australian National Parks and Wildlife Service, and private landholders. Management activities conducted in the 'Operation Bounceback' footprint include aerial 1080 baiting, shooting, and mustering of feral animals, as well as weed control and revegetation works (Figure 3; Bounceback Celebrating 20 Years, Report, 2012). The success of 'Operation Bounceback' has underpinned the successful reintroduction of several species (western quolls, *Dasyurus geoffroii*, brush-tailed possums, *Trichosurus vulpecula*) and the re-establishment and expansion of the existing local fauna populations (echidnas, *Tachyglossus aculeatus*, sand goannas, *Varanus gouldii*, Flinders Ranges carpet pythons, *Morelia spilota metcalfei*), which have historically been highly restricted by fox and cat predation.

The umbrella species, however, and the reason 'Operation Bounceback' was established originally, was the YFRW. Since the beginning of 'Operation Bounceback', YFRW abundance has steadily increased throughout most of their distribution with populations now expanding and occupying areas where they haven't been recorded previously in European history. Their recovery is mostly attributed to the lack of foxes in the landscape, but the control of feral goats and rabbits has also played a significant role in their recovery (Tyndale-Biscoe 2005; Bounceback Celebrating 20 Years, Report, 2012; Sharp *et al*. 2014). In the Coturaundee Range, New South Wales, when foxes were controlled (baited), the YFRW population increased by 45% over six years (Sharp *et al.* 2014), suggesting that fox control is vital component for recovering threatened populations. This relationship between fox control and population recovery has also been observed in black-footed rock-wallaby (*Petrogale lateralis*) colonies (Kinnear *et al*. 1988; 1998; 2010). The YFRWs recovery since the early 1990's has been so remarkable that they now no longer need listing as a nationally threatened species whilst fox control measures remain in place (Woinarski *et al*. 2023).

Current and Future Threats:

As foxes are currently under control across the Bounceback footprint (Figure 3), competition with exotic and native herbivores appears to be the greatest limiting factor for YFRW populations (Lim *et al*. 1987; Lethbridge *et al*. 2019; Tyndale-Biscoe 2005). Feral goats, rabbits, and overabundant euros (*Osphranter robustus erubescens*) can actively compete with YFRWs for food and shelter resources (Dawson & Ellis 1979), as has also been observed with other *Petrogale* species (Black-footed rock-wallaby; Creese *et al*. 2019). This is especially relevant in times of drought when resource availability is already low and competition for food and water resources is high within habitats (Dawson & Ellis 1979).

But as climate change progresses, there is likely to be a whole new suite of threats projected upon YFRWs. As their distribution is exclusively restricted to semi-arid regions of Australia, their risk of population decline caused by climate change impacts, is high. Most threats will likely be associated with decreases in annual rainfall, and the decreased reliability of summer rainfall. With lower annual rainfall (Head *et al.* 2014; Department for Environment and Water 2022), vegetation moisture content is likely to be below 20% for longer periods, forcing YFRWs to access free water more frequently. However, as the summer rainfall which provides YFRWs with access to free water becomes unreliable, the lack of water sources, coupled with prolonged low vegetation moisture, may become a major limiting factor on population abundance.

Climate change also threatens to further restrict recruitment and dispersal events, further exacerbating genetic isolation, diet variability and potentially, their gut microbiome. The effects that these changes

will have on YFRW metabolism, biology and behaviour are currently unknown, but they could potentially pose a threat to the longevity and fecundity of populations across their distribution.

Thesis Aims:

As many aspects of the YFRWs ecology make them susceptible to climate change impacts, this project aims to advance our understanding of YFRW ecology in habitats with different connectivity, to help inform on-ground species management. Specifically, this thesis will examine: 1) seasonal changes in body condition, breeding, blood biochemistry and antioxidant levels, 2) genetic diversity and kinship, 3) rainfall effects on diet and dietary overlap with goats and euros, 4) baseline faecal microbiome characteristics and interactions with diet, and 5) the species' predicted future distribution in South Australia and New South Wales under climate change.

Currently, our understanding of these aspects of YFRW ecology are either outdated, assessed using old techniques, have not been conducted at all, or have only been assessed at a small spatial scale. In each chapter of this thesis, comparisons between a YFRW population in connected (Italowie Gap in the Vulkathunha-Gammon Ranges National Park) or isolated (Bimba Hill in the Bimbowrie Conservation Park) habitat will be examined and discussed. The basis of successful conservation programs is an in-depth ecological knowledge of a species, and as such, this project will improve our understanding of these factors and also contribute new knowledge which will help prepare management plans for YFRW populations at risk from climate change.

Chapter One

"Population demographics, health and breeding of yellow-footed rock-wallabies (*Petrogale xanthopus xanthopus*) in connected and isolated habitat in semi-arid South Australia"



"*The rock-wallaby is by far the most abundant of animals, and yet it is a much persecuted creature*"

– Fountain 1907

Statement of Authorship

Title of Paper	Population demographics, health and breeding of yellow-footed rock-wallabies (*Petrogale xanthopus xanthopus*) in connected and isolated habitat in semi-arid South Australia
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Principal Author

Name of Principal Author (Candidate)	Lauren Werner		
Contribution to the Paper	Study design, field work, sample collection, laboratory preparation (blood samples), statistical analyses, writing manuscript.		
Overall percentage (%)	90		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	14/10/2024

Co-Author Contributions

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

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

Name of Co-Author	Ms Shannon Kleemann		
Contribution to the Paper	Field work, sample collection, statistical analysis, editing manuscript.		
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Contribution to the Paper	Field work, sample collection.		
Signature		Date	14/10/2024

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Contribution to the Paper	Primary supervisor. Study design, sample collection, editing of manuscript.		
Signature		Date	14/10/2024

Population demographics, health and breeding of yellow-footed rock-wallabies (*Petrogale xanthopus xanthopus*) in connected and isolated habitat in semi-arid South Australia

Werner L.E, Kleemann S.L, Dix S.K, Taggart D.A

Abstract:

Population monitoring is vital to inform conservation management programs for vulnerable wildlife species. The yellow-footed rock-wallaby (YFRW; *Petrogale xanthopus xanthopus*) a medium-sized, semi-arid adapted marsupial, was captured periodically between 2021-2022 to compare the effects of habitat connectivity and rainfall on population demographics, health, and breeding in two YFRW colonies in South Australia. Morphological measurements and biological samples were collected from each captured YFRW (n = 52) to assess differences within and between study sites. YFRWs from Italowie Gap (n = 24), a highly connected, mountainous colony site in the Vulkathunha-Gammon Ranges National Park, appeared healthy, regardless of having a high prevalence of ectoparasites, and had a consistently good body condition index (BCI) throughout the study, although BCI did decrease significantly during an extremely dry period. Births occurred throughout the year, but a marked peak was observed during autumn (March/April) following historic patterns of high summer rainfall in this region. For YFRWs from Bimba Hill (n = 28) occupying isolated, rocky jumbles on shallow hills in the Bimbowrie Conservation Park, there were more sub-adults captured than at Italowie Gap, and BCI remained consistent throughout the study, reflecting their overall good health and low ectoparasite prevalence. Births at Bimba Hill occurred throughout the year, but in comparison to Italowie Gap, a minor peak was observed in July, reflecting the consistent year-round rainfall pattern of this region. Blood biochemical parameters were generally within the normal reference ranges for the species, with some variations observed between sites and/or sampling times, presumably associated with variations in water availability and plant nutritional content. The results from this study indicate that this species is well adapted to its harsh, semi-arid habitat, with most captured animals maintaining good health in an environment with highly erratic rainfall. The differences observed between sites suggest that habitat-specific species management plans may be beneficial for YFRWs. Further monitoring is required to assess how climate change will impact this iconic semi-arid species and continue to inform their conservation management into the future.

Introduction:

Globally, the number of wildlife species which are threatened by habitat loss, illegal hunting, human interference, diseases, exotic pests and/or climate change is increasing (Daszak *et al*. 2000; Eigenbrod *et al*. 2008; Wilson 2019; Weiskopf *et al*. 2020; Sethi 2022; Camprasse *et al*. 2023; Morini *et al*. 2023). As such, establishing baseline information on wildlife species' ecology and health, together with regular monitoring, is important to help guide species conservation management actions. This is particularly relevant for threatened species to aid in reducing their risk of extinction, and to increase the success of conservation actions, such as reintroductions (Schultz *et al*. 2011; Bergman *et al*. 2018; Norkaew *et al*. 2018; Ferreira *et al*. 2019; McCaffrey *et al*. 2023).

Currently, Australia has the worst mammal extinction rate, with native mammal species declining by 35% since European settlement (Burbidge *et al*. 2008; Woinarski *et al*. 2015; Saltré *et al*. 2019; Bayraktarov *et al*. 2021; Tulloch *et al*. 2023). Additionally, many other native species are also considered Vulnerable or Endangered (Woinarski *et al*. 2015). The main causes of species extinctions and population declines are habitat loss and fragmentation, and the introduction of feral pests (Cane toads, *Rhinella marina*; feral goats, *Capra hircus*; European rabbits, *Oryctolagus cuniculus*; feral cats, *Felis catus*; red foxes, *Vulpes vulpes*) (Woinarski *et al*. 2015; Doherty *et al*. 2016). In semi-arid regions of Australia, where many threatened native mammal species reside, feral cats and red foxes are a major threat to native wildlife viability (Sharp *et al*. 2014; Woinarski *et al*. 2015).

The yellow-footed rock-wallaby (YFRW; *Petrogale xanthopus xanthopus*), the focus of this study, is a Vulnerable (EPBC Classification; Woinarski *et al*. 2014; Baker & Gynther 2023), medium sized, macropodid marsupial which, in South Australia, is found in the semi-arid Flinders, Gammon, Gawler and Olary Ranges (Copley 1981 & 1983; Lim & Giles 1987; Lim *et al.* 1987; Copley & Alexander 1997). Historically, this species was described as the most abundant mammal in the Flinders Ranges (Fountain 1907) but as a consequence of extensive hunting for the fur trade (Lydekker 1894) and the establishment of the red fox, YFRW numbers declined dramatically, resulting in the local extinction of some populations, with many more becoming threatened with extinction.

Operation Bounceback, a landscape-scale pest control program initiated in the early 1990's to address the population declines of YFRWs and other native species, aimed to control foxes, feral goats, cats, and rabbits in the Flinders, Gammon, Gawler and Olary Ranges, South Australia (Bounceback Celebrating 20 Years, Report, 2012). Since this program was implemented some 30+ years ago, the YFRW and many other local fauna and flora species, have begun recovering and are once again common in the landscape. The recovery of the YFRW has been so significant that it has now been recommended to be removed from the threatened species list (Woinarski *et al*. 2023).

A study by Sharp & McCallum (2015), examined the population dynamics of YFRWs, revealing that survival rates are often determined by forage availability nearby to refuge sites. This was also supported by a study in New South Wales, where YFRW population increases were correlated with high rainfall in the preceding year (Sharp & Norton 2000). As YFRWs have relatively low rates of dispersal (Sharp 1997a), especially in isolated colony sites (Smith *et al*. 2023), this is likely further restricting access to the forage resources required for population growth and stability.

Currently, detailed monitoring data is limited for YFRW populations, with reference data only available from short-term studies on two reintroduced populations in the northern Flinders Ranges (Aroona Dam) and south-western Queensland (Lapidge 2001), or from the southern Flinders Ranges (Lim *et al*. 1987). To address the gap in contemporary population knowledge, this study examined YFRW population demographics, health and breeding to determine how these parameters were affected by habitat connectivity and rainfall. This information is important for guiding the future management of YFRWs and how conservation programs should be regionally tailored to improve the success of management actions.

Methods:

Ethical Statement:

This study was approved via a Scientific Research Permit from the Department of Environment and Water, South Australia (#Y26992-2), and animal ethics approval obtained from the University of Adelaide's Animal Ethics Committee (S-2020-097).

Study Sites:

This study was conducted in South Australia at Italowie Gap in the Vulkathunha-Gammon Ranges National Park, and in the Bimba Hill region of the Bimbowrie Conservation Park during 2020-2022 (Figure 1).

Italowie Gap is approximately 500km north of Adelaide in the Gammon Ranges region. The YFRW habitat at Italowie Gap is a combination of complex and simply stratified geological features formed from sandstone, siltstone, and quartzite, within a steep mountain range (Balcanoona Range). YFRWs are found along the length of this range which stretches for approximately 20 kilometres (see Appendix 1, Figure S1 pg. 212), making this site highly connected to other colonies. At Italowie Gap, vegetation is mostly confined to creek beds, dominated by river red gum trees (*Eucalyptus camaldulensis*), but on the hill slopes rock fuchsia bush (*Eremophila freelingii*), *Acacia* species, and white cypress pine (*Callitris glaucophylla*) are present. During times of high rainfall, the lower slopes

and plateaus are dominated by rock sida (*Sida petrophila*) and desert lantern-bush (*Abutilon leucopetalum*) with small herbaceous plants and grasses growing amongst the rocks. The average annual rainfall is 256.6mm, however this region was in drought for the four years preceding this study (2017-2020), receiving a total of 470.6mm across this period, before drought-breaking rainfall occurred in 2020 (Bureau of Meteorology). Across the 15-month period of this study, 469mm of rainfall was recorded (Figure 2; Bureau of Meteorology).

The Bimba Hill region in Bimbowrie Conservation Park is approximately 350km north-east of Adelaide in the Olary Ranges (Figure 1). YFRW habitat at Bimba Hill is shallow, isolated monzogranite outcrops, scattered with large complex boulder jumbles, flat sheets of rock, and earthen slopes. The Bimba Hill region has some nearby YFRW colonies (within 5 kilometres), but most other colonies in the park are separated by approximately 10 kilometres of mostly open ground (see Appendix 1, Figure S2 pg. 213). However, even colonies near to Bimba Hill are not connected by suitable YFRW habitat, making this site quite isolated. The vegetation in this region is dominated by rock fuchsia bush (*Eremophila freelingii*), with occasional mulga (*Acacia aneura*) and dead finish (*Acacia tetragonophylla*) present. After high rainfall events, earth slopes and areas amongst the boulders are dominated by rock sida (*Sida petrophila*) and *Solanum* species, with herbaceous plants and grasses growing along the base of boulders or in rock crevices. The average annual rainfall for this region is 203.2mm, however this area was also in drought in the four years preceding this study (2017-2020) when only 409mm of rainfall were recorded (Bureau of Meteorology). During the 15-month period of this study, 317.7mm of rainfall was recorded for the region (Figure 2; Bureau of Meteorology).

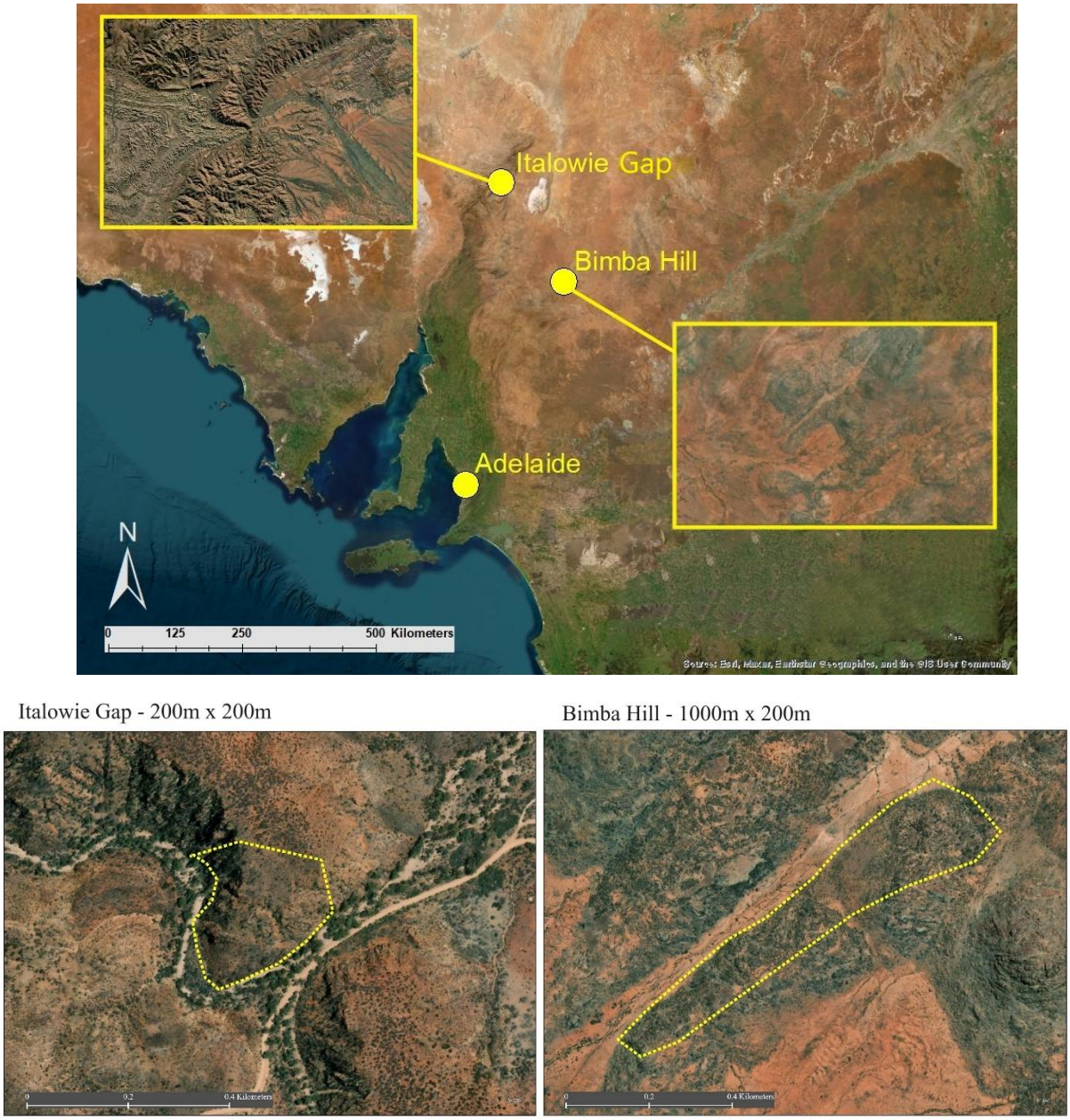


Figure 1: *Top*: A satellite image showing the location of trapping sites for yellow-footed rock-wallabies (*Petrogale xanthopus xanthopus*) at Italowie Gap in the Vulkathunha-Gammon Ranges National Park and at Bimba Hill in the Bimbowrie Conservation Park. *Inset images:* Satellite view of each region where trapping was conducted (top left- Italowie Gap, bottom right- Bimba Hill). *Bottom*: The approximate area where yellow-footed rock-wallabies were trapped, indicated with a yellow outline, at Italowie Gap (*left*) and Bimba Hill (*right*).

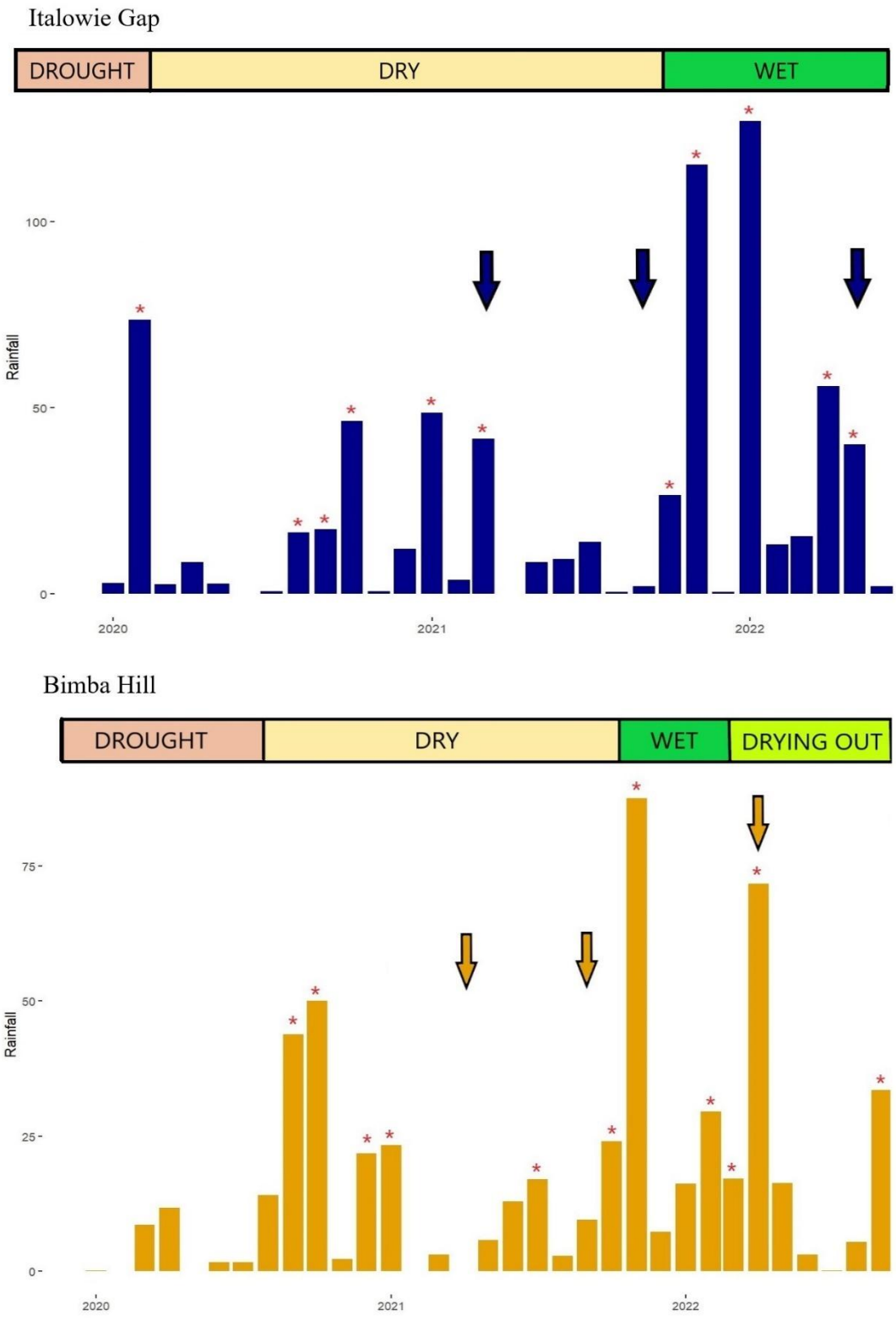


Figure 2: The total monthly rainfall (mm) received preceding and during the study period at Italowie Gap (*top*) and Bimba Hill (*bottom*), South Australia. Months of higher-than-average rainfall (*), conditions at each site (drought, dry, wet or drying out), and yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) trapping times (downward arrow) are indicated. Rainfall data obtained from the Bureau of Meteorology.

Trapping and Sampling:

Trapping for YFRWs was conducted across an area of ~200m x 200m at Italowie Gap, and ~200m x 1000m at Bimba Hill (Figure 1). Trapping was conducted at each site at three time points: March/April 2021, September 2021, and April/May 2022. An additional trapping event was conducted at Italowie Gap in September 2023, however, only breeding data from this time is included in this study.

Approximately 4-6 weeks prior to active trapping, 10 traps were placed at each trapping site in areas deemed to be occupied by YFRWs (i.e. near rock crevices, high scat presence). Soft-sided steel traps, made specifically for capturing YFRWs and other *Petrogale* species, were used (Crestware Industries, South Australia). Traps were wired open for free-baiting for 4-8 weeks prior to each trapping trip, where a mixture of kangaroo lucerne pellets (Lauke Mills), carrot, apple, and sweet potato was placed into and surrounding each trap approximately once a week. For each site, active trapping was conducted for four nights per trip. Traps were checked and cleared of any captured YFRWs by 8am the following morning, where any captured animals were transferred into close-weave heavy duty cotton drill fabric bags and labelled with the trap number. Further information regarding trap setup and trapping preparation can be found in the *Supplementary Chapter* of this thesis.

Morphometric measurements, body weight, pouch conditions, scrotal diameter, presence of ectoparasites, and biological samples (i.e. tissue, blood, faecal samples, microbiome swabs) were collected from each captured YFRW, where possible. If ectoparasites were encountered on the captured animal, samples were collected, stored in 90-100% ethanol and sent for identification by a Parasitologist at the University of Adelaide. The number of adult females which were carrying pouch young at the time of capture was recorded, and to examine trends in birth rates, the age of pouch young and young sub-adults (n = 36) was calculated from head length measurements using the values provided in Poole *et al*. (1985). Age was determined by counting back from the date of capture to estimate the date of birth, assuming a normal YFRW gestational period of 31-32 days (Poole *et al*. 1985). The number of births per month were then visualised with rainfall data obtained from the Bureau of Meteorology (www.bom.gov.au) to assess the timing of increased birth rates. The size of reproductively mature YFRW was also examined, where for females, sexual maturity was assessed using body weight and head length in relation to pouch characteristics and young presence. For male YFRWs, body weight and scrotal diameter was used to assess sexual maturity, as these measures have been shown previously in another *Petrogale* species to be indicative of being reproductively viable (Taggart *et al*. 2005).

To assess the physical condition of captured YFRWs a body condition index (BCI) was used, and was developed from the residuals of a body weight by head length linear regression to examine changes in condition at different sampling times.

For blood biochemical and antioxidant analysis, blood samples were collected from the lateral caudal tail vein using a 2mL syringe and 22G needle. Blood was then placed in a 2mL heparin blood tube and centrifuged for 10 minutes at high speed to separate blood components. Blood plasma was then removed and transferred into a 1.5mL Eppendorf tube using a single-use transfer pipette and kept frozen until analysis.

When applicable, a unique ear tag and microchip was given to each individual YFRW processed for future identification. After processing, each YFRW was released close to their site of capture. All animal processing and sampling procedures are described in the *Supplementary Chapter* of this thesis.

Blood Biochemical Analysis:

Blood samples (n = 51) were sent to the biochemistry laboratory at Adelaide Zoo for processing using a Beckman Coulter AU480 Chemistry Analyser. General blood biochemistry and antioxidant capacity measures, specifically, trolox equivalent antioxidant capacity (TEAC; Re *et al*. 1999), ferric reducing ability of plasma (FRAP; Benzie & Strain 1996) and thiobarbituric acid reactive substances (TBARS; Esterbauer & Cheeseman 1990), were conducted using the corresponding recommended protocols and where necessary, with modified absorbance colorimetric assays.

Retinol (Vitamin A) and α-tocopherol (Vitamin E) levels were assessed using high-performance liquid chromatography (HPLC; modified from Catignani & Bieri 1983). Reverse phase HPLC analysis was performed using a Waters 600 Pump and 600E Controller coupled to a Waters 2489 UV/Visible Detector (Waters Corporation, Milford MA). Apollo columns (Alltech, Australia) were used as the stationary phase with a guard column (C18, 75 x 4.6mm: Particle size 5µm) positioned ahead of the analytical column (C18, 250 x 4.6mm: Particle size 5µm). An isocratic mobile phase consisting of 75% acetonitrile and 25% methanol was applied at a flow rate of 2mL.min^{-1} for 17 minutes. The detector was set at 326nm for 6 minutes then changed to 220nm for the last 11 minutes. Retinol was detected at 326nm after approximately 3.5 minutes and d-α-tocopherol at 220nm after approximately 13 minutes. Reagents were of analytical grade and solvents of HPLC grade. Chromatograph output was processed using the Delta Chromatography Data Systems software.

Calibration curves for reference standards of retinol and d-α-tocopherol (Sigma-Aldrich) were constructed by plotting area under the curve (AUC in uV.s^{-1}) versus retinol or d-α-tocopherol concentration (µg.mL^{-1}). Least squares regression was performed to determine the slope, intercept and coefficient of determination for both standards. Assay concentrations were calculated using the following formula:

$$Analyte\ (\mu g.\,mL^{-1}) = \frac{AUC\ for\ assay\ \times\ standard\ concentration\ \times\ sample\ dilution}{AUC\ for\ standard\ \times\ sample\ weight\ (g)}$$

Animal hydration can generally be determined by examining urea and creatinine blood plasma ratios in mammal species (Morgan *et al*. 1977; Akimoto *et al*. 2011; Robert & Schwanz 2013; Lin *et al*. 2016). However, as macropodids utilise urea for microbial synthesis for microbial fermentation (Armati *et al*. 2006), it is an unreliable measure. Therefore, this study used creatinine as the primary indicator of hydration status.

Statistical Analysis:

YFRW capture data (n = 65) was entered into Microsoft Excel for visualisation and statistical analysis. An initial assessment (i.e. mean, standard errors) showed a high level of variation between adult and sub-adult values, and therefore, individuals were separated into age cohorts before informative analysis was conducted. Adult and sub-adult YFRWs were differentiated based on pouch characteristics and the presence of a pouch young (females), or scrotal diameter and epididymis development (males). The sex ratio for each trapping period and the entire study was calculated examining the proportion of males captured using the equation:

$$number\ of\ males\ \div (number\ of\ males + number\ of\ females)$$

The calculated sex ratio for each time point and site was then compared for deviations from 0.5 (i.e. 1F:1M) using a chi-square test.

An average BCI was calculated for adult and sub-adult/juvenile YFRW separately to account for morphological variation between the age classes. Statistically significant differences in body condition between sampling times, and between study sites, was determined using a two-sample t-test, assuming unequal variances, using a significance level of 0.05.

YFRW blood biochemistry data was firstly analysed using the RefVal add-in to identify abnormal blood values indicative of a problems occurring during blood sampling or biochemical analyses. This revealed abnormal values in blood results of three sub-adult YFRWs from Bimba Hill and one adult YFRW from Italowie Gap. Subsequently these four individuals were removed from further analysis. As with morphological analyses, blood analyses were also conducted in two age cohort groupings (adult and sub-adult/juvenile) due to differences in nutrient requirements and growth status. Each blood biochemical parameter was tested for normality and homogeneity of variances using SPSS (IBM, U.S.A). The results from these tests then determined if the values for individual blood biochemical parameters were compared using a two-sample t-test assuming unequal variances or a one-way ANOVA, to assess differences between study sites and sampling time, both using a 0.05 significance level.

Results:

Trapping:

A total of 52 individual YFRWs (23F, 29M) were captured across the three trapping times from both sites. Specifically, twenty-four YFRWs (16F, 13M) were trapped at Italowie Gap, and 28 (15F, 21M) at Bimba Hill.

Only sub-adult and juvenile YFRWs were captured at Bimba Hill during autumn 2021 (Table 1). This was due to smaller, inappropriately sized traps being used at this time. In subsequent trapping trips larger rock-wallaby traps were available for use, which facilitated the capture of YFRWs from all age cohorts. During all subsequent trapping trips to Bimba Hill, adult YFRWs were the most common cohort captured (Table 1).

The number of YFRWs captured per trapping trip generally increased as trapping progressed, with 17% and 25% of individuals recaptured on subsequent trips at Italowie Gap and Bimba Hill, respectively (Table 1). The sex ratio of captured YFRWs throughout this study at Italowie Gap was 0.45 (proportion of males sampled; 1F:0.8M), and at Bimba Hill was 0.58 (1F:1.4M). Although at Italowie Gap the proportion of males sampled was lower than the expected 0.5, and at Bimba Hill it was higher, there was not a statistically significant difference between the study sites (Chi-square test, $p = 0.32$).

Table 1: Yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) trapping data during trips to Italowie Gap and Bimba Hill in 2021-22. The number of captured individuals is reported per age cohort (adults, sub-adults, juvenile), and number of each sex captured with the overall sex ratio provided. Recaptures refers to individual YFRWs which were captured across multiple trapping trips.

	Autumn 2021		**Spring 2021**		**Autumn 2022**		**Total**	
	Italowie Gap	Bimba Hill	Italowie Gap	Bimba Hill	Italowie Gap	Bimba Hill	Italowie Gap	Bimba Hill
Adults	5	0	6	5	7	10	18	15
Sub-adults	1	6	3	4	0	5	4	15
Juvenile	1	2	3	2	3	2	7	6
Total	7	8	12	11	10	17	29	36
Recaptures	-		3	0	2	8	5	9
Sexes	3F, 4M	3F, 5M	6F, 6M	4F, 7M	7F, 3M	8F, 9M	16F, 13M	15F, 21M
Sex ratio	0.57	0.63	0.5	0.64	0.3	0.53	0.45	0.58

Breeding:

A total of 12 adult female YFRWs were captured at both sites, six from both Italowie Gap and Bimba Hill.

At Italowie Gap, all adult females had a pouch young at the time of capture. Two females were captured during all three trapping trips, where one of these females had a different young during each trip. The other female captured during all trapping trips had the same young in the pouch during autumn and spring 2021 trapping trips, with a new young present in autumn 2022. Both of these observations, in conjunction with pouch young birth date calculations, indicate that these females were breeding continuously, with no anoestrus period between young.

Of the nine times an adult female was captured at Bimba Hill, there was pouch young present eight times. Two adult females initially captured in spring 2021, had new pouch young when captured in autumn 2022, suggesting there had been little to no anoestrus period between young, also indicating continuous breeding was occurring.

After estimating the age of pouch young and young sub-adult YFRWs (n = 36) from both sites, no obvious trends in the timing of births were observed in response to localised high rainfall events (Figure 3). There was variation between study sites in which months had the highest rate of YFRWs births (Figure 4). At Italowie Gap, a marked peak in births occurred in early autumn (March/April – 42% of births recorded), with the remainder spread relatively evenly across the other months of the year. This peak in births corresponded with historically high rainfall months at Italowie Gap (i.e. April; Figure 4), rather than being in response to opportunistic rainfall events (Figure 3; Figure 4). In contrast, births at Bimba Hill were spread quite evenly across the months of the year, with a slightly higher birth rate in July (Figure 4), but also showed no response to localised high rainfall events (Figure 3).

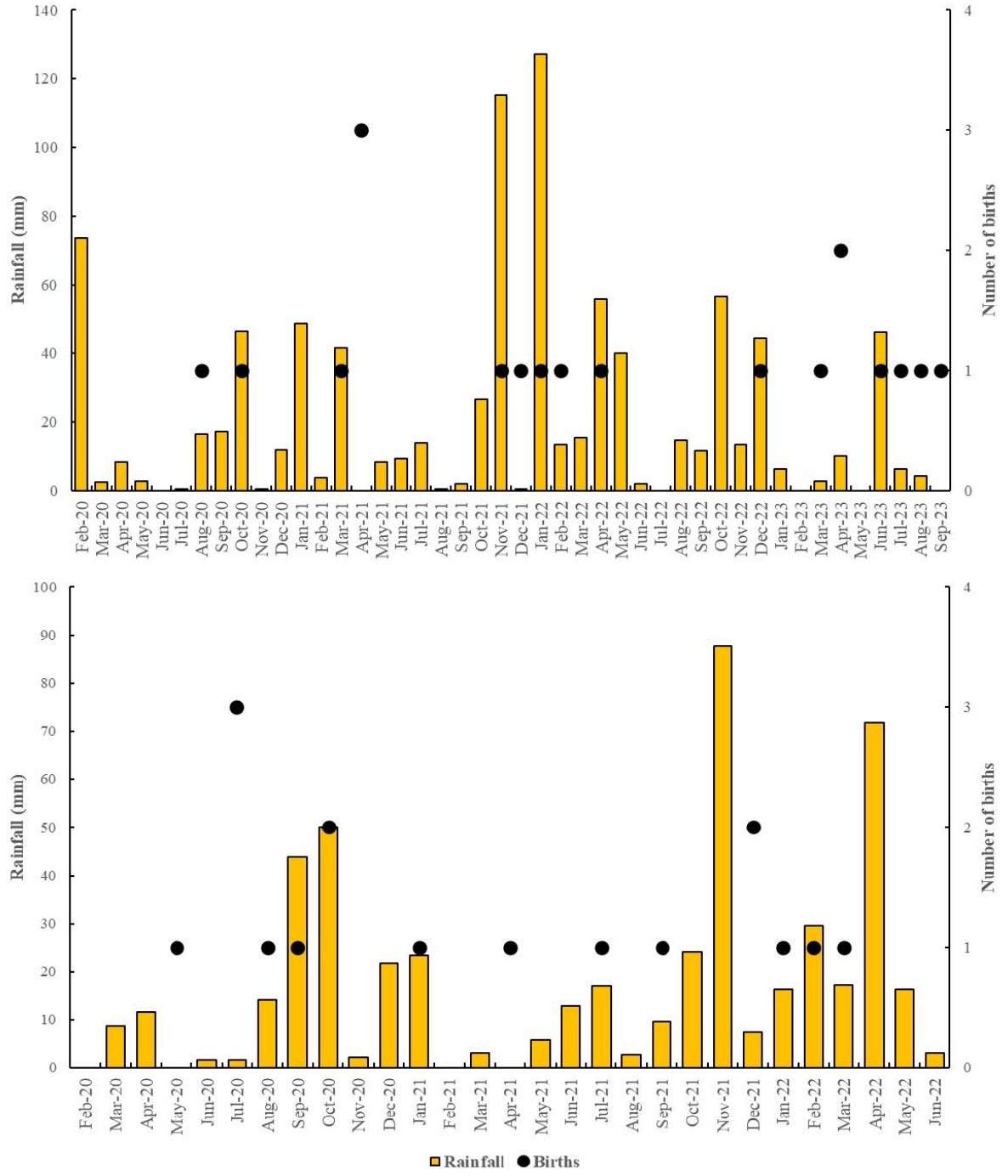


Figure 3: The number of births (●) of yellow-footed rock-wallabies (*Petrogale xanthopus xanthopus*) at Italowie Gap (*top*), and Bimba Hill (*bottom*), South Australia, in relation to localised rainfall (Data acquired from Bureau of Meteorology) shown in millimetres.

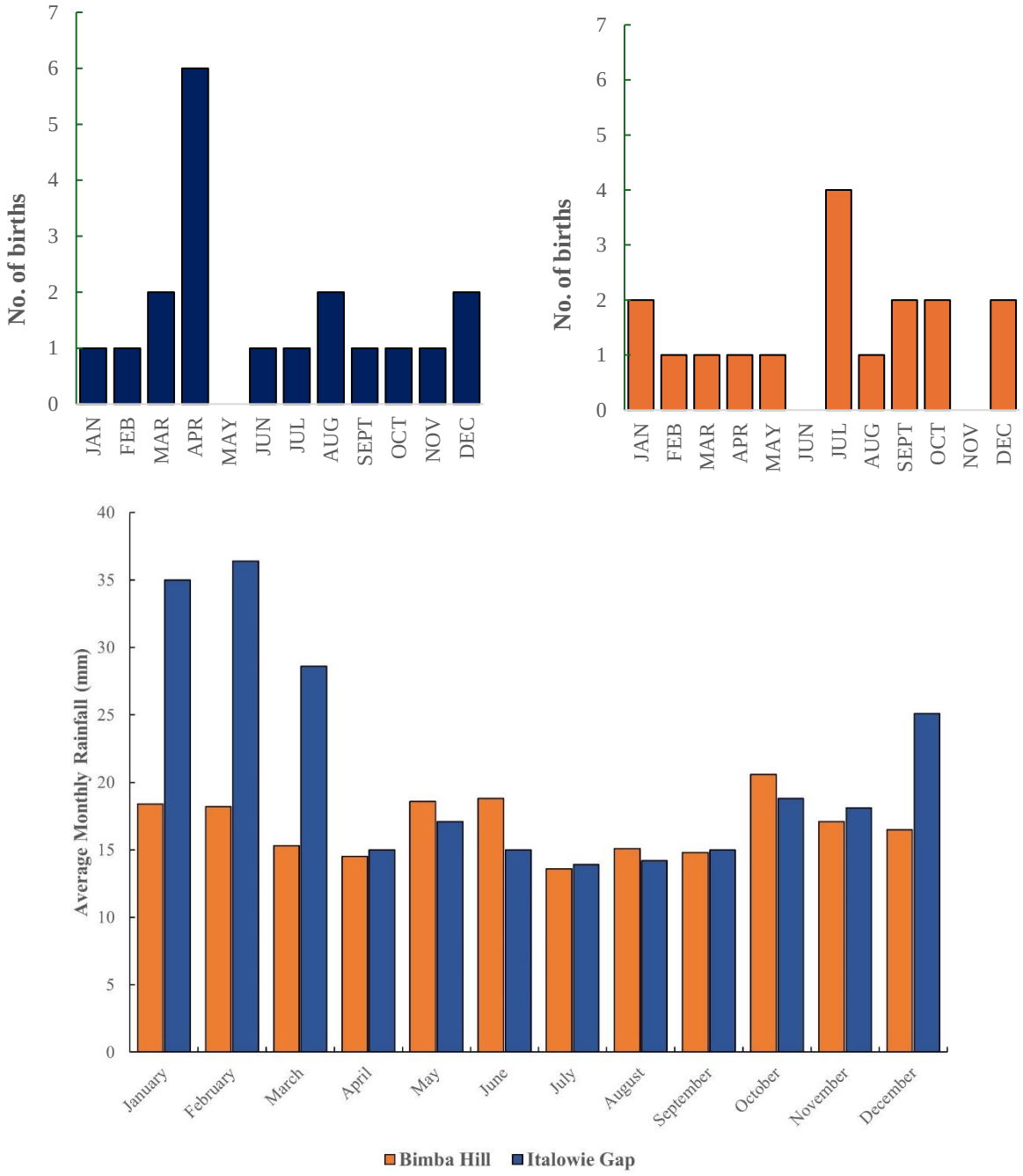


Figure 4: The number of births of yellow-footed rock-wallabies (*Petrogale xanthopus xanthopus*) at Italowie Gap (*top left*), and Bimba Hill (*top right*), South Australia. Time of birth was determined from head length measurements of pouch young and young sub-adult rock-wallabies (as per Poole *et al.* 1985). *Bottom*: the 100-year average rainfall per month (Bureau of Meteorology) for Italowie Gap (blue) and Bimba Hill (orange), South Australia.

Sexual Maturity:

Females:

A deep pouch with dark pouch secretions, elongated teats, a swollen mammary gland, and/or pouch young presence were characteristics used to identify sexually mature female YFRWs. Whereas, sub-adult females typically had a shallow, dry pouch, with small, undeveloped teats. Based on these criteria, the data gathered from female YFRWs captured across this study suggests that females reach sexual maturity at approximately 4.5kg, with a head length of approximately 100mm (Figure 5).

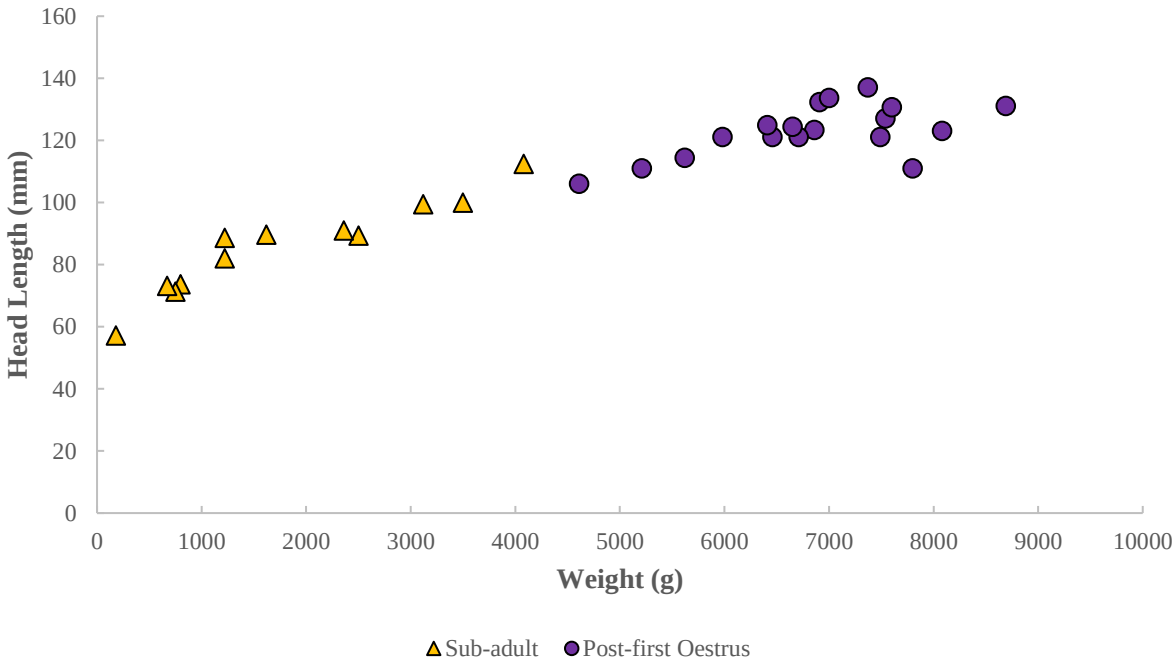


Figure 5: The relationship between head length, body weight and sexual maturity in female yellow-footed rock-wallabies (*Petrogale xanthopus xanthopus*). ▲ = sub-adult females, ● = adult, sexually mature females (post first oestrus).

Males:

Analysis of data collected from male YFRWs captured on 29 occasions indicated that scrotal diameter increased slowly until ~25mm, when males weighed around 4kg. After this time a rapid increase in scrotal diameter was observed until it started to plateau at ~40mm, when males weighed 6-7kg (Figure 6). This suggests that the onset of sexual maturity in male YFRWs occurs at approximately 6-7kg when the scrotal diameter reaches 40-50mm.

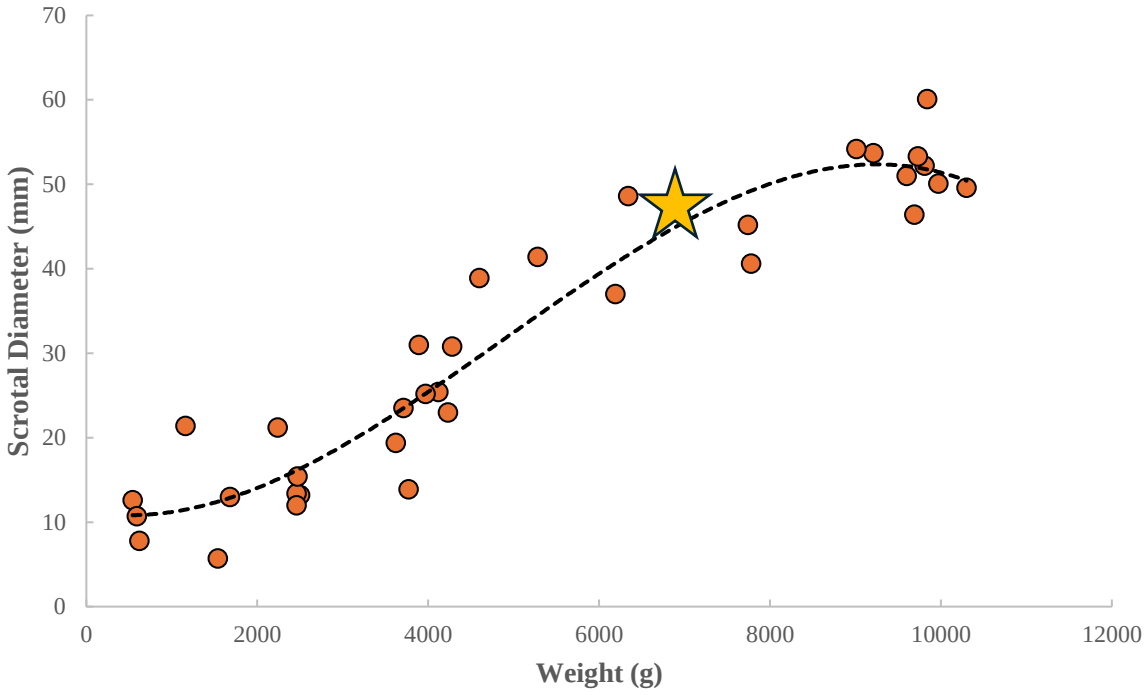


Figure 6: The relationship between scrotal diameter, weight and the onset of sexual maturity (orange ☆) in male yellow-footed rock-wallabies (*Petrogale xanthopus xanthopus*).

Physical Condition:

Italowie Gap:

The mean body condition of adult YFRWs at Italowie Gap declined significantly (18.6% decline; p = 0.04; Figure 7) from autumn 2021 (n = 5, mean = 79.17, S.D = 11.9) to spring 2021 (n = 6, mean = 64.45, S.D =, 7.5) when the site was in extremely dry conditions. Substantial rainfall fell between November 2021 and January 2022 (242.4mm; Figure 2), resulting in the BCI of the adult YFRWs increasing by autumn 2022 (n = 7, mean = 68.52, S.D = 6.8), however this increase was not deemed statistically significant (p = 0.33; Figure 7).

The BCI of sub-adult/juvenile YFRWs declined significantly at Italowie Gap between spring 2021 (n = 6, mean = 41.15, S.D = 14.6) and autumn 2022 (n = 3, mean = 22.93, S.D = 7.4) (22.1% decline; p = 0.04; Figure 7). Adult and sub-adult/juvenile mean BCI was significantly different in spring 2021 (p = 0.01) and autumn 2022 (p = 0.0007) where adult YFRWs were in better condition. Although this difference in BCI was not statistically significant in autumn 2021 (p = 0.13), this is likely attributed to the low sample size of sub-adults/juvenile YFRWs during this time (Table 1).

Ectoparasites were only observed during spring 2021 and autumn 2022 trapping trips, where kangaroo ticks (*Amblyomma triguttatum*) and trombiculid mites (Family *Trombiculidae*) were detected. During spring 2021, 43% of adult YFRWs had ectoparasites, whilst in autumn 2022, all adult YFRWs had prominent ectoparasite infestations.

Bimba Hill:

No significant differences were observed in adult BCI at Bimba Hill between spring 2021 (n = 5, mean = 64.67, S.D = 3.5) and autumn 2022 (n = 10, mean = 62.14, S.D = 6.5) trapping periods (p = 0.63; Figure 7), despite drought breaking rainfall occurring in September/October 2020 (93.8mm; Figure 2) and November 2021 (87.6mm; Figure 2). The only significant difference in BCI was observed between autumn 2021 (n = 8, mean = 44.97, S.D = 6.02) and autumn 2022 (n = 10, mean = 62.14, S.D = 6.5) for sub-adult/juvenile YFRWs (p = 0.04; Figure 7). As was observed at Italowie Gap, adult BCI was significantly greater than sub-adults/juvenile YFRWs in both spring 2021 (p = 0.01) and autumn 2022 (p = 0.007) trapping periods. Ectoparasites were only detected during autumn 2022 and were much less prevalent than at Italowie Gap, with only two sub-adults mildly infected with chewing lice (*Heterodoxus* species).

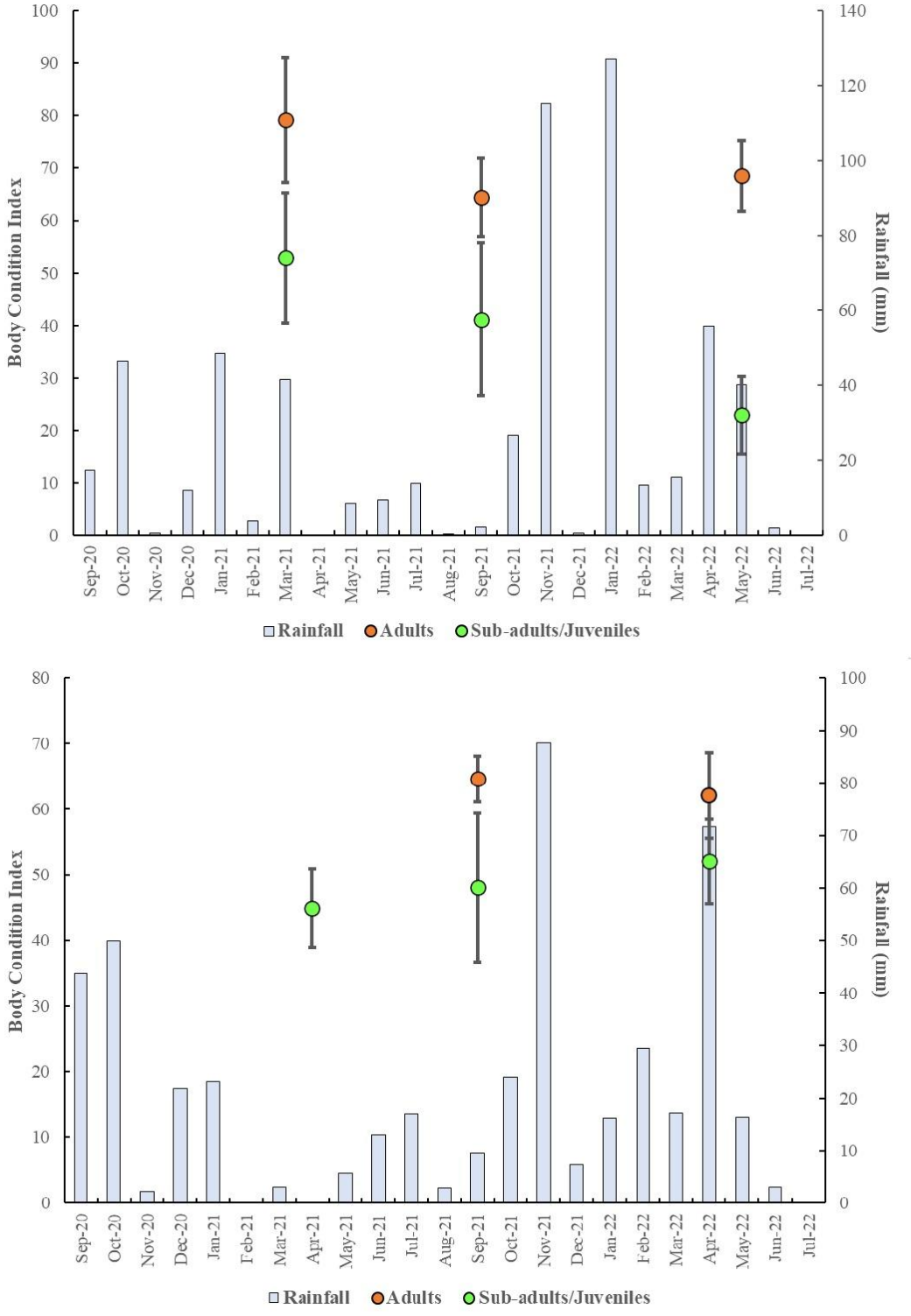


Figure 7: The relationship between rainfall and body condition of yellow-footed rock-wallabies (*Petrogale xanthopus xanthopus*) at Italowie Gap (*top*) and Bimba Hill (*bottom*). Body condition index values shown are the average values for all individuals in each age cohort (adults and sub-adult/juveniles) with standard deviation indicated, per time point. Rainfall data is shown in millimetres (Acquired from the Bureau of Meteorology).

Blood Biochemistry:

The blood biochemical parameters of urea, total protein, albumin, cholesterol, and triglycerides were examined to determine the nutritional status of YFRWs in this study, whilst creatinine was used to assess hydration status. No statistically significant differences were found in total protein, albumin, and cholesterol ($p > 0.05$; Table 2) levels across this study.

Urea levels in adult YFRWs at Italowie Gap however, were significantly higher in spring 2021 (n = 5, mean = 11.98mmol/L, S.D = 1.5) compared to autumn 2022 (n = 6, mean = 9.45mmol/L, S.D = 1.62). Urea levels also differed significantly between study sites in autumn 2022 ($p = 0.002$; Table 2) where urea levels were lower at Italowie Gap. At Bimba Hill creatinine levels increased significantly ($p = 0.03$) between spring 2021 (n = 5, mean = 90.6µmol/L, S.D = 14.01) and autumn 2022 (n = 10, mean = 110.3 µmol/L, S.D = 11.4), and also varied significantly between sites in autumn 2022 ($p = 0.0002$; Table 2) where creatinine levels were lower at Italowie Gap (n = 6, mean = 76.5 µmol/L, S.D = 11.7). During spring 2021, triglycerides were significantly lower ($p = 0.01$) at Italowie Gap (n = 5, mean = 0.43, S.D = 0.1) compared to Bimba Hill (n = 5, mean = 0.88, S.D = 0.3) but were not significantly different at any other sampling time.

Antioxidant indices, FRAP and TBARS, differed significantly in adult YFRWs between sites in autumn 2022 (FRAP $p = 0.02$; TBARS $p = 0.03$), with values of both parameters at Italowie Gap being significantly lower (n = 6; FRAP mean = 0.54 mmol/L, S.D = 0.06; TBARS mean = 1.65µmol/L, S.D = 0.2) than the values observed at Bimba Hill (n = 10; FRAP mean = 0.94mmol/L, S.D = 0.4; TBARS mean = 2.05µmol/L, S.D = 0.5) (Table 2). Differences in adult TBARS results were also observed within each site between spring 2021 and autumn 2022, where TBARS levels significantly decreased ($p = 0.03$ for both sites) during autumn 2022 (Italowie Gap; spring 2021: n = 5, mean = 2.54µmol/L, S.D = 0.6; autumn 2022: n = 6, mean = 1.65µmol/L, S.D = 0.2) (Bimba Hill; spring 2021: n = 5, mean = 2.82µmol/L, S.D = 0.5; autumn 2022: n = 10, mean = 2.05µmol/L, S.D = 0.5) (Table 2).

At Bimba Hill, a significant increase ($p = 0.01$) in the vitamin E levels of adult YFRWs was also observed in autumn 2022 (n = 10, mean = 8.71µg/mL, S.D = 1.7) compared to spring 2021 (n = 5, mean = 5.91 µg/mL, S.D = 1.7) (Table 2). A general increase in vitamin E was also observed at Italowie Gap during this time but was not statistically significant ($p = 0.08$).

No significant differences in TEAC, ascorbic acid (vitamin C) or vitamin A levels between sampling times or study sites were observed during this study (Table 2).

Table 2: The average blood biochemical and antioxidant values for adult yellow-footed rock-wallabies (*Petrogale xanthopus xanthopus*) from Italowie Gap and Bimba Hill, South Australia. (*) significant difference between sites, $p < 0.05$. (**) significant difference with site between spring 2021 and autumn 2022, $p < 0.05$. ± Standard deviation.

	Italowie Gap		Bimba Hill		Reference range (95% CI)
	Spring 2021	**Autumn 2022**	**Spring 2021**	**Autumn 2022**	
Urea (mmol/L)	11.98 (±1.5) **	9.45 (±1.62) *, **	12.06 (±0.51)	13.8 (±0.96) *	11.08 – 13.15
Creatinine (µmol/L)	97.2 (±8.41)	76.5 (±4.8) *	90.6 (±14.01) **	110.3 (±11.4) *, **	89.08 – 103.3
Total Protein (g/L)	60.9 (±1.79)	59.25 (±0.87)	59.56 (±2.26)	57.29 (±1.25)	57.38 – 60.37
Albumin (g/L)	41.88 (±1.76)	39.33 (±1.08)	40.62 (±2.0)	39.17 (±0.73)	38.72 – 41.14
Cholesterol (mmol/L)	3.45 (±0.2)	3.205 (±0.27)	4.008 (±0.19)	3.895 (±0.33)	3.38 – 4.0
Triglycerides (mmol/L)	0.43 (±0.1) *	0.66 (±0.4)	0.88 (±0.3) *	0.85 (±0.5)	0.58 – 0.88
FRAP (mmol/L)	0.6 (±0.02)	0.54 (±0.06) *	0.7 (±0.05)	0.94 (±0.4) *	0.63 – 0.84
TEAC (mmol/L)	2.28 (±0.04)	2.28 (±0.01)	2.27 (±0.04)	2.26 (±0.02)	2.24 – 2.29
TBARS (µmol/L)	2.54 (±0.6) **	1.65 (±0.2) *, **	2.82 (±0.5) **	2.05 (±0.5) *, **	1.96 – 2.44
Vit. C (µmol/L)	20.60 (±1.55)	20.62 (±1.23)	20.46 (±1.29)	23.14 (±0.86)	20.38 – 22.73
Vit. A (µg/mL)	0.03 (±0.01)	0.06 (±0.02)	0.08 (±0.03)	0.115 (±0.04)	0.04 – 0.12
Vit. E (µg/mL)	5.46 (±1.61)	9.2 (±0.59)	5.91 (±1.7) **	8.71 (±1.7) **	6.68 – 8.64
Bicarbonate (mmol/L)	27.2 (±2.2) *, **	30.33 (±2.6) *, **	23.2 (±1.6) *	24 (±2.5) *	24.55 – 27.29
Magnesium (mmol/L)	0.76 (±0.1) *, **	1.06 (±0.2)**	0.91 (±0.08) *	1.004 (±0.15)	0.88 – 1.02
Sodium (mmol/L)	139.17 (±3.5)	141.5 (±3.02) *	141.2 (±1.8) **	135.8 (±2.4) *, **	137.5 – 140.27
Calcium (mmol/L)	2.02 (±0.11)	2.4 (±0.11) *	2.29 (±0.13)	2.28 (±0.07) *	2.26 – 2.35

Adult YFRW bicarbonate levels increased significantly at Italowie Gap ($p = 0.01$) and Bimba Hill ($p = 0.0007$) between spring 2021 (Italowie Gap; n = 5, mean = 27.2mmol/L, S.D = 2.2; Bimba Hill; n = 5, mean = 23.2 mmol/L, S.D = 1.6) and autumn 2022 (Italowie Gap; n = 6, mean = 30.33 mmol/L, S.D = 2.6; Bimba Hill; n = 10, mean = 24 mmol/L, S.D = 2.5) (Table 2). Magnesium levels were significantly lower ($p = 0.04$) at Italowie Gap (n = 5, mean = 0.76 mmol/L, S.D = 0.1) than at Bimba Hill (n = 5, mean = 0.91 mmol/L, S.D = 0.08) in spring 2021. However, the magnesium level at Italowie Gap had increased significantly ($p = 0.02$) from spring 2021 to autumn 2022 (n = 6, mean = 1.06 mmol/L, S.D = 0.2) (Table 2).

During autumn 2022, Italowie Gap had significantly higher levels of sodium and calcium compared to Bimba Hill ($p = 0.003$ and $p = 0.04$, respectively). The significant difference in sodium levels between sites was the result of a decrease in sodium at Bimba Hill between spring 2021 (n = 5, mean = 141.2 mmol/L, S.D = 1.8) to autumn 2022 (n = 10, mean = 135.8 mmol/L, S.D = 2.4) ($p = 0.0006$; Table 2).

The average values and reference ranges (95% CI) for all blood biochemical parameters assessed in adult and sub-adult/juvenile YFRWs in this study are outlined in Appendix 1 (see Table S1; Table S2 pg. 214-215).

Discussion:

Building our knowledge the effects of habitat connectivity and rainfall on YFRW population demographics, health and breeding will provide important insights into the current status of YFRWs in South Australia. This will inform how climate change may impact this species into the future, and help conservation agencies tailor management strategies for the species across the species' distribution.

The information gained from trapping was used to assess population demographics at each site. Similar numbers of YFRWs were captured at both Italowie Gap (n = 24) and Bimba Hill (n = 28). However, only four sub-adults were captured at Italowie Gap during the entire trapping period, and were not recaptured at any time. In comparison, 15 sub-adults were captured at Bimba Hill with three subsequently recaptured. An explanation for this may be that juvenile recruitment at Italowie Gap is lower than at Bimba Hill, possibly in response to resource abundance (Sharp & McCallum 2015) or high competition rates with feral herbivores (i.e. feral goat). Alternatively, in highly connected habitat, such as that seen at Italowie Gap, may be facilitating dispersal events and breeding group fluctuations (Sharp 1997a; Hazlitt *et al*. 2006), whereas isolated outcrop habitat, such as that seen at Bimba Hill, may limit dispersal opportunities.

Although the proportion of males captured during trapping was generally lower at Italowie Gap compared to Bimba Hill, no significant differences in the sex ratio of trapped individuals were detected between sites and were at parity. This may be due to male-biased dispersal being favoured in connected habitat, as has been observed in other rock-wallaby species, such as the short-eared rock-wallaby (*Petrogale brachyotis*) (Potter *et al*. 2012). While a larger sample size may be required to determine if this trend holds true, habitat connectivity and the ability to disperse would likely be an explanation for the difference in male abundance.

In this study, all breeding age females captured had a pouch young. This was not surprising as YFRWs are continuous breeders which use embryonic diapause, a process suggested to help maximise recruitment during favourable environmental conditions (e.g. high resource availability) (Lim *et al*. 1987; Robinson *et al*. 1994). Previous findings that birth rates in YFRW colonies are not affected by localised high rainfall events were confirmed in this current study (Sharp & Norton 2000). Although births occurred in all months of the year at both sites, and the highest rate of births peaked at different times, appearing to be correlated with trends in the 100-year average rainfall data for each region.

Lim *et al*. (1987) found that YFRWs at Middle Gorge, an area receiving high summer and winter rainfall, had an increased frequency of births during autumn and late winter/early spring, with additional low birth rates throughout the rest of the year. High birthing rates coinciding with rainfall and the resulting pulse of resource abundance have been reported in other species (Bronson 1985; Ogutu *et al*. 2013; Jordan *et al*. 2017). The high rainfall period at Italowie Gap typically occurs in summer, with limited rainfall in winter (Corbett 1980; Smith & Morton 1990; Morton 2022). In contrast, Bimba Hill receives a consistent 15-20mm of rain year-round, although occasional extreme summer rainfall events can also occur (Bureau of Meteorology). This indicates that in primarily summer rainfall zones, such as Italowie Gap, birth rates increase during autumn, coinciding with historic increases in resource abundance following summer rainfall. Whereas, in regions of consistent year-round rainfall, YFRW births may be more favourable during the winter when free water sources are more readily available, the water content of feed is high, and no physiological heat-stress is occurring.

Body condition, which provides an insight into an animal's health (Caron *et al*. 2003; Turner *et al*. 2012; Robert & Schwanz 2013), was assessed for YFRWs captured at both sites. A significant decline in adult YFRW BCI was observed at Italowie Gap from autumn 2021 to spring 2021, but no significant increase in BCI was observed following extremely high rainfall. This suggests that if plant biomass had been assessed in this study, it may have indicated that the increase in vegetation biomass following high rainfall had minimal effect on YFRW body condition. This contrasts with a previous study which found YFRW body condition at the Aroona Dam Sanctuary population was highly dependent on rainfall and vegetation abundance (Lapidge 2005). In another arid-dwelling *Petrogale*

species, the black-footed rock-wallaby (*Petrogale lateralis lateralis*), body condition followed a seasonal trend reaching a maximum in early summer and a minimum in early spring, and was found to significantly increase 3-6 months after high rainfall (Willers *et al*. 2011). The decrease in BCI at Italowie Gap in spring 2021 may therefore be explained by low rainfall in the preceding three-months (24mm of rainfall). While during times of inferred high plant biomass following high rainfall (85mm, prior to autumn 2022 trapping) it appears that a 'normal' BCI was restored rather than exponentially gaining condition. Further monitoring during periods of variable rainfall would be needed to conclusively support this.

Additionally, the condition of sub-adult YFRWs differed from adults at both sites, suggesting that whilst sub-adults are undergoing a period of rapid growth and energy expenditure, they are more sensitive to resource availability. Our data suggests that adult YFRW BCI appears to be mainly influenced by periods of low rainfall, rather than high rainfall, but for sub-adult YFRWs, high rainfall resulting in increased resource abundance may be a major factor influencing survival.

The hydration and nutritional status of YFRWs at each site across the study period was examined through changes in the concentration of blood components. Creatinine is associated with muscle mass and hydration, where an increase in creatinine corresponds to high muscle mass gain or dehydration (Ali *et al.* 2012; Lo *et al*. 2022; Le Page *et al*. 2024). Creatinine values in this study (65-125 µmol/L) were all within the reference range for wild YFRWs (Lapidge 2001) and brush-tailed rock-wallabies (Barnes *et al*. 2008). As BCI was relatively consistent throughout, it indicates that the significant increase in creatinine observed at Bimba Hill between spring 2021 and autumn 2022, was due to a lower hydration status rather than an increase in muscle mass. Similar observations have been made in the western grey kangaroo (*Macropus fuliginosus*), where body condition remained stable during summer and autumn months while blood urea and creatinine levels fluctuated (Arnold 1987). However, as creatinine levels were within the normal range, it suggests that YFRWs at both sites were maintaining their required hydration level from either free water sources or their diet (Lapidge & Munn 2011). Permanent free water sources are available at Italowie Gap, but there are no permanent water sources surrounding the colony at Bimba Hill (*pers comm.* P. Watkins), and the YFRWs here likely rely more heavily on dietary water or ephemeral water sources to maintain hydration.

Similarly, all indicators of nutritional status (total protein, albumin, cholesterol, triglyceride, and urea) were within the reference ranges for captive or wild YFRWs (Lapidge 2001; *unpubl. data.* P. McCarthy), indicating that YFRWs were meeting their nutritional requirements. However, the values in this study were higher than those reported in northern hairy-nosed wombats (*Lasiorhinus krefftii*; Reiss *et al*. 2008) and tammar wallabies (*Notamacropus eugenii*; Robert & Schwanz 2013), and lower than those reported in black-footed rock-wallabies (Ruykys *et al*. 2012). The high rainfall recorded at Italowie Gap in the summer of 2021/22 likely resulted in feed containing low protein and high-water

content (Herman & Larkins 1999). A low protein diet has been associated with low urea production in macropodids (Algar *et al*. 1988; Stirrat 2003), and additionally has been observed to result in a decrease in body condition and reproductive output in hamsters (*Tscherskia triton*, Zhao *et al*. 2022). Although there were no observable decreases in BCI or reproductive output during autumn 2022, a low protein diet may have contributed to the significantly lower urea values observed at Italowie Gap during a time of high resource abundance.

This study also examined blood antioxidant levels (FRAP, TEAC, TBARS, Vitamin A and E) in adult YFRWs at both sites. Existing YFRW antioxidant reference ranges only exist for vitamin E and TBARS, and during this study most values for these parameters were within their normal reference ranges (Vitamin E 0.1 - 8.43mg/L; Lapidge 2001; TBARS 1.75 - 5.2 µmol/L; *unpubl. data.* P. McCarthy). Vitamin E and TBARS values reported in this study were also similar to those observed in black-footed rock-wallabies (Vitamin E 1.3 – 9.2mg/L; TBARS 0.04 – 3.19µmol/L; Ruykys *et al*. 2012). While no YFRW reference range exists for FRAP, values in this current study were also similar to those reported in black-footed rock-wallabies (FRAP 0.53 - 1.53mmol/L; Ruykys *et al*. 2012). However, some of these measures (FRAP, TBARS and vitamin E) can also be influenced by the antioxidant content of plant tissue ingested (Lapidge 2005; Aksoz *et al*. 2020). This may explain why some results during the pulse of resource abundance in autumn 2022 were outside of their normal reference range, such as vitamin E at Bimba Hill which exceeded the range. As TBARS is a measure of the products of reactive oxygen species, the substance which causes oxidative damage to cells, the low value at Italowie Gap during autumn 2022 suggests that physiological stress was also low during this time. As such, the significant seasonal variations in antioxidant values in this study were likely influence by resource abundance and plant nutrient content, indicating that these factors in conjunction with rainfall may influence blood antioxidant levels in YFRWs.

Essential ions in adult YFRW blood, such as magnesium, calcium and sodium, were all within the reference ranges for this species (0.56 – 1.08mmol/L; 2.16 – 3.2mmol/L, 136 – 157mmol/L, respectively; *unpubl. data.* P. McCarthy). The values for ion levels observed in this study were similar to those reported in the northern hairy-nosed wombat (Reiss *et al*. 2008) and Lumholtz's tree-kangaroo (*Dendrolagus lumholtzi*; Shima *et al*. 2021), suggesting that the values in the current study are within a normal marsupial range. Differences in ion levels observed between sites were likely directly related to dietary intake (Jahnen-Dechent & Ketteler 2012), suggesting that the nutritional content of plants differed between sites. The bioavailability of calcium can be hindered by the presence of oxalates with which it is often complexed (Weaver *et al*. 1987; Heaney *et al*. 1989; Wilson & Clifford 1990; Linder 1991), and may explain the low calcium level at Bimba Hill. Sodium, however, is naturally available in soil and water and can be incorporated into the tissue of salt tolerant plants (Flowers *et al*. 1977; Bommer *et al*. 1982; Srivarathan *et al*. 2024). As such, the site and seasonal differences observed here may have resulted from ingesting plants with oxalates present, a

decrease in the intake of sodium storing plants, or an increase in water intake diluting plasma ion levels. Dietary analysis would be needed to confirm this.

Evidence of muscle damage was observed in blood liver parameters obtained from most YFRWs captured (determined from creatine kinase [CK], alanine aminotransferase, potassium, aspartate aminotransferase, and lactate dehydrogenase results). Values for CK were highly variable and above captive YFRW reference values (60 - 540mmol/L; *unpubl. data.* P. McCarthy). High blood liver parameters, including CK, in response to physical restraint has been described in grizzly bears (*Ursus arctos horribilis*; Cattet *et al*. 2003). Further investigations are needed to determine if high liver parameters for YFRWs at these study sites can be attributed solely to muscle damage from being trapped and physically restrained, or if liver disease/damage is occurring, possibly as a result of ingesting toxic plants (Fletcher *et al*. 2009; Woolford *et al*. 2020; Gilbert *et al*. 2021; Stegelmeier & Davis 2023).

Conclusions:

Trapping data from this study revealed insights into population demographics, breeding characteristics, and the health status of YFRWs at Italowie Gap and Bimba Hill, South Australia, and their relationship with rainfall and habitat connectivity during a period encompassing months of above and below average rainfall. Although no statistically significant male-biased sex ratio was detected, habitat connectivity is likely affecting the population demographics of YFRW colonies through influencing male-biased dispersal opportunities.

Births were occurring year-round, reflecting the continuous breeding strategies of YFRWs. Subsequently, all adult females were found with pouch young throughout the study, with a relationship detected between birth rate and long-term, historic monthly rainfall patterns. A significant increase in birth rate was detected at Italowie Gap during March/April, corresponding to the period following historically high summer rainfall, whereas at Bimba Hill birth rates rose slightly during July, a time when physiological stress is less, and water and food resources are more reliable. This result suggests that regionally tailored management programs (e.g. timing of pest control, or supplementation of resources) may be beneficial for YFRWs to enhance survival and increase population recruitment rates during dry times and drought.

The body condition of adult YFRWs remained relatively constant at Bimba Hill. However, after a prolonged dry period, the body condition of YFRWs at Italowie Gap significantly decreased, but was restored following a period of high rainfall, which resulted in a pulse of resource abundance. This suggests that YFRWs have a natural reliance on high rainfall events for maintaining their physical health.

Blood biochemistry results showed that extreme rainfall events did not extensively alter values outside of the normal reference ranges for the species, and showed that YFRWs from both sites were not experiencing hydration or nutritional stress throughout the study period. However, when significant differences were observed in blood parameters or antioxidant levels, it was likely the result of high rainfall driving changes in vegetation water and nutrient content, or geographical differences in plant diversity. Again, these results indicate that high rainfall events are vital for maintaining YFRW health, especially after prolonged dry periods.

Climate change threatens to alter the environmental characteristics which the YFRW and many other semi-arid dwelling species rely upon to provide resources (i.e. food, water, shelter). Access to long-term datasets, like that now being established for the YFRW, will greatly assist wildlife agencies in identifying issues of concern early, and to optimize management actions that support the species' survival and persistence into the future.

Chapter Two

"Population genetics of two yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) colonies in connected and isolated habitat in semi-arid South Australia"



"*From the eastern portion of the State it will almost certainly disappear before very many years are past, since its pelt is far too attractive to permit it to survive as long as the fur trade exists*"

– Prof. Wood Jones 1924

Statement of Authorship

Title of Paper	Population genetics of two yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) colonies in connected and isolated habitat in semi-arid South Australia
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☑ Unpublished and Unsubmitted work written in manuscript style
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Principal Author

Name of Principal Author (Candidate)	Lauren Werner		
Contribution to the Paper	Study design, field work, sample collection, laboratory preparation, statistical analyses, writing manuscript.		
Overall percentage (%)	90		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	14/10/2024

Co-Author Contributions

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

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

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Contribution to the Paper	Study design, laboratory supervision, editing manuscript.		
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Contribution to the Paper	Primary supervisor. Study design, sample collection, editing of manuscript.		
Signature		Date	14/10/2024

Population genetics of two yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) colonies in connected and isolated habitat in semi-arid South Australia

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Abstract:

The yellow-footed rock-wallaby (YFRW; *Petrogale xanthopus xanthopus*) is found in a range of rocky habitat within semi-arid regions of South Australia, New South Wales and Queensland, Australia. Currently, many populations are restricted to isolated habitat patches which has the potential to impact gene flow, genetic diversity, and levels of inbreeding. This study aimed to establish baseline population genetic data for YFRWs occupying two different habitats: simple, connected, mountainous rocky habitat (Italowie Gap, Gammon Ranges, South Australia), and complex, isolated, low rocky outcrops (Bimba Hill, Olary Ranges, South Australia). Tissue samples were acquired from trapping these sites (n = 50) and from a captive colony for comparison (Gorge Wildlife Park; n = 9), and were analysed using DArTSeq (Diversity Arrays Technology, University of Canberra). The genetic diversity observed at Italowie Gap was higher than that at Bimba Hill, suggesting gene flow via connected habitat may be influencing genetic diversity in YFRWs (Italowie Gap; H_O = 0.226, uH_E = 0.226, A_R = 1.653, Bimba Hill; H_O = 0.182, uH_E = 0.172, A_R = 1.463). Inbreeding values were similar between sites (Italowie Gap; F_{IS} = 0.006, Bimba Hill; F_{IS} = -0.44), but kinship values were significantly higher at Italowie Gap compared to Bimba Hill (Italowie Gap = 0.152, Bimba Hill = 0.104; p = 0.02) indicating that regardless of habitat connectivity, the YFRWs at Italowie Gap were more closely related. These results suggest that habitat connectivity is influencing breeding group structure and migration, potentially contributing to the YFRW genetic diversity at these sites. Developing baseline population genetic values for YFRWs is important as their ecology make them highly vulnerable to drought and climate change induced resource depletion, and as a consequence, significant population fluctuations. Both the YFRW colonies examined here do not currently require genetic management, but continued monitoring at these sites and sites across the YFRW distribution more broadly will be vital to track genetic declines, and help prepare for the impacts that increased drought frequency and severity may have on their long-term genetic viability.

Introduction:

Genetic monitoring of wildlife populations is a vital part of conservation management, especially in the face of climate change where extinction risk is predicted to increase (Parmesan 2006; Lee *et al*. 2015; Urban 2015; Naujokaitis-Lewis *et al*. 2021; Paniw *et al*. 2021).

Australia already has the worst rate of mammal extinctions in the world due to the introduction of feral predators (feral cat, *Felis catus*; red fox, *Vulpes vulpes*), competition with introduced herbivores (feral goat, *Capra hircus*; European rabbit, *Oryctolagus cuniculus*), and habitat degradation (Woinarski *et al.* 2015; Tulloch *et al*. 2023). It is also a continent of climatic extremes, and therefore future climate change impacts on wildlife are expected to be significant (Lee *et al*. 2015; Urban 2015).

The yellow-footed rock-wallaby (YFRW; *Petrogale xanthopus*) is a medium sized, macropodid marsupial found in semi-arid regions of South Australia, western New South Wales, and south-western Queensland, Australia (Copley 1981 & 1983; Maynes & Sharman 1983; Lim *et al*. 1987; Gordon *et al.* 1993; Pope *et al*. 1996; Dovey *et al*. 1997). In South Australia, YFRWs (*P. x. ssp. xanthopus*) are found in the Flinders, Gammon, Gawler and Olary Ranges amongst rocky gorges, escarpments, and outcrops. At the time of European colonisation, the YFRW was considered the most abundant animal in the Flinders Ranges of South Australia (Fountain 1907, as reported in Copley 1981 & Lim *et al*. 1987). Excessive hunting by Europeans for the fur-trade, predation by the introduced red fox, and competition with introduced (feral goats and rabbits) or overabundant native herbivores (euros, *Osphranter robustus erubescens*) led populations of YFRWs to decline rapidly by the 1990's resulting in local extirpation across their distribution and threatening the species with extinction (Hume *et al.* 2004; Tyndale-Biscoe 2005; Hayward *et al.* 2011; Abbott *et al*. 2014; Sharp *et al.* 2014; Michael & Lindenmayer 2018).

Today, the species has recovered considerably due to 'Operation Bounceback', a landscape-scale conservation program focussed on restoring natural ecosystems across some of South Australia's semi-arid biodiversity hotspots through the control of feral pest species and revegetation programs (Bounceback Celebrating 20 Years Report 2012; Garnett *et al*. 2018). After nearly 30 years of intensive fox baiting and goat control programs, the YFRW has recovered to a point that it has been recommended to be removed from the threatened species list (Woinarski *et al*. 2023).

YFRWs are habitat specialists that require access to rock crevices or rocky jumbles for protection from predation, climatic extremes, and to minimise metabolic water loss (Wilson *et al*. 1976; Gordon *et al*. 1978; Copley 1981; Sharp 1997b). The areas they inhabit are highly variable in complexity, size, lithology, topography, elevation, soil type, and resource abundance (Copley 1981 & 1983; Lim & Giles 1987; Lim *et al*. 1987; Sharp 1997a & 1997b; Smith & Allen 2021). Additionally, their habitat

also varies in its connectivity, which is an important aspect for improving species resilience to both short and long-term climatic or environmental change (Pearson *et al*. 2014), which is particularly relevant to habitat specialists (Jarzyna *et al*. 2015; Oliver *et al*. 2015; Holyoak & Heath 2016). As climate change progresses, it is anticipated that species which either have specific habitat requirements or exist where available habitat is naturally fragmented or limited, like the YFRW, will face reduced resource availability and/or limited dispersal opportunities (Crompton *et al*. 2008; Rudnik *et al.* 2012; Shi *et al*. 2015; Kutschera *et al*. 2016). Furthermore, the impacts of limited habitat availability or habitat fragmentation have been identified to lead to declines in population genetic diversity, resulting in genetic bottleneck events, population declines, and increased levels of inbreeding (Gibbs 2001). These impacts have been observed previously in other species such as the black-footed rock-wallaby (*Petrogale lateralis pearsoni*; Jones Lennon *et al*. 2011), the tammar wallaby (*Notamacropus eugenii*; Miller *et al*. 2011), and the northern quoll (*Dasyurus hallucatus*; Spencer *et al*. 2017).

However, the impact of the YFRWs historic decline in abundance on their genetic diversity, and how their preferred, often isolated, habitat has exacerbated this, is not well known across their distribution. A study by Smith *et al*. (2023a) begun to build our understanding on how habitat fragmentation impacts YFRW genetic diversity, revealing that colonies in fragmented habitat had lower genetic diversity than those in connected landscapes.

As such, the aim of this current study was to continue building detailed data on the genetic parameters of YFRW colonies by comparing a colony from connected, simple, rocky mountain escarpment habitat, with that of a colony in an isolated, complex, rocky outcrop habitat, and to consider how habitat connectivity may affect future population viability as climate change progresses.

Methods:

Ethical Statement:

Trapping of YFRWs was approved under the University of Adelaide's Animal Ethics Committee (S-2020-097), and Scientific Research Permit approved from the Department of Environment and Water, South Australia (#Y26992-1).

Study Sites:

Trapping was conducted at Italowie Gap in the Vulkathunha-Gammon Ranges National Park, and at the Bimba Hill region of the Bimbowrie Conservation Park (Figure 1) in March/April 2021, September 2021, and April/May 2022.

Italowie Gap is 500km north of Adelaide in the Gammon Ranges region of South Australia (-30.558691, 139.164285). YFRW habitat at this site is characterised by steep escarpments with simple stratified sandstone, siltstone, and quartzite rock formations, interspersed with loose earth and shale scree slopes. This site is located on the southern end of the Balcanoona Range, and represents a highly connected habitat due to YFRW colonies being found along the approximately 20km span of the range, and with many other colonies in the surrounding areas (see Appendix 1, Figure S1 pg. 212). During a population survey in this region in 2019, the average density of YFRWs was 4.7 per km^2 (Lethbridge *et al*. 2019). At Italowie Gap, the vegetation is mostly confined to adjacent creek beds, dominated by river red gum trees (*Eucalyptus camaldulensis*), with rock fuchsia bush (*Eremophila freelingii*) and white cypress pine (*Callitris glaucophylla*) scattered across the slopes. During periods of high rainfall, rock sida (*Sida petrophila*) and desert lantern-bush (*Abutilon leucopetalum*) become abundant on the slopes and plateaus, with small herbaceous plants and grasses growing between the rocks. Trapping was conducted over an area of approximately 200m x 200m amongst the rocky portion of the site (Figure 1). The long-term average rainfall in the Vulkathunha-Gammon Ranges National Park is 256.6mm, but across the study period of 15 months the region received 469.9mm (Bureau of Meteorology, data from closest weather station, Arkaroola).

The Bimba Hill region in the Bimbowrie Conservation Park is approximately 350km north north-east of Adelaide, in the Olary Ranges of South Australia (-31.983341, 140.211817). The majority of rock-wallaby habitat here is comprised of low elevation, isolated monzogranite outcrops, consisting of large, complex boulder jumbles with interspersed earth slopes. The Bimba Hill trapping site is part one of the largest YFRW populations in the Bimbowrie Conservation Park. There are some nearby colonies (<5-10km), however, all of them are separated by mostly open grassland and shallow hills (see Appendix 1, Figure S2 pg. 213). As such, this site represents isolated habitat with low connectivity to other colonies due to dispersal needing to occur across high predation risk and low resource areas. During a population survey in this region in 2019, the average density of YFRWs was 8.9 per km^2 (Lethbridge *et al*. 2019), nearly double that observed in the Gammon Ranges region. The vegetation in this area is characterised by rock fuchsia bush (*Eremophila freelingii*), with occasional mulga trees (*Acacia aneura*) and dead finish bushes (*Acacia tetragonophylla*). After rainfall, the slopes are dominated by rock sida (*Sida petrophila*) and *Solanum* species, with herbaceous plants and grasses growing in between rocks or at the base of boulders. Trapping was conducted over an area of approximately 200m x 1000m (Figure 1). The long-term average rainfall in this region is 203.2mm, however during the study period of 15 months received 310.5mm (Bureau of Meteorology, data from Bimbowrie weather station).

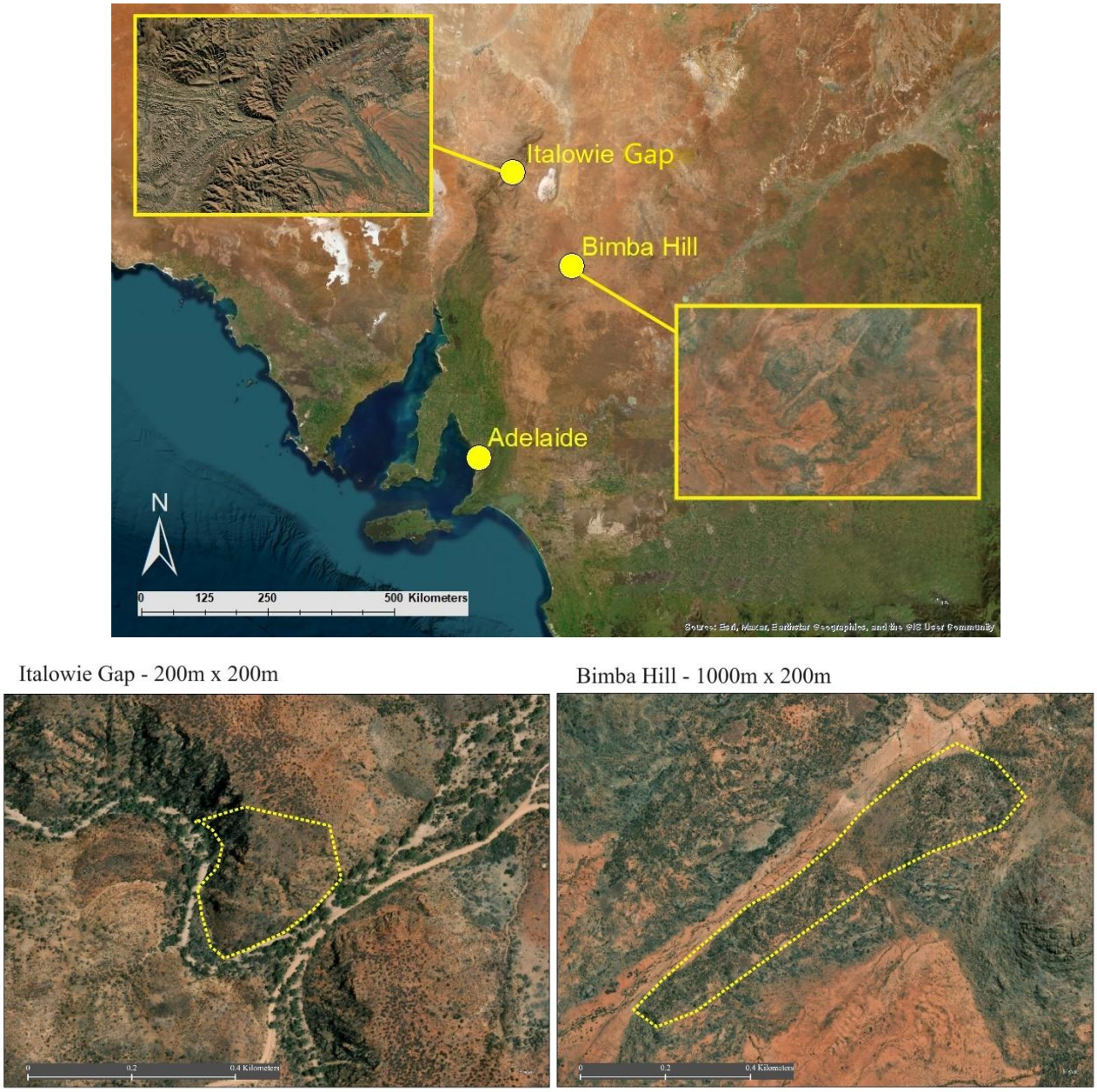


Figure 1: *Top*: A satellite image showing the study sites, Italowie Gap in the Vulkathunha-Gammon Ranges National Park (connected, simple, rocky escarpment habitat) and Bimba Hill in the Bimbowrie Conservation Park (isolated, complex, rocky outcrops), in relation to Adelaide, South Australia. *Bottom*: Satellite imagery of both of the study sites, Italowie Gap (*left*) and Bimba Hill (*right*), with the areas which were trapped for yellow-footed rock-wallabies (*Petrogale xanthopus xanthopus*) indicated in yellow.

Sample Acquisition:

Ear biopsies (n = 50) were obtained from YFRWs during a trapping program. Trapped animals were held in heavy duty close-weave cotton bags upon removal from traps and throughout the duration of processing. Ear biopsy samples were taken using a leather hole punch (4mm punch) and then stored in 90% ethanol in 1.5mL Eppendorf tubes, and kept frozen until laboratory processing. Further details on trapping and sample collection can be found in the *Supplementary Chapter* of this thesis.

Further samples were obtained from a captive YFRW colony (n = 9; 8F, 1M) at Gorge Wildlife Park, South Australia, to be used comparatively as an artificially genetically isolated population. This captive colony originated from the Adelaide Zoo YFRW collection which was founded in the late 1800's and early 1900's. Records on specific founder numbers and sex ratios are lacking due to poor record keeping at the inception of the captive collection (*pers. comms.* L. Andrews, J. Buchecker). However, only three known wild individuals have been added to the collection during the 1980-90's from the lower Flinders Ranges (*pers comms.* D. Schultz).

DNA Extraction and Quantification:

DNA from tissue samples (n = 59) was extracted using the Bioline Isolate II Genomic DNA Kit (Meridian Bioscience, USA). Half of the tissue sample was cut into small pieces using a scalpel blade, and was digested overnight (10-12 hours) in an Eppendorf Thermomixer C at 56ºC on 600rpm before continuing with the extraction protocol. The recommended extraction protocol for tissue DNA extraction was followed, except for separating the final elution stage into two 50µL steps.

Extracted tissue DNA was quantified using a Quantus Fluorometer (Promega, USA) before being transferred into screw-top 1.5mL tubes and kept frozen (-20ºC) until further analysis.

Analysis and Statistics:

Extracted DNA samples were sent to Diversity Arrays Technology (DArT; University of Canberra, A.C.T) for analysis through their DArTSeq platform. Filtering of the resulting single nucleotide polymorphisms (SNPs) was conducted using the *dartR* v.2.9.7 package (Gruber *et al*. 2018; Mijangos *et al*. 2022) in *R* (R Core Team 2023) to remove SNPs which could influence data accuracy and to improve the quality of results (dartR – Guide to Basic Filtering v1.8.3). The dataset was filtered for Hardy-Weinberg equilibrium, call rate (>95%), monomorphic loci, secondary loci, and reproducibility (>99%). Specific filtering rates aligned with *dartR*'s recommended values.

Before further analysis was conducted, known pouch young were removed from the dataset (n = 7) to remove biasing of results, creating a final dataset comprised of 52 individuals: 27 from Bimba Hill, 16 from Italowie Gap, and nine from Gorge Wildlife Park.

A principal coordinates analysis (PCoA) was conducted using *dartR* to assess the level of population differentiation between sites. Mean heterozygosity (observed and unbiased expected heterozygosity; H_O and uH_E) and the inbreeding (F_{IS}) values were calculated in *dartR* using the *gl.report.heterozygosity* function. Allelic richness (A_R) was analysed using the *PopGenReport* v4.3.2 package (Adamack & Gruber 2014; Gruber & Adamack 2015).

Kinship parameters were examined using the *SNPRelate* v1.34.1 package (Zheng *et al*. 2012). Pairwise kinship values were determined using identity-by-descent analysis utilising the KING method (IBD-KING) as per Onley *et al*. (2021). Kinship relationships were visualised using a Fruchterman Reingold layout to examine kinship links between individuals in each wild population. To examine the statistical significance of kinship values, a t-test assuming unequal variances using a significance level of 0.05 was conducted examining the kinship between male-female kin (M-F), male-male kin (M-M), and female-female kin (F-F) from the analysis output. Kinship coefficient values were interpreted from those indicated in Lopes *et al*. (2013) which proposes values for parent-offspring or full siblings (0.25), and half-siblings (0.15) relationships.

Effective population size (N_E) was estimated using the *NeEstimator* software (Do *et al*. 2014) using the linkage disequilibrium model with random mating assumed, minimum allele frequencies were set to 0.05, 0.02 and 0.01, and jackknife confidence intervals used.

Results:

Sample Collection and SNP Data:

A total of 52 YFRWs were caught at Italowie Gap (n = 24) and Bimba Hill (n = 28). A detailed description of the population demographics of captured YFRWs at each site is provided (see Appendix 2, Table S1 pg. 216). Due to one sample being misplaced and one sample being labelled incorrectly, a total of 50 tissue samples from trapped wild YFRWs were available for genetic analysis. Samples from captured pouch young were also removed from the genetic dataset (n = 7) to minimise biased results based on their close kinship to the mother. The overall sex ratio of YFRWs caught across both sites was approximately 1F:1.2M (23F, 29M), however, at Italowie Gap it was 1F:0.8M, compared to 1F:1.4M at Bimba Hill.

The raw DArTSeq data contained 12,211 SNP identified from the tissue samples sent for analysis from wild (Italowie Gap and Bimba Hill) and captive (Gorge Wildlife Park) YFRWs. SNPs which

were monomorphic (n = 1,228) or not reproducible (n = 3,377) represented approximately a quarter of the initial SNPs observed, whilst loci with low call rate (n = 883) or secondary loci (n = 360) were less prevalent. Once filtering had been conducted, the total number of SNPs available for informative analysis was 6,363.

Genetic Diversity:

The PCoA showed genetic differentiation between all three colonies (Figure 2). All values for YFRW genetic diversity at Italowie Gap (n = 16, H_O = 0.226, uH_E = 0.226, A_R = 1.653, F_{IS} = 0.006) were higher than those observed at Bimba Hill (n = 27, H_O = 0.182, uH_E = 0.172, A_R = 1.463, F_{IS} = -0.44) (Table 1). The values for effective population size (N_E) ranged from 6.4 to 26 for Italowie Gap, to 12.5 to 64.9 for Bimba Hill. The genetic values observed for Gorge Wildlife Park YFRWs were similar to those seen for Bimba Hill YFRWs across all parameters, except for N_E (Table 1).

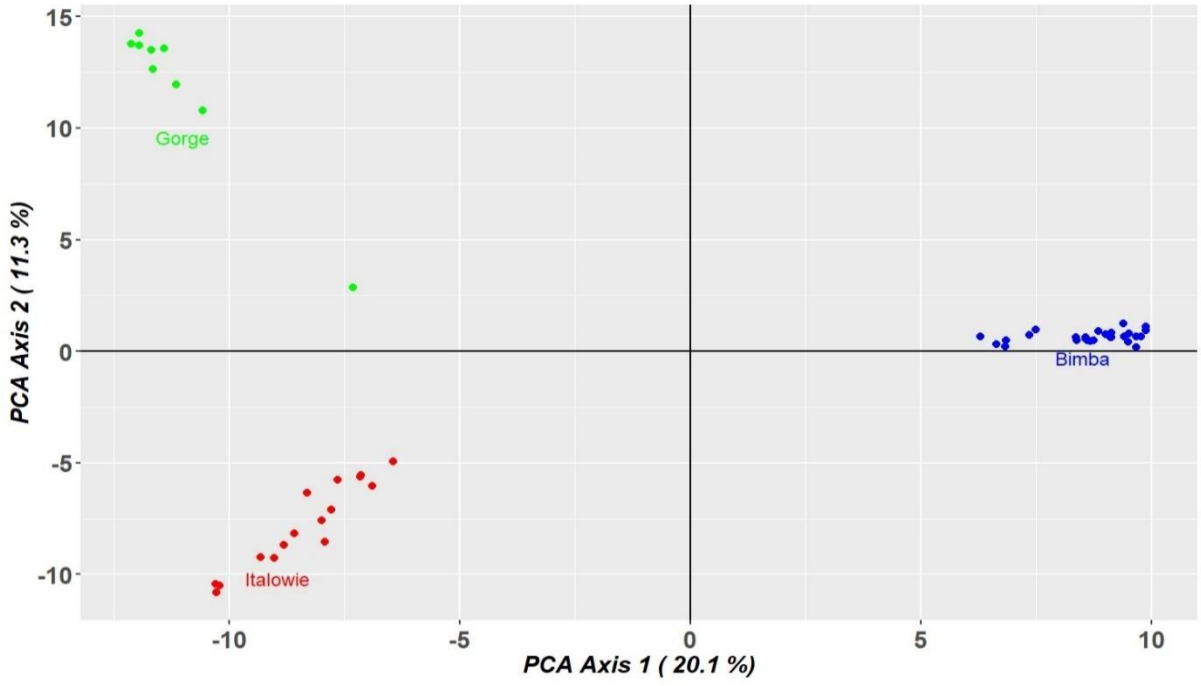


Figure 2: A principal coordinates analysis showing the genetic differentiation between yellow-footed rock-wallabies (*Petrogale xanthopus xanthopus*) sampled from Italowie Gap in the Vulkathunha-Gammon Ranges National Park (Italowie), Bimba Hill in the Bimbowrie Conservation Park (Bimba), and a captive colony from Gorge Wildlife Park (Gorge), South Australia.

Table 1: The genetic diversity values of yellow-footed rock-wallabies (*Petrogale xanthopus xanthopus*) from Italowie Gap, Bimba Hill, and Gorge Wildlife Park as determined from single nucleotide polymorphism data. The analysis parameters are effective population size (N_E) with lower and upper confidence intervals (C.I), observed heterozygosity (H_O), unbiased expected heterozygosity (uH_E), allelic richness (A_R) and the inbreeding value (F_{IS}). S.D = standard deviation.

	N_E	Lower C.I	Upper C.I	H_O (± S.D)	uH_E (± S.D)	A_R	F_{IS}
Italowie Gap	11.9	6.4	26	0.226 (0.2)	0.226 (0.18)	1.653	0.006
Bimba Hill	23.9	12.5	64.9	0.182 (0.22)	0.172 (0.2)	1.463	-0.44
Gorge Wildlife Park	2.7	1.9	6.5	0.185 (0.23)	0.179 (0.2)	1.478	-0.02

Kinship Analysis:

Kinship values ranged from 0.035 to 0.26 at Italowie Gap and 0.032 to 0.28 at Bimba Hill. Overall, analyses showed that Italowie Gap had a significantly higher level of kinship between the YFRWs captured compared to Bimba Hill ($p = 0.02$ Table 2). However, no significant differences were observed between average sex-pairwise kinship values between sites. The average pairwise kinship values for both Italowie Gap and Bimba Hill were within the range of 'half-siblings' (as per Lopes *et al*. 2013), despite kinship at Italowie Gap being significantly greater than at Bimba Hill.

Although genetic diversity parameters aligned the captive YFRW colony with Bimba Hill (Table 1), kinship results showed that kinship of the captive colony was closer to Italowie Gap (Average kinship = 0.15 ± 0.05, data not shown).

Table 2: The pairwise kinship values of yellow-footed rock-wallabies (*Petrogale xanthopus xanthopus*) from Italowie Gap and Bimba Hill as determined from single nucleotide polymorphism data using the identity-by-descent KING method. F-F = female to female kinship, M-M = male to male kinship, M-F = male-female kinship. *p-values* were determined using a t-test and significance level of $p < 0.05$. Statistically significant results are indicated with an asterisk (*).

	Average Kinship (μ ± S.D)	F-F Kinship (μ ± S.D)	M-M Kinship (μ ± S.D)	M-F Kinship (μ ± S.D)	M-M vs F-F (*p-value*)
Italowie Gap	0.152 (0.08)	0.139 (0.08)	0.149 (0.1)	0.161 (0.08)	0.92
Bimba Hill	0.104 (0.07)	0.122 (0.09)	0.076 (0.06)	0.11 (0.08)	0.24
Between sites (*p-value*)	*0.02**	0.78	0.49	0.06	

The number of pairwise kinship relationships between YFRWs at each site varied. At Italowie Gap, a total of 24 close kin relationships were comprised of 12 M-F, two M-M, and 10 F-F pairwise kinship relationships. At Bimba Hill, close kin relationships were substantially more, with a total of 43 close kin relationships, comprised of 24 M-F, 11 M-M, and eight F-F relationships. Visualisation of these kinship relationships revealed that there appeared to be a core group of nine YFRWs which were close kin at Italowie Gap, with seven individuals unrelated to this core group also having been sampled from (Figure 3). Whereas most YFRWs at Bimba Hill were kin to another individual although ~50% of these linkages were weak, and only two individuals were unrelated to the rest of the group (Figure 3).

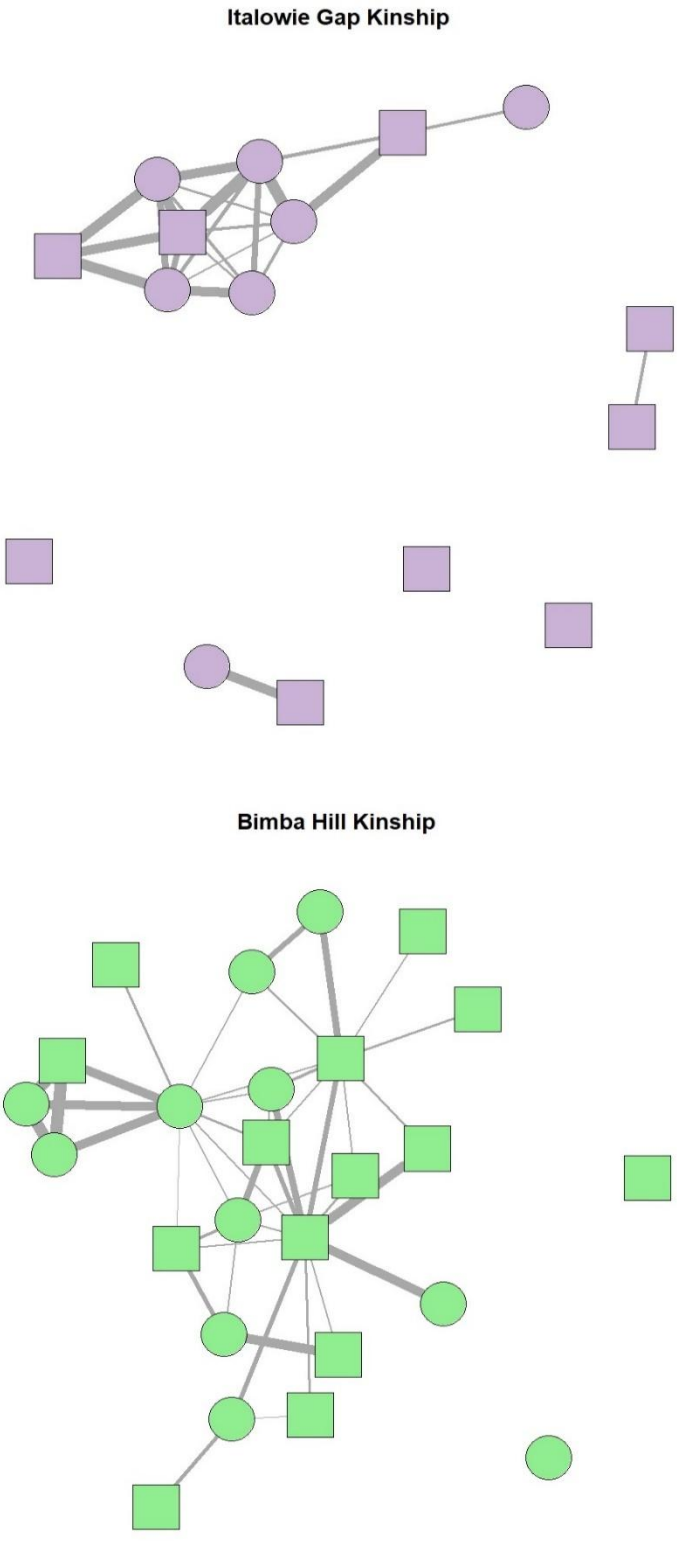


Figure 3: A Fruchterman Reingold layout graph showing the kinship linkages between male (squares) and female (circles) yellow-footed rock-wallabies (*Petrogale xanthopus xanthopus*) at Italowie Gap (*top/purple*), and Bimba Hill (*bottom/green*) determined using SNP data. Italowie n = 16, Bimba n = 25. Link thickness increases with the strength of kinship.

Discussion:

This study examined the genetic diversity of two YFRW colonies with different connectivity (connected, simple rock structure, mountainous habitat, versus, isolated, complex rock outcrop, relatively flat habitat) within its current distribution to help determine how habitat connectivity affects genetic diversity, inbreeding and kinship.

Low to moderate levels of genetic diversity were observed at both YFRW colonies. The genetic diversity of the YFRWs at the connected Italowie Gap colony however, was found to be higher than that observed at the isolated Bimba Hill colony. In fact, the genetic diversity of YFRWs at Bimba Hill was more similar to that of a captive YFRW colony. This supports the theory that isolated populations are likely to have lower levels of diversity and higher inbreeding parameters, such as those seen in island populations of rock-wallabies (Eldridge *et al*. 1999; Jones Lennon 2011). This was also supported by the findings reported in Smith *et al*. (2023a) where small YFRW colonies in less connected habitat (Gawler and Grey Ranges) had lower genetic diversity levels compared to a connected colony. Similar results have also been found in other mammal species, such as the Gee's golden langur (*Trachypithecus geei*) where populations in small, isolated habitat fragments had less genetic diversity than populations in larger habitat fragments (Ram *et al*. 2016). Together, this suggests that habitat isolation is likely having a negative effect on the genetic diversity of YFRW colonies. Although, due to the lack of comparative genetic values for these study populations, the impact of historic YFRW population declines may also be contributing to a lack of genetic diversity.

In Smith *et al*. (2023a), the values for heterozygosity (H_O = 0.254), allelic richness (A_R = 1.596) and inbreeding (F_{IS} = 0.017) in a connected Flinders Ranges YFRW population, were all similar to those observed in the current study at the Italowie Gap colony (H_O = 0.226, A_R = 1.653, F_{IS} = 0.006). Additionally, similarities were also evident between the heterozygosity (H_O = 0.193 and 0.201) and allelic richness (A_R = 1.448 and 1.427) of isolated colonies in Queensland (Smith *et al*. 2023a), which were comparative to those values observed at Bimba Hill (H_O = 0.182; A_R = 1.463) and the captive population (H_O = 0.185; A_R = 1.478) in this current study. These findings suggest that the interaction between habitat connectivity and the genetic diversity of YFRWs is likely consistent across their distribution, where genetic values are markedly higher in connected habitat.

The factor associated with habitat connectivity which is likely facilitating the maintenance of genetic diversity is greater dispersal ability. In short-eared rock-wallabies (*Petrogale brachyotis*), habitat connectivity has been found to play a significant role in promoting male-biased dispersal between colonies which was linked to maintaining population genetic diversity (Potter *et al*. 2012). Male-biased dispersal is also been described in YFRWs, with males moving between breeding groups and thus, maintaining or enhancing the genetic diversity within colonies (Sharp 1997a). It has also been suggested that YFRWs are more mobile within connected mountain ranges than was previously

reported by Sharp (1997a), with evidence now indicating that individuals can potentially migrate 13kms from their origin (Potter *et al*. 2020; Smith *et al*. 2023a).

The high degree of habitat connectivity at Italowie Gap and low male sex ratio (n = 24; 1F:0.8M), supports the idea that the region's habitat connectivity facilitates greater male-biased migration, aiding in maintaining the colonies' genetic diversity. However, the difference in sex ratio between sites was not deemed to be statistically significant (Chi-square test, $p = 0.32$) and with a comprehensive census, the abundance of males may not be substantially different between sites. Regardless, as the Italowie Gap colony is located amongst approximately 20km of rocky range habitat with YFRWs found along its span, the findings of Potter *et al*. (2012) likely also apply here. This is further strengthened through kinship analyses, where six genetically unique male YFRWs were captured at Italowie Gap which showed no kinship linkage to the 'core' genetic group of the site. In contrast, at the Bimba Hill colony, where surrounding colonies are few and are separated by 5-10 kilometres of open land, the ratio of males captured was much higher (n = 28; 1F:1.4M) suggesting limited male-biased dispersal. However, Smith *et al*. (2023a) found migration events had occurred even in isolated habitats where colonies were separated by 5km of unsuitable habitat. The results from the current study overall support the idea that the degree of habitat connectivity affects the YFRWs ability to migrate and the frequency of migration events, but evidence from Smith *et al.* (2023a) suggests that periodic dispersal events are likely occurring in isolated colonies, such as Bimba Hill, to maintain a moderate level of genetic diversity.

Interestingly, all average pairwise kinship values (M-F, F-F, and M-M) were higher at Italowie Gap than at Bimba Hill, which appears in opposition to theorised hypotheses where the isolated YFRW colony at Bimba Hill were predicted to have lower genetic diversity and higher kinship values. However, this is likely caused by the close genetic linkage of the 'core' group at Italowie Gap, as kinship pairings were less (24 kinship pairings) than at Bimba Hill (43 kinship pairings). This suggests that there are more individuals at Italowie Gap which are closely related, often akin to a half sibling relationship, whilst at Bimba Hill, all but two individuals were related to another individual in the colony with ~50% of linkages being quite weak. While this may be a reflection of the impact of habitat connectivity, it may also indicate that YFRWs trapped at Italowie Gap were from a single highly related breeding group, with some transient, dispersing males present. This follows the typical structure of a *Petrogale* colony which is composed of numerous breeding groups, consisting of a dominant male, numerous breeding females, sub-adult females/males, and juveniles, with some transient individuals which move between groups (Sharp 1997a; Hazlitt *et al*. 2004; 2006). Within breeding groups, female philopatry is generally favoured (Sharp 1997a) as is also true for other *Petrogale* (Jarman & Bayne 1997; Hazlitt *et al*. 2004; 2006) and mammal species (i.e. greater stick-nest rats, *Leporillus conditor*; Onley *et al*. 2021). However, sampling at Bimba Hill occurred over a much larger area than at Italowie Gap, and therefore may have encompassed multiple breeding

groups. Further research would be needed, with a greater sample size and over a larger area, to assess if the trend observed here is true over a broad-scale, or if there are differences in YFRW philopatric behaviours occurring depending on the level of habitat connectivity.

Interestingly, one pairwise kinship pairing at Bimba Hill resulted in a value of 0.45 and only differed at 5.5% of SNPs (data not shown). This value would be consistent with monozygotic twins, or the result of sampling or laboratory errors (Lopes *et al*. 2013). Sampling error, as in sampling from the same individual twice, is highly unlikely as in this trapping program individuals were given a unique microchip and ear-tagged to allow for future identification. Additionally, due to the same sampling process being implemented for all captured YFRWs, evidence of previous sampling would have been evident as both individuals were caught in the same trip, only two days apart. Laboratory error is also highly unlikely as each DNA sample was processed in different extraction runs. Twins have previously been observed in macropodid marsupials, including another *Petrogale* species (*P. assimillis*), and are considered a rare event making up less than 1% of births (Spencer & Marsh 1997; Norbury 1987; van Oorschot 1989). Genetic data relating to YFRW twins was detected in the Smith *et al*. (2023a) study, but due to a lack of microchipping/ear-tagging of pouch young, was not able to be irrefutably supported and may have been caused by sampling or laboratory errors. Twins have also previously been recorded in captive colonies of YFRWs (Collins 1973). If accurate, this result may provide one of the first records of twins in a wild-living YFRW colony, and provide evidence that both twins can survive to, and beyond, weaning age.

In this study, the negative inbreeding (F_{IS}) value at Bimba Hill indicated that there was an excess of heterozygotes. The mechanism behind a heterozygosity excess and its significance is poorly understood, but it has often been attributed to small population size and binomial sampling error caused by differences in allele frequencies between male and female breeding animals, creating a heterozygote excess in their offspring (Robertson 1965; Luikart & Cornuet 1999; Waples 2015). Some studies in birds and reptiles discuss the presence of heterozygote excess and indicate that this may be caused by an inbreeding avoidance mechanism (Stow & Sunnucks 2004; Szulkin *et al.* 2009). Inbreeding avoidance is unproven in *Petrogale* species, but for other species which live in highly fragmented or limited habitat, this phenomenon is mostly evident in mate selection (Costello *et al*. 2008; Becker *et al*. 2012; Smorkatcheva 2021), and through dispersal (Hu *et al*. 2017). However, as a negative value was only detected at Bimba Hill, further investigation is required to confirm that this was due to an inbreeding avoidance strategy.

Conclusions & Further Research:

This study has found that genetic diversity, kinship, and inbreeding in YFRW colonies in two different habitats in South Australia are likely affected by habitat connectivity, and possibly influenced by breeding group structure and philopatric behaviour. Although, the impacts of historic population declines may also be contributing to regional differences in genetic diversity. If these genetic results are consistent across connected and isolated YFRW colonies, it suggests that isolated colonies may be more vulnerable to climate change than their connected habitat counterparts, due to lower baseline genetic diversity values and their reduced ability to disperse. As such, isolated populations will require on-going monitoring and appropriately tailored genetic management in the future. However, currently, we suggest that there is no need for genetic intervention at either of the study sites, as genetic parameters were not extremely low. However, as there are now baseline genetic information available for these colonies, further future genetic monitoring is recommended to assess the rate of genetic erosion to at each site.

A previous study in the northern Flinders Ranges suggested that individuals in YFRW colonies can be quite transient (*pers. obs.* P. Hornsby), with colony sizes fluctuating by almost 50% across a three-month period (Copley 1981). How YFRW population fluctuations and breeding group demographics differ in regions of different habitat connectivity has not been examined previously and requires further investigation. This would allow for a more complete ecological understanding to be applied to future genetic investigations of this species and provide the necessary insights required to optimise conservation management practices.

Further broad-scale investigations should also be conducted to examine dispersal and migration dynamics in habitats with differing connectivity, with an emphasis on the impact of resource abundance. This information would help build a better understanding of how environmental conditions may restrict migration, especially during poor conditions which are likely to become exacerbated with climate change, and be used to inform human-mediated genetic management.

Population size may also be influencing these results as genetic variation and population size have previously been shown to have a positive correlation (Frankham 1996). Aerial surveys which were conducted in 2019 found that the density of YFRWs in the Olary Ranges region was more than double that of the Gammon Ranges (Lethbridge & Strauss 2007; Lethbridge *et al*. 2019). As the Italowie Gap trapping site is only a small area in a vast, highly connected mountain range (~20 kilometres in length) where other YFRW colonies are known to inhabit, changes in population size and dynamics are likely to be occurring more frequently than at Bimba Hill, where the habitat is not continuous. Therefore, further research into the influence of YFRW population size on genetic diversity, inbreeding and kinship is recommended to determine populations requiring increased conservation effort.

Long-term datasets for Australian wildlife are few and far between, especially for species which have undergone a population expansion. Furthermore, these datasets are vital for staying ahead of the curve and for establishing timelines for when intervention must be initiated. Continued monitoring at these study sites will start to build a long-term dataset for the YFRW in these regions and work toward ensuring the species' longevity and persistence into the future.

Chapter Three

"The diet of the yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) and dietary overlap with sympatric herbivores in semi-arid South Australia"



"*Although only known to science for c.150 years, P. xanthopus or 'Andu' has long been entrenched in aboriginal culture and the traditions of the Adnyamathanha or Hills People who occupied the Flinders Ranges*"

– Lapidge 2001

Statement of Authorship

Title of Paper	The diet of the yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) and dietary overlap with sympatric herbivores in semi-arid South Australia
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☑ Unpublished and Unsubmitted work written in manuscript style
Publication Details	To be prepared for journal submission after completion.

Principal Author

Name of Principal Author (Candidate)	Lauren Werner		
Contribution to the Paper	Study design, field work, sample collection, laboratory preparation, statistical analyses, writing manuscript.		
Overall percentage (%)	90		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	14/10/2024

Co-Author Contributions

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

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

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The diet of the yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) and dietary overlap with sympatric herbivores in semi-arid South Australia

Werner L.E., Kleemann S.L, Biffin E, van Dijk K, Waycott M, Taggart D.A.

Abstract:

The yellow-footed rock-wallaby (YFRW; *Petrogale xanthopus xanthopus*) is found in rocky ranges, gorges and isolated rocky outcrops in the semi-arid region of South Australia and western New South Wales, where herbivore species are likely to experience marked changes in their diet as climate change impacts vegetation diversity and abundance. This study examined the diet of YFRWs and two sympatric herbivores (the feral goat, *Capra hircus*, and euro, *Osphranter robustus erubescens*) in two different habitats in South Australia: a steep, highly connected mountain range (Italowie Gap, Gammon Ranges), and a low, open, isolated rocky outcrop habitat (Bimba Hill, Olary Ranges). Plant DNA was extracted from scat samples collected across multiple time points in 2020-2022, and genetically sequenced using hybridization capture with custom myBaits® probes to identify specific plant species in scat samples. Results indicated that diversity in the YFRW diet was similar between study sites across all sampling periods (14-48 species). Dietary diversity was commonly related to rainfall, where an increase in dietary diversity and shrub species detected in samples during dry periods at both sites. Herbaceous plants were most commonly detected in sequencing reads (Italowie Gap – 32.61% to 71.43%, Bimba Hill – 53.84% to 85.71%), with *Portulaca oleracea (Portulacaceae*) highly prevalent (Italowie Gap - 15.6% to 66.4%, Bimba Hill - 19.6 to 75.9%) in samples during dry periods. Additionally, sympatric herbivore dietary overlap during dry periods was high at both sites, with three of the four major plant species detected in YFRW samples also detected in goat and euro samples at Italowie Gap (*Acacia*, *Malvastrum*, *Sigesbeckia australiensis*), and *P. oleracea* detected in all sympatric species samples (YFRW 76%, euro 54%, goat 29%) at Bimba Hill. Currently, YFRWs are resilient to dry times and drought, however, our findings suggest that during and following these unfavourable conditions competition for food resources from feral goats and euros is heightened. Critically, long-term monitoring of YFRW diet is required to determine how dietary preferences are altered by vegetation changes and lower annual rainfall with climate change. Additionally, the continued control of competitor species (feral goats and euros) is required to minimise competition with YFRWs for vital, sporadically available resources in these regions.

Introduction:

As climate change progresses, vegetation abundance and diversity available to herbivorous species is expected to change significantly (Svenning & Sandel 2013; Wu *et al*. 2015; Yuan *et al*. 2019; Mohammed & Wang 2023). As such, the prevalence of diet studies on Australian fauna are increasing for threatened or high conservation priority species, such as possum or glider species (Smith 1982; Turner 1984; Jones *et al.* 2006; Pestell & Petit 2007; Mathieson *et al*. 2020), *Petrogale* species (Short 1989; Horsup & Marsh 1992; Telfer & Bowman 2006; Tuft *et al*. 2011; van Eeden *et al*. 2011; Creese *et al*. 2019; White & Fleming 2021), the Tasmanian bettong (*Bettongia gaimardi*; Taylor 1992) and carnivorous marsupials (Fisher & Dickman 1993; Warnecke *et al*. 2012; Andersen *et al*. 2017; Fancourt *et al*. 2018). However, the ability for herbivores to adapt to differences in vegetation abundance and diversity will depend on their adaptive plasticity and the extent of localised climate change effects (Davis *et al*. 2005; Mills 2012; Höglund *et al*. 2019).

As a result of historic and current habitat loss, predation from introduced predators (i.e. red fox, *Vulpes vulpes* and feral cat, *Felis catus*), and competition with exotic herbivores (i.e. feral goat, *Capra hircus* and European rabbits, *Oryctolagus cuniculus*) (Woinarski *et al.* 2015; Tulloch *et al*. 2023), the arid and semi-arid regions of Australia contain many threatened species. Unfortunately, climate change is expected to cause severe changes in weather patterns within these regions (Department of Environment and Water 2022). For herbivore species in these regions which are already severely reduced and/or declining in abundance, vegetation changes driven by climate change will further threaten their future viability.

The focus of this study is to characterise the dietary composition of the semi-arid adapted yellow-footed rock-wallaby (YFRW; *Petrogale xanthopus xanthopus*), a marsupial macropodid currently classified as Vulnerable (EPBC classification, Woinarski *et al*. 2014; Baker & Gynther 2023), a result of over-hunting following European settlement, predation by the introduced red fox, and competition with exotic herbivores (Copley 1981; Copley 1983; Lim *et al*. 1987). With climate change threatening to significantly impact the regions which YFRWs inhabit, a highly adaptable dietary composition in response to local environmental conditions will be vital to minimise the threat of a decreased colony health status due to nutritional changes or a lack of food resources. Changes in vegetation nutrient content have already been documented previously in response to climatic changes, such as having lower nitrogen (Cotrufo *et al*. 1998), phosphorus (Gifford *et al*. 2000) and protein (Robinson *et al*. 2012). As vegetation nutrient availability continues to decline and/or change, altering from YFRWs current adaptations, significant negative implications may be experienced in colonies across their distribution, threatening the species' future viability.

Generally, the metabolic rate of macropodids is lower than other eutherian mammals, with their energy requirements met from short-chain fatty acid production via foregut fermentation of often low-

quality vegetation obtained from grazing and/or browsing (Sanson 1989; Smith 2009). Previous YFRW dietary studies have been conducted using broad, micro-histological methodologies, revealing that YFRWs preferentially select grass and forbs species following high rainfall and have a higher proportion of shrub intake during drought times (Dawson & Ellis 1979; Lim *et al*. 1987; Copley & Robinson 1983; Lapidge 2000). With new technologies now available for in-depth dietary assessments, and climate change impacts intensifying, a comprehensive study of the YFRW diet is required to fill important contemporary knowledge gaps for informing species management.

This project used a targeted DNA hybridization approach with custom angiosperm chloroplast myBaits® (Waycott *et al*. 2021) for the dietary assessment of YFRWs and their main competitive sympatric herbivore species (feral goats and euros, *Osphranter robustus erubescens*), to examine the relationship between localised rainfall with dietary composition and resource competition. This contemporary dietary assessment will build a detailed understanding of current dietary preferences of YFRWs in two colonies in different geographical regions, how preferences differ in response to rainfall, and identify the ideal timing of sympatric species control programs.

Methods:

Ethical Statement:

The collection of all scats samples for this project was approved under a larger YFRW trapping program which was approved by the University of Adelaide Animal Ethics Committee (S-2020-097), and a scientific permit approved from the Department for Environment and Water, South Australia (Y26992-1).

Study Sites:

This study was conducted at two sites in the YFRWs distribution in semi-arid South Australia. Italowie Gap in the Vulkathunha-Gammon Ranges National Park (-30.558164, 139.165001) is located approximately 500km from Adelaide (Figure 1). The average annual rainfall in this region is 254.7mm, and during the study period of 25 months (2020-2022) received 679.8mm (Bureau of Meteorology, data from closest weather station, Arkaroola). YFRW habitat at Italowie Gap is comprised of steep sandstone, siltstone and quartzite rock formations of simple rock formations, ledges, and fissures above an ephemeral creek bed. The majority of flora present at this site is located on the lower slopes or in the creek bed, consisting of mature river red gums (*Eucalyptus camaldulensis*) and various shrubs (e.g. *Senna artemisioides*, *Acacia* species, *Pittosporum angustifolium*) in the creek, *Callitris* trees, *Acacia* species and rock fuchsia bush (*Eremophila*

freelingii) on the slopes, and a variety of small herbaceous plants and grasses growing high amongst the rocks. During periods of high rainfall, this site is dominated by plants with stellate trichomes (*Abutilon leucopetalum* and *Sida petrophila*).

Bimba Hill in the Bimbowrie Conservation Park (-31.984824, 140.209636) is located approximately 350km north-east of Adelaide (Figure 1). The average annual rainfall is 202.3mm and during the study period of 25 months (2020-2022) received 429.3mm (Bureau of Meteorology, data from Bimbowrie weather station). YFRW habitat at Bimba Hill is shallow, isolated, rocky outcrops with large monzogranite boulders forming complex jumbles amongst earthen hills interspersed with simple sheets of flat rock. Floral communities are spread throughout the site at all terrain levels, and mostly consist of rock fuchsia bush, mulga (*Acacia aneura*), dead finish (*Acacia tetragonophylla*) and lemon-scented grass (*Cymbopogon ambiguus*), with small herbaceous species present amongst boulders. After periods of high rainfall, this site is also dominated by plants with stellate trichomes (*Abutilon leucopetalum* and *Sida petrophila*), and *Solanum* species amongst the rocks.

The rainfall received during the 3-month period prior to scat sampling varied between sites (Table 1). Three-monthly rainfall amounts were chosen as an indicator of local vegetation conditions due to previous investigations showing that rock-wallaby body condition and vegetation growth increment (green vegetation) are correlated with rainfall in the preceding 3-6 months (Dawson & Ellis 1996; Willers *et al*. 2011). Both of the current study sites were in drought conditions from 2017 until February (Italowie Gap) or September (Bimba Hill) 2020, however, conditions remained dry until extremely high rainfall occurred between November 2021 and January 2022 (Table 1; Figure 2).

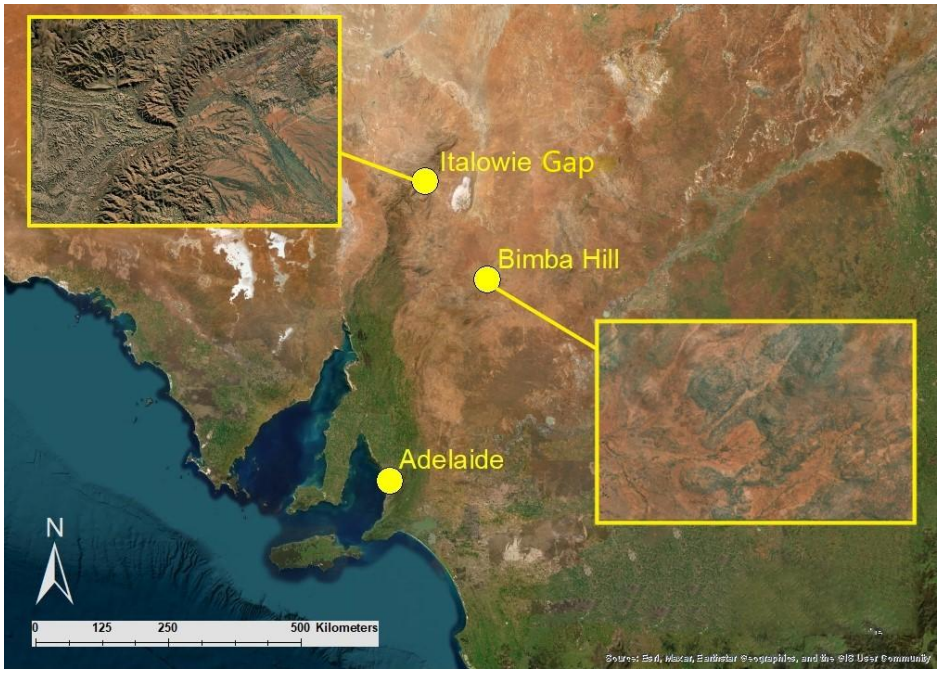


Figure 1: A satellite image showing the study sites, Italowie Gap in the Vulkathunha-Gammon Ranges National Park and Bimba Hill in the Bimbowrie Conservation Park, in relation to Adelaide, South Australia.

Table 1: The total rainfall (mm) during the three-month period prior to collecting yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) scats from Italowie Gap (*top*) and Bimba Hill (*bottom*), South Australia, at five different time periods.

Italowie Gap					
	Summer 2020	**Autumn 2021**	**Winter 2021**	**Summer 2022**	**Spring 2022**
Rainfall in preceding 3-month period to sampling	34.4	64.4	31.6	140.9	83
Bimba Hill					
	Spring 2020	**Autumn 2021**	**Winter 2021**	**Autumn 2022**	**Spring 2022**
Rainfall in preceding 3-month period to sampling	96	45.1	35.6	111.1	122.1

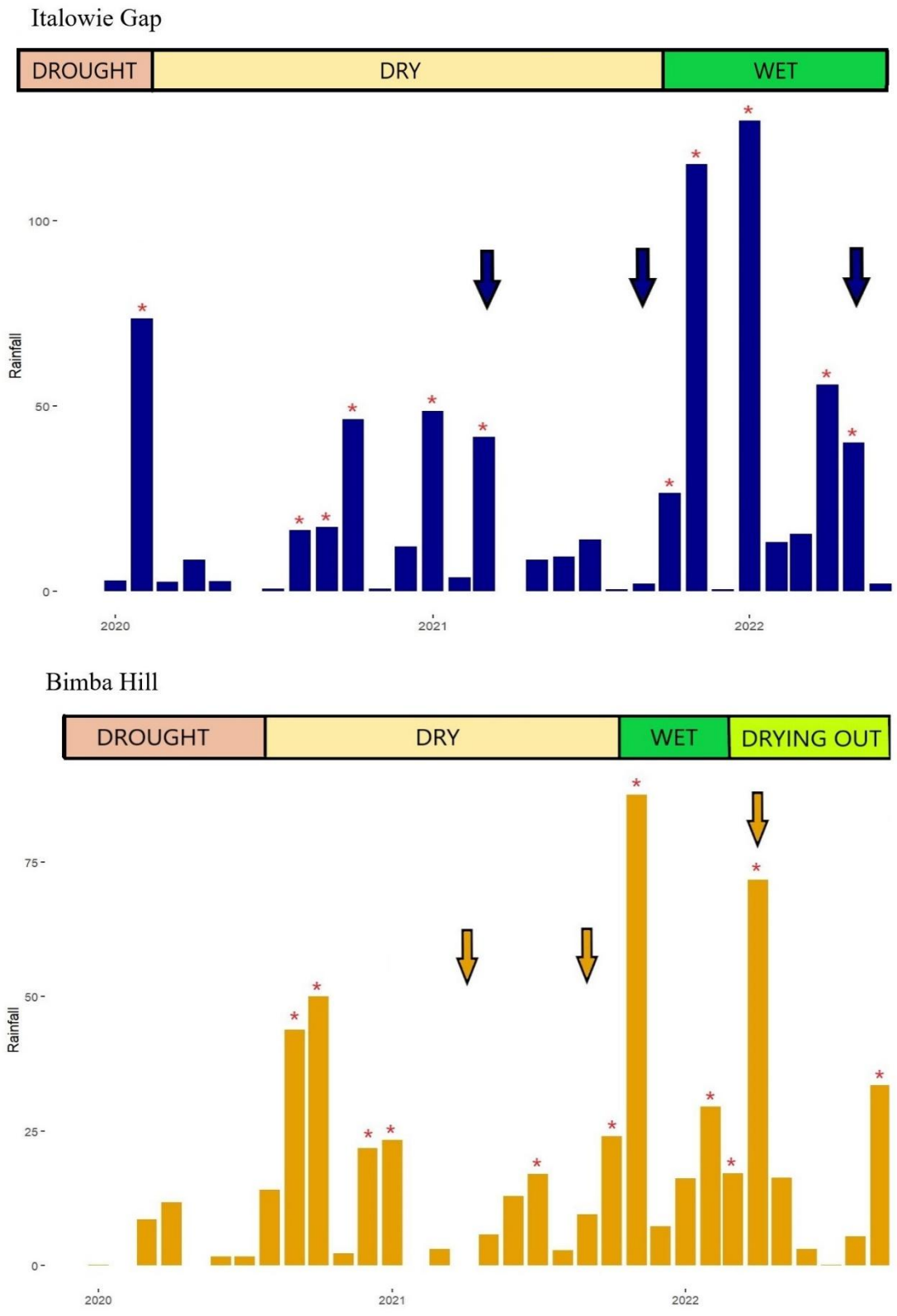


Figure 2: The total monthly rainfall (mm) experienced in the Vulkathunha-Gammon Ranges National Park (*top*) and Bimbowrie Conservation Park (*bottom*), South Australia. On each graph, months of above monthly average rainfall are indicated (*), scat sampling times (downward arrows), and the condition of the habitat and vegetation status (drought, dry, wet, drying out).

Sample Acquisition:

Scat samples (n = 244) from YFRWs were collected from random walking surveys at the two study sites during October/December 2020, March 2021, August 2021, February/March 2022 and November 2022 (Figure 2), from now on are referred to as spring/summer 2020, autumn 2021, summer/autumn 2022, and spring 2022, respectively.

When available, scats from euros (n = 34) and feral goats (n = 42) were also acquired by random surveying, but collections from these species were limited by abundance fluctuations at the study sites. Feral goat control was being conducted at both sites across this study with hundreds of goats removed each year (through 'Operation Bounceback', *pers. comm.* S. Johnson and P. Watkins). Goat presence was low throughout at Italowie Gap with scat samples only collected in spring 2022. However, goat scats were consistently detected at Bimba Hill, facilitating the collection of samples during all sampling periods at this site. During the drought (2017-2020) euro numbers declined dramatically. As a consequence, extremely low euro abundance was observed across both study sites and fresh scats were encountered infrequently. Therefore, examination of the diet of all three species was only possible at Bimba Hill immediately following the drought (summer 2020) and at Italowie Gap 10-11 months following high rainfall (spring 2022) (Figure 2).

All scats were collected using a plastic snap-lock bag inverted over the collector's hand to avoid contamination, and recorded with location, date, and species identification. Identifying scats from the three species was determined by visual inspection due to each species' scats have distinct characteristics (Figure 3). YFRW scats are described as being 'torpedo' shaped with one slightly pointed end (Lim *et al*. 1987; Triggs 2004). Comparatively, feral goat scats are small, oval pellets, and euro scats are generally larger, more rounded, and have a smaller particle size than YFRW scats (Triggs 2004). Only scats which had black colouration were collected to ensure the quality of plant DNA for extraction. A black colouration corresponds to scats that were deposited within seven days of collection (*pers. obs.* L. Werner and D. Taggart). Scats were kept frozen at -20ºC until laboratory processing.

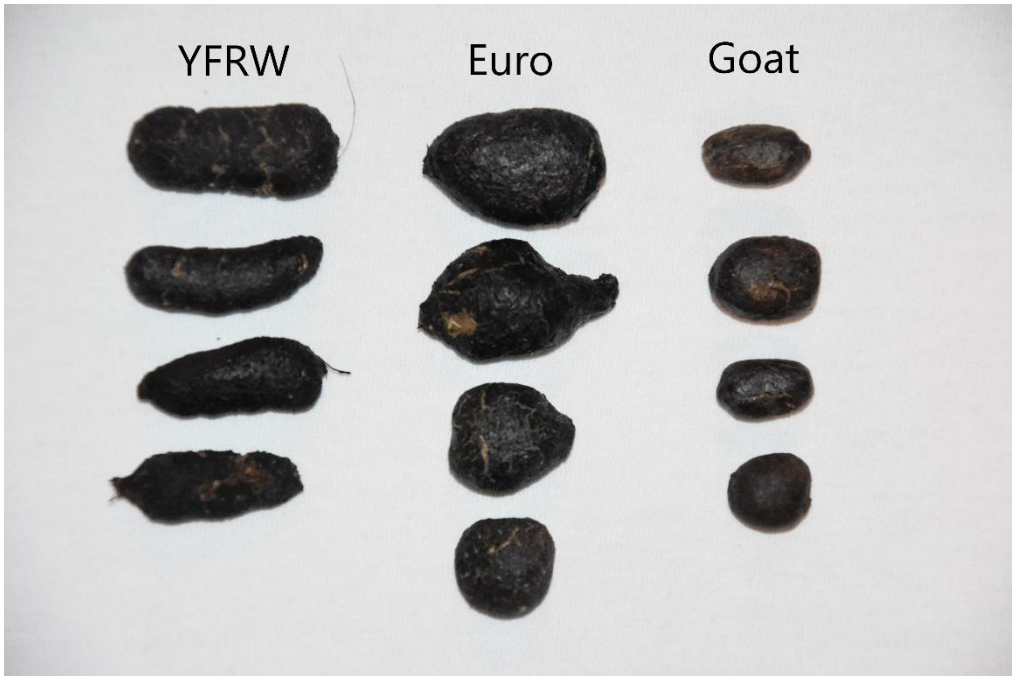


Figure 3: Photograph showing the morphological difference between yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*), euro (*Osphranter robustus erubescens*) and feral goat (*Capra hircus*) scats.

Sample Preparation and DNA Extraction:

Individual scat samples (n = 320) were prepared for DNA extraction using a Labconco FreeZone 4.5L -50^{0}C freeze-drier (Labconco Corporation, USA) for 48-72 hours. Freeze-dried samples were then separated into individuals (one scat) or grouped into bulks consisting of either 4-5 scats (small bulk) or 8-15 scats (large bulk), to maximise the information gained from analysis. The final samples (n = 170) were then pulverised using a mortar and pestle to form a powder. Subsets of the pulverised samples were then processed using the Isolate II Plant DNA kit (Meridian Bioscience, USA) following the standard protocol and lysis buffer PA1. The eluted DNA was then quantified using a Quantus Fluorometer (Promega, Australia) before being stored frozen at -20ºC.

Library Preparation and Hybridisation Capture with myBaits®:

Genomic libraries compatible with Illumina sequencing were generated using the extracted DNA (n = 170) and the NEBNext® Ultra™ II FS DNA Library Prep Kit (New England Biolabs, Australia). In brief, DNA was fragmented enzymatically, end-repaired, a-tailed, and custom stubby Y-adaptors ligated to the size selected inserts. All preparation was performed using an Eppendorf epMotion 5075 (Eppendorf, USA) liquid handler. A detailed description of the methodology can be found in Waycott

et al. (2021). Libraries were PCR amplified over 21 cycles and the resulting intermediate-stage library was then visualised on a 1.5% Agarose gel.

The library gel images were assessed visually for intensity to determine the relative fractions of each sample added into a pool. Pools were then purified with MagNA beads (Rohland & Reich 2012) at a 1:1x ratio and eluted in 35µL of 1x TE.

Target capture was performed using the OzBaits_Cp bait set (Waycott *et al*. 2021) on the library pools following the manufacturer's instructions (myBaits® targeted NGS capture; Manual version 4.01, Arbor Biosciences, USA), and hybridization conducted at 65ºC for 24 hours. The bait design targets approximately 50 regions of the chloroplast genome and can capture these regions for any vascular plant species (Waycott *et al*. 2021). After hybridization and clean-up, the resulting enriched libraries were finalised (made compatible with Illumina sequencing flow cell and sequencing primers) by performing a PCR for 18 cycles with indexed P5 and P7 fusion primers (Waycott *et al*. 2021). Following this PCR stage, the products were purified with MagNA beads at a 1:1 ratio.

The finalised libraries were then analysed on an Agilent TapeStation 4150 (Agilent Technologies, USA) with high-sensitivity tapes to assess molarity and fragment size distribution (region setting set to 300-700bp). All libraries were then pooled in equimolar concentration based on TapeStation results. The pooled library was then size selected on a Pippin Prep (Sage Science) using a 2% Agarose gel cassette with marker 'L' and range set to 300-600bp. The final library was again visualised on the Agilent TapeStation 4150 to ensure the quality and molarity of the sequencing products.

The final pooled library was then sent to the Australian Genome Research Facility, Melbourne, for sequencing using one lane of the Illumina NovaSeq X 10B flow cell for 2 x 150bp.

Sequencing Data Processing:

Sequencing of samples returned between 15K -2.4M paired-end sequence reads. Reads were then transferred to the Phoenix HPC and demultiplexed using Axe (Murray and Borevitz 2018) by recognition of the internal barcodes imbedded in the ligated stubby Y-adaptors, and was followed by quality and adaptor trimming using FASTP (Chen 2023). Trimmed paired-end reads were imported into CLC Genomics Workbench v. 20.0.2 (Qiagen) and *de novo* assembled (settings: length and similarity fraction = 0.9, minimum contig length = 300). The contigs generated from the *de novo* assemblies were then imported into Geneious v 2022.0.12 (https://www.geneious.com). Each contig was then compared with a plant DNA reference database, which includes sequences of chloroplast gene regions from native and introduced flora found across South Australia (https://data.environment.sa.gov.au/Content/Publications/vascular-plants-bdbsa-taxonomy.xlsx), with all flora represented to the generic level. To identify matches within the database, BLASTn was used

with an expect value of 1e-20, and 10 hits for each matching contig were saved. In Geneious, the sequence classifier tool, 'Classify Sequences' (http://assets.geneious.com/documentation/geneious/Sequence_Classifier_Manual.pdf), was then used to compare the contigs with positive hits within the database of pooled BLASTn outputs obtained from the previous step. The Classify Sequences tool matches the query and database sequences using pairwise alignments and reports the identity for the proportion of overlap between the two sequences. The Classify Sequences tool uses a GenBank taxonomy string (https://www.ncbi.nlm.nih.gov/taxonomy/) attached to each database sequence to decide the appropriate levels of classification based upon user defined thresholds. Here, we used a 95% overlap identity threshold to record a match and a ≥98% identity overlap to classify a query sequence to genus. If there was a single match above this threshold, it was identified to species but for multiple near equal matches at ≥98% identity, the sequence was classified using the lowest common ancestor from the taxonomic string (e.g. two or more congeneric species with the same identity would be classified at the genus level, and species from different genera in the same family would be classified to a family level).

Informative Analysis:

Analysis was based on the number of sequencing reads for each plant species detected in scat samples which was used as an estimate of the relative abundance, however this method is not indicative of total dietary biomass intake.

All plant species identified (n = 204) in sequencing data were cross-referenced with the Australasian Virtual Herbarium (https://avh.chah.org.au) and the Atlas of Living Australia (https://www.ala.org.au) to determine the species' presence at the study sites. Where sequences were classified to a species level but were determined as absent from the site, and when multiple species from the same genus were present, they were each reclassified for ease of visualisation (represented as '*Genus* sp.'). Each species/genus identified in sequencing results were classified by plant type (grass, herb [annual], herb [perennial], shrub, and tree) in accordance with classifications obtained from the Electronic Flora of South Australia website (www.flora.sa.gov.au), or other online or paper resources.

The proportion of sequencing reads per sample was determined by calculating the proportion of each species in relation to the total number of sequencing reads:

$$(x \div Total\ Number\ of\ Sequencing\ Reads\ of\ Sample) \times 100$$

This same equation was used to determine the proportions of plant types (grass, annual herb, perennial herb, shrub, and tree) and individual plant species for each sampling time.

Statistically significant changes in plant species between time points was determined using a t-test for paired means with a 0.05 significance level. To compare the total number of plant species detected, an ANOVA test was used using a 0.05 significance level. For ease of visualisation, plant species which had <5% of total sample sequencing reads were grouped into their corresponding plant type (i.e. other grasses, other herbs, other shrubs, other trees). A full list of plant species detected throughout the study are included in Appendix 3 (pg. 217).

Results:

Yellow-footed Rock-wallaby Diet at Italowie Gap:

A total of 85 individual plant species were detected in YFRW samples throughout the study at Italowie Gap, with the mean number of plant species detected across the five sampling points being 33. Significantly, the total number of plant species detected decreased from a high of 48 species in winter 2021 to a low of 14 species in autumn 2022 ($p = 0.02$) (Table 2). Grasses, annual and perennial herbs, and shrubs were detected in samples at all time points with the trees absent during both autumn sampling times. During all sampling periods the detection of herb species was highest, composing 35-85% of reads per sampling time (Table 2; Figure 4). The number of shrub species detected in autumn (2021 and 2022) were considerably lower than winter and spring sampling times (Table 2). During spring 2020, grass species were detected more than any other time, and the number of grass sequencing reads were in similar proportions to that of herb and shrub species (Table 2; Figure 4).

During dry the driest sampling times (spring 2020, autumn 2021 and winter 2021) the dominant plants detected in the YFRW diet were *Acacia* species and *Portulaca oleracea* (common purslane) (Figure 4). During these times, these two plants constituted 48.2% (spring 2020), 66.4% (autumn 2021) and 69.8% (winter 2021) of the total sequencing reads. After high rainfall during the summer of 2021/22 (240mm of rainfall, Table 1; Figure 2), the prevalence of plant species in the samples changed dramatically across a six-month period. The dominant plant species detected in YFRW samples changed to *Malvastrum* (false mallow; herb) and *Abutilon* (mallow; shrub) species, where *Malvastrum* constituted 39.6% (autumn 2022) and 35.8% (spring 2022), and *Abutilon* 24% (autumn 2022) of the total number of reads detected for the given time points (Figure 4).

Table 2: The number of plant species which were detected in yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) scats collected from Italowie Gap, South Australia. Plant species have been separated into plant types; grass, herb (annual), herb (perennial), shrub and tree. Samples were collected in spring 2020, autumn 2021, winter 2021, autumn 2022 and spring 2022.

	Spring 2020	**Autumn 2021**	**Winter 2021**	**Autumn 2022**	**Spring 2022**
Grass	13	6	7	1	3
Herb (Annual)	7	3	11	3	7
Herb (Perennial)	8	7	11	7	11
Shrub	14	5	17	3	14
Tree	4	0	2	0	1
Total	46	21	48	14	36

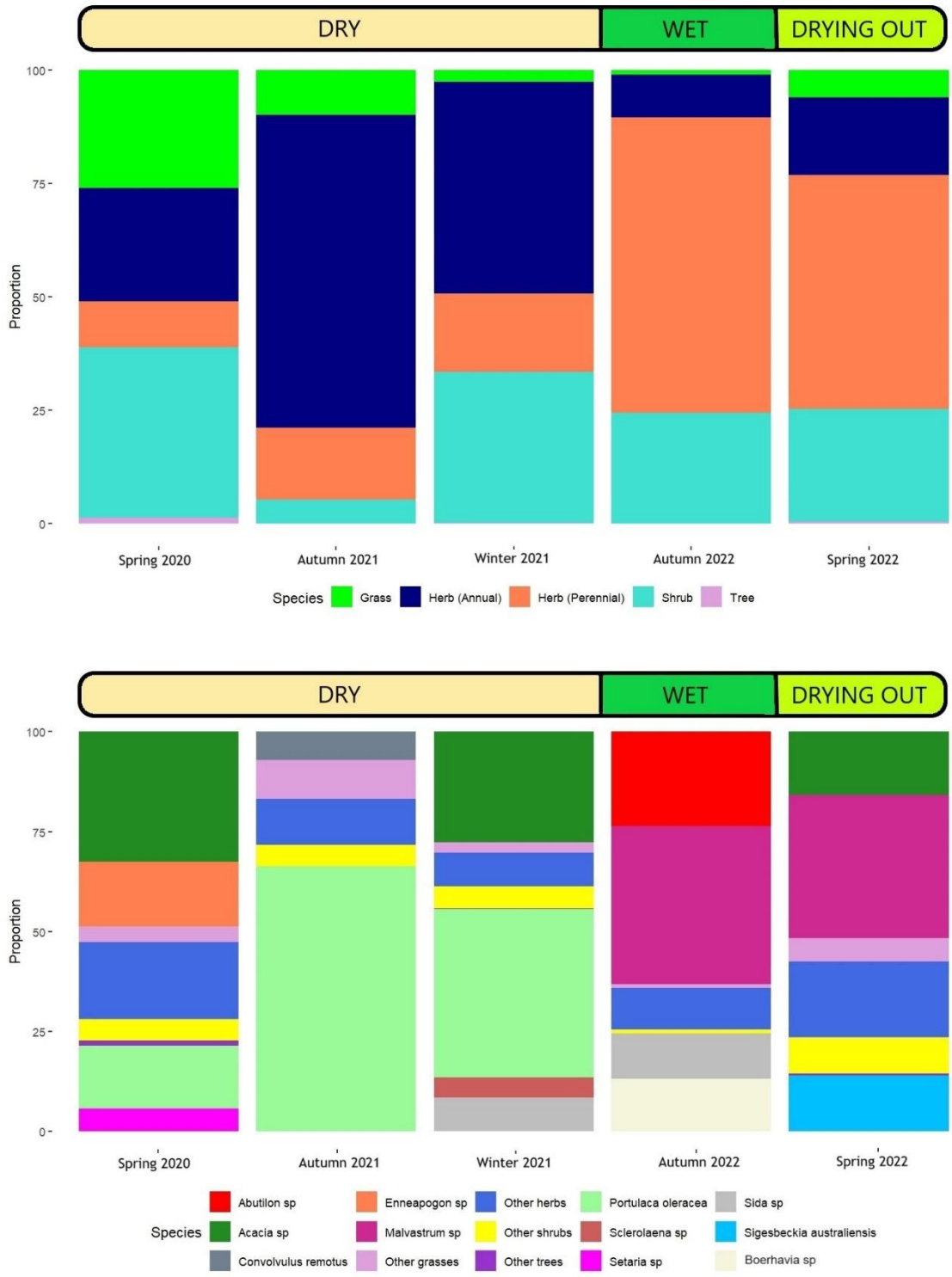


Figure 4: The proportion (%) of sequencing reads of each plant type (*top*) and individual plant species (*bottom*) detected in yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) scats collected from Italowie Gap, South Australia. Plant types (*top*) are defined as grass, herb (annual and perennial), shrub or tree. Scat collections were conducted during spring 2020, autumn 2021, winter 2021, autumn 2022 and spring 2022.

Dietary Overlap with Sympatric Herbivores at Italowie Gap:

A significant overlap in dominant plant species was observed between YFRW, feral goat and euro samples collected during spring 2022. Significantly less plant species was detected in the diet of euros (12 species) compared to YFRWs (36 species) and goats (31 species) (ANOVA $p = 0.03$; Table 3). However, the plant types consumed by YFRWs, goats and euros at Italowie Gap were similar, with the exception of trees, which were not detected in the diet of euros (Figure 5). Results showed that reads from perennial herbs (51.5%) and shrubs (24.8%) in YFRW samples represented over three-quarters of the plant species detected (Figure 5). Goat sample sequencing results also indicated that samples were comprised by many annual herbs and shrubs (41%), whilst the majority of species in euro samples were comprised of perennial herbs and shrubs (89%; Figure 5), suggesting that all three sympatric herbivores were consuming similar proportions of herbaceous and shrub species.

This was supported by examining the proportion of sequencing reads for individual plant species detected in samples from each herbivore species (Figure 5). The most prevalent plant species detected in YFRW samples were *Acacia* species, *Malvastrum* species, and *Sigesbeckia australiensis*. *Acacia* species were the only genera detected in all three species' samples during this time (Figure 5). However, the overlap between YFRW, goat and euro samples were evident by the presence of the three dominant plant species of the YFRW diet (65.6% of sequencing reads) being shared also by the goat and/or euro (*Acacia* species [all species], *Malvastrum* species [euro diet], and *Sigesbeckia australiensis* [goat diet]).

Table 3: The number of species for each plant type which were detected in scat samples collected from yellow-footed rock-wallabies (YFRW; *Petrogale xanthopus xanthopus*), feral goats (*Capra hircus*) and euros (*Osphranter robustus erubescens*) at Italowie Gap, South Australia, as determined from scat samples. Scat samples were collected in spring 2022. Plant types have been classified as grass, herb (annual), herb (perennial), shrub and tree.

	Spring 2022		
	YFRW	**Feral Goat**	**Euro**
Grass	3	2	1
Herb (Annual)	7	10	2
Herb (Perennial)	11	5	3
Shrub	14	9	6
Tree	1	5	0
Total	36	31	12

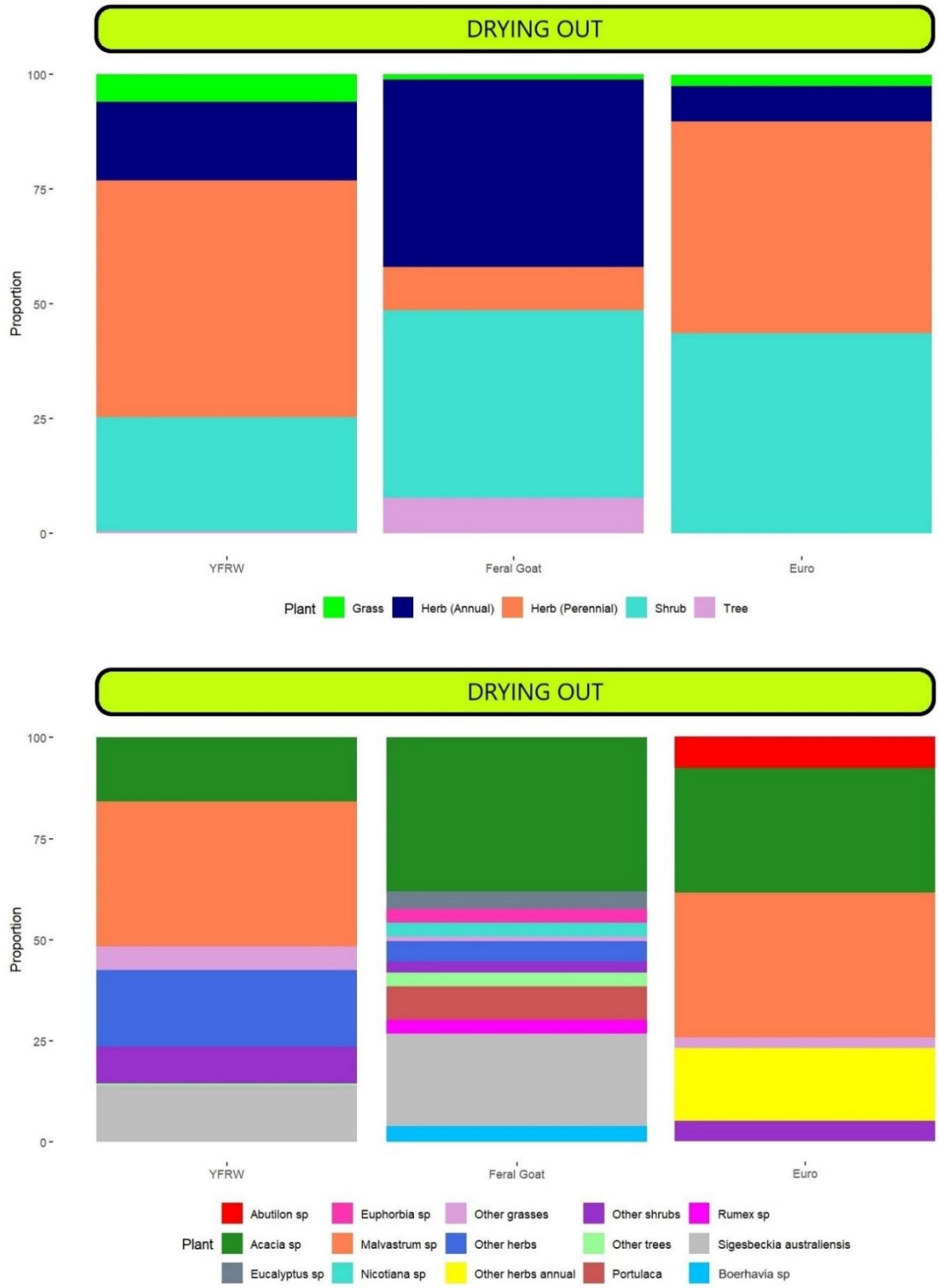


Figure 5: The proportion (%) of sequencing reads of plant types (*top*) and individual plant species (*bottom*) detected in yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*), feral goat (*Capra hircus*) and euro (*Osphranter robustus erubescens*) scats at Italowie Gap, South Australia, collected during spring 2022. Plant types are defined as grass, herb (annual and perennial), shrub and tree.

Yellow-footed Rock-wallaby Diet at Bimba Hill:

A total of 77 individual plant species were detected in YFRW samples from Bimba Hill, with the mean number of plant species detected across the five sampling periods being 26. The number of species varied from a low of 14 in summer 2020 to a high of 42 in winter 2021, but this variation was not deemed statistically significant ($p > 0.05$) (Table 4). Herbs (annual and perennial) and shrubs were commonly detected in YFRW diet samples, whereas trees were only detected twice (Table 4). Grass species were detected in YFRW samples in the three sampling times between autumn 2021 and summer 2022, which also corresponded with the periods when more plant species were detected in samples (Table 4; Figure 6). The main plant type detected throughout all sampling periods was herbs, constituting 62.5-99.6% of the total number of reads (Figure 6).

A high proportion of annual herbs were detected in samples during dry sampling periods (summer 2020 and autumn 2021) where they constituted 70.9% (autumn 2021) to 80.3% (summer 2020) of the total number of reads. The proportion of shrubs was highest during winter 2021 which was the driest sampling period with the lowest total rainfall received in the three-month period prior to sampling (35.6mm; Figure 2) (Figure 6).

During two of the driest sampling periods, the main species detected in YFRW samples was *P. oleracea* represented in summer 2020 (75.9%) and autumn 2021 (48.1%). However, this species was absent from samples in winter 2021 when shrub detection rates had increased. After significant rainfall in November 2021, prior to the summer 2022 sampling (Table 1; Figure 2), *P. oleracea* was again detected in the diet of YFRWs (19.6%). Sequencing reads of *Boerhavia* (tarvines) and *Solanum* (nightshades) species were uncommon or absent during the driest periods (summer 2020, autumn 2021 and winter 2021) but were commonly detected after the significant rainfall in November 2021 occurred (Figure 2; Figure 6).

Table 4: The number of plant species which were detected in scat samples of yellow-footed rock-wallabies (*Petrogale xanthopus xanthopus*) collected from Bimba Hill, South Australia. Plant species have been separated into plant type; grass, herb (annual), herb (perennial), shrub and tree. Samples were collected across five separate time points; summer 2020, autumn 2021, winter 2021, summer 2022 and spring 2022.

	Summer 2020	**Autumn 2021**	**Winter 2021**	**Summer 2022**	**Spring 2022**
Grass	0	5	3	3	0
Herb (Annual)	7	7	13	11	6
Herb (Perennial)	5	7	10	10	7
Shrub	2	7	15	4	5
Tree	0	0	1	1	0
Total	14	26	42	29	18

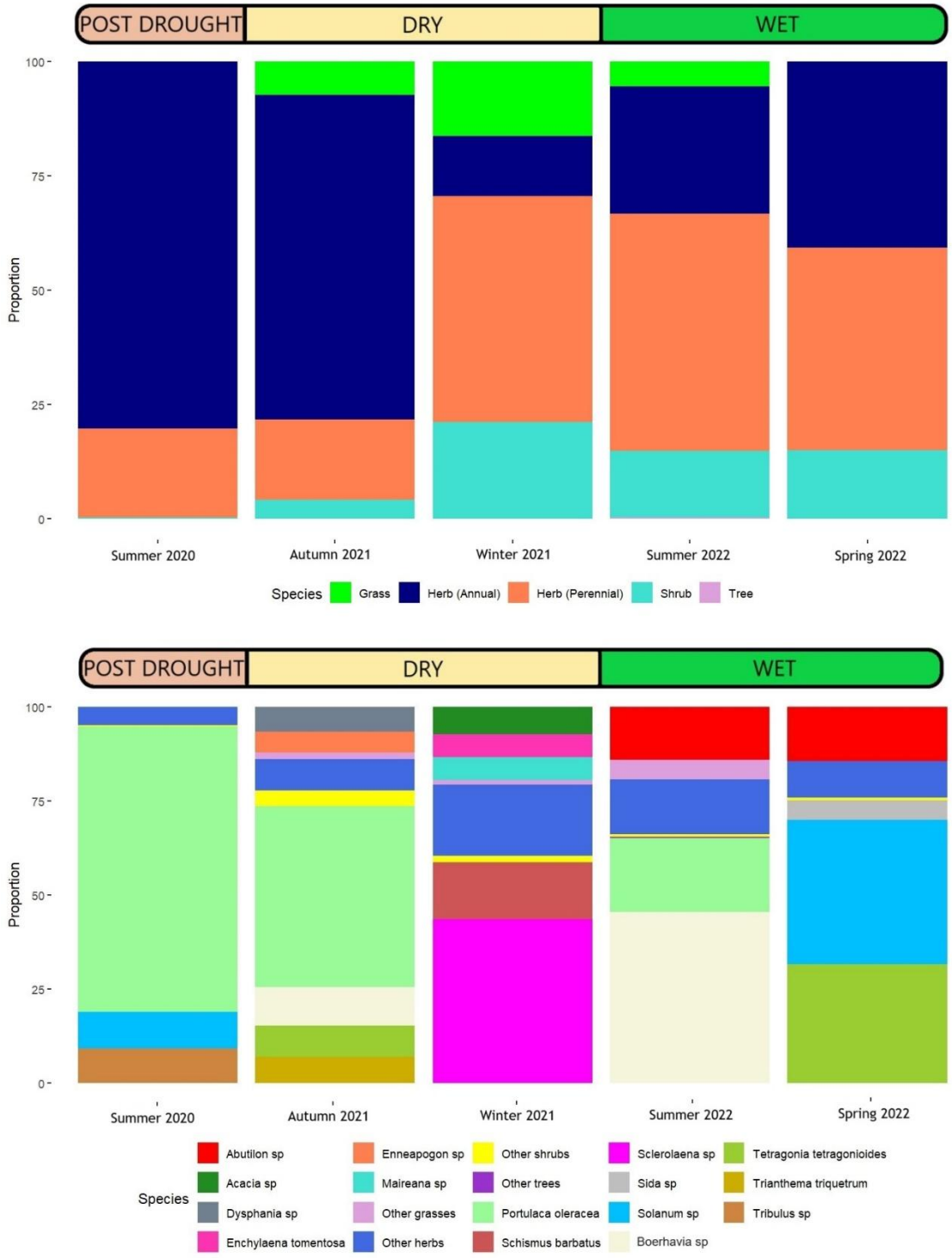


Figure 6: The proportion (%) of sequencing reads of plant types (*top*) and individual plant species (*bottom*) detected in yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) scats collected from Bimba Hill, South Australia. Plant types are defined as grass, herb (annual and perennial), shrub or tree. Scats collections were conducted during summer 2020, autumn 2021, winter 2021, summer 2022 and spring 2022.

Dietary Overlap with Sympatric Herbivores at Bimba Hill:

During summer 2020, a time immediately following a drought period, the number of plant species detected was similar for YFRWs and goats, with the majority of species detected being herbs or shrubs, whereas euros had a higher proportion of grasses (10%) detected (Table 5; Figure 7). During this time, *P. oleracea* represented approximately 76% of the total reads detected in YFRW samples, and was also heavily detected in euro (54%) and goat (29%) samples (Figure 7). When annual herb species were detected in goat samples during summer 2020 and autumn 2021 it was associated with the presence of *Euphorbia* species (spurges) (31% and 23% of total sequencing reads, respectively), a plant species which was not detected at any time in YFRW samples (Figure 7). When herbs were lowest in YFRW samples (62%, winter 2021), it corresponded to goat samples comprising <2% of sequencing reads from herb species, when a high proportion of shrub species was present instead. As sampling progressed goat samples were comprised mainly of shrubs (*Acacia* species), with detection rates increasing from 28.8% (summer 2020) to 85.4% (spring 2022) (Figure 7).

Table 5: The number of species for each plant type detected in scat samples collected from yellow-footed rock-wallabies (*Petrogale xanthopus xanthopus*), feral goats (*Capra hircus*) and euros (*Osphranter robustus erubescens*) from Bimba Hill, South Australia. Samples were collected in summer 2020, autumn 2021, winter 2021, summer 2022 and spring 2022. Plant types have been classified as grass, herb (annual), herb (perennial), shrub and tree.

	Summer 2020			**Autumn 2021**		**Winter 2022**		**Summer 2022**		**Spring 2022**	
	YFRW	**Goat**	**Euro**	**YFRW**	**Goat**	**YFRW**	**Goat**	**YFRW**	**Goat**	**YFRW**	**Goat**
Grass	0	0	14	5	0	3	1	3	2	0	0
Herb (Annual)	7	11	10	7	5	13	1	11	0	6	0
Herb (Perennial)	5	3	8	7	4	10	1	10	0	7	5
Shrub	2	11	5	7	9	15	6	4	8	5	5
Tree	0	1	0	0	3	1	3	1	3	0	2
Total	14	26	37	26	21	42	12	29	13	18	12



Figure 7: The proportion (%) of sequencing reads of plant types (*top*) and individual plant species (*bottom*) detected in yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*), feral goat (*Capra hircus*) and euro (*Osphranter robustus erubescens*) scats from Bimba Hill, South Australia. Plant types have been classified as grass, herb (annual and perennial), shrub and tree. Scat samples were collected during summer 2020, autumn 2021, winter 2021, autumn 2022 and spring 2022.

Discussion:

Historically, knowledge of the diet of YFRWs was based on the classification of plant fragments in faecal pellets using micro-histological examination (Dawson & Ellis 1979; Lim *et al*. 1987; Copley & Robinson 1983; Lapidge 2000). In this current study, genetic identification of plant material from faecal pellets was used for the first time to determine the plant species present in YFRW scats. Here we used scat samples to assess the diet of YFRWs from two different regions, collected across multiple periods of varying rainfall, and additionally compared these results with dietary samples obtained from two sympatric herbivores to assess dietary overlap and identify the level of resource competition.

Genetic sequencing of scat samples alone cannot determine the exact plant biomass intake of individual plant species prior to scat deposition due to a multitude of factors. For example, the digestibility of herbaceous species is higher than other, more woody species due to their high water and low fibre content (Bråthen *et al*. 2021), and therefore the number of reads in sequencing results may be an underrepresentation of their absolute biomass intake. As such, this study has used the proportion of sequencing reads for each sample as an indicator of plant biomass intake, but we do not suggest that this comprehensively represents the amount of plant material ingested. Further investigation into the passage time and degradation of plant DNA through the digestive system of YFRWs would be needed to develop correction formulas for individual plant species to be able to conclude total dietary biomass intake from genetic sequencing results.

However, the results from this study have allowed for the accurate identification of specific plant species in scat samples through the use of a plant genetic library. The results revealed similarities in YFRW dietary composition between the two study sites. Of the 162 plants detected in YFRW scat samples, approximately 29% occurred at both sites. Plants were consumed from a range of growth forms (i.e. herbs, shrubs, grasses) aligning with previous notions that YFRWs are opportunistic, mixed feeders (Dawson & Ellis 1979; Copley & Robinson 1983; Lim *et al*. 1987). An apparent selection for herbaceous plant species was observed throughout the current study at both sites, also supporting the earlier findings from YFRW diet studies (Dawson & Ellis 1979; Copley & Robinson 1983). Herbaceous plant species are recognised as having high palatability, fast growth rates, and high nitrogen and phosphorous levels (Bråthen *et al*. 2021), and therefore are preferred dietary components of many herbivore species, such as swamp wallabies (*Wallabia bicolor*; Davis *et al*. 2008), sambar deer (*Rusa unicolor*; Quin *et al*. 2023), black-footed rock-wallabies (*Petrogale lateralis lateralis*; Creese *et al*. 2019) and allied rock-wallabies (*Petrogale assimilis*; Horsup & Marsh 1992).

High detection rates of *P. oleracea*, an annual herb, were evident at each site for most sampling periods. *P. oleracea* is a native succulent which has high nutrient richness (Petropoulos *et al*. 2016; Iranshahy *et al*. 2017) and high moisture content in the leaves (91.8-93.4%) (Pinela *et al*. 2017). The

species is capable of tolerating drought conditions (Ferrari *et al*. 2020), which would suggest that it is an ideal food source for herbivores in semi-arid regions, and would be preferentially selected for when available. The high detection rate of *P. oleracea* suggests that YFRWs at both sites are indeed selecting this plant when available, and are utilising it as a major food source for its high water and nutrient content during dry conditions.

The YFRW has been previously stated to preferentially selecting grass during good environmental conditions as a major component of their diet (Copley & Robinson 1983; Lim *et al*. 1987). Grass species have also been observed to comprise the majority of the diet of brush-tailed rock-wallabies (*Petrogale penicillata*) in East Gippsland, Victoria (van Eeden *et al*. 2011). Although, in our study the detection rate of grass species was relatively low at both sites following high rainfall periods (7-10%). In contradiction to previous YFRW studies, the proportion of grass species detected in fact increased during drier periods at both study sites (16.3-25.9%). However, the northern Flinders Ranges has lower annual rainfall and experiences different seasonal rainfall patterns than southern regions, where the previous YFRW diet studies were based. This was supported in Copley & Robinson (1983) where YFRW dietary composition differed between northern and southern regions, and may explain the results of differing dietary preferences observed here between study sites. Additionally, the diet of Olary Ranges YFRWs has not been examined previously, and therefore no comparative values are available for this region. Grasses are known to have low nitrogen and high fibre content during dry conditions and are often not highly represented in herbivore diets during these conditions (Lim *et al*. 1987; Lapidge 2000). Therefore, during the dry times when grasses were being ingested at the study sites, they likely had low nutritional content, and may have only been consumed by YFRWs when other nutritious plant species were depleted.

The increase in grass intake also corresponded to a general increase in YFRW dietary diversity during dry times. While the number of plant species detected in scats at Italowie Gap was significantly higher across both winter and spring, high plant species diversity was only detected in winter at Bimba Hill, with moderate levels of plant diversity throughout the rest of the seasons. Similar findings were observed by Dawson and Ellis (1979) in western New South Wales where YFRW dietary diversity was greater during dry/drought times, where plant species consumed approximately doubled compared to higher-than-average rainfall periods. These changes fit with the optimal foraging theory, which has been observed in other species (Stephens & Krebs 1986; Sih & Christensen 2001; Svanbäck & Bolnick 2007; Pyke 2010), where at high population density or low food resource abundance, dietary diversity increases. In the semi-arid region, vegetation cover changes in response to seasonal rainfall peaks, where cover is generally highest during the summer/autumn months, and consistently decreases until reaching a minimum during early spring (Dawson & Ellis 1979; Corbett 1980; Smith & Morton 1990; Morton 2022). As such, the results of our study suggest that the YFRW

has seasonal dietary preferences, such as selecting mostly herbaceous species during high plant biomass, but has the ability to broaden their diet as the preferred vegetation becomes depleted.

Changes in the diet of other species, such as the African elephant (*Loxodonta africana*; Woolley *et al*. 2009), Atlas deer (*Cervus elaphus barbatus*; Ismaili *et al*. 2018), South African giraffe (*Giraffa camelopardalis giraffa*; Gordon *et al*. 2016), and several savannah herbivores (Abraham *et al*. 2019) has been observed in response to drought or dry conditions. This is especially true when examining the feeding habits of mixed feeders where the feeding behaviour of grazers shifts to browsing during dry/drought periods (Abraham *et al*. 2019). This trend was also observed in the dietary selection of YFRWs in this study, where browse detection rates increased as conditions became drier at both sites.

During dry periods, *Acacia* species were prevalent in YFRW samples from both sites. The high proportion of shrubs in YFRW samples from Italowie Gap was associated solely with *Acacia* species, whereas at Bimba Hill, the high proportion of shrubs was associated with *Enchylaena tomentosa* (ruby saltbush), *Maireana* species, and *Acacia* species. *Acacia* species are not known to be a preferred dietary item of YFRWs or other macropodids as tannins in the foliage make it unpalatable and can reduce the bioavailability of nutrients (Irlbeck & Hume 2003). However, during drought periods when the foliage has died, tannin levels are low, making it more palatable (Irlbeck & Hume 2003). As such, the increased detection of shrub species during dry times, such as *Acacia*, was likely due to ingesting dead foliage due to a lack of availability of more palatable plant species.

Through dietary overlap analyses, it was identified that the main plant species detected in samples from all three sympatric herbivores were similar during spring 2022 at Italowie Gap and summer 2020 at Bimba Hill. At Italowie Gap the three plant species present in high proportions in YFRW samples which were also represented in either the goat or euro samples (*Acacia* species, *Malvastrum* species, and *Sigesbeckia australiensis*). This trend was also reflected at Bimba Hill where the main plant species detected in YFRW samples, *P. oleracea*, was also detected in goat and euro samples. This indicated that during these sampling times when vegetation was scarce or with declining nutritional content, and the landscape was recovering from drought conditions, there was a high degree of dietary overlap between YFRWs, goats and euros.

Other studies have also shown that exotic herbivores often compete with native fauna for food resources such as the guanaco (*Lama guanicoe*) and the introduced feral donkey (*Equus asinus*), or the Walia ibex (*Capra walie*) and the domestic goat (Reus *et al*. 2014; Gebremedhin *et al*. 2016). In Australia, the feral goat is a prominent exotic herbivore whose diet, through micro-histological studies, has generally shown an overlap with sympatric native fauna due to their broad dietary diversity (Dawson *et al*. 1975; Dawson & Ellis 1979; Dawson & Ellis 1996; Creese *et al*. 2019). However, the native, herbivorous euro can become overabundant during periods of high resource

availability or when provided with water ad lib, and can also compete with native wildlife (Dawson & Ellis 1979; Copley 1983; Parkes *et al*. 1996).

The results from previous studies comparing the diet of rock-wallabies and the same sympatric herbivores identified goats as the main resource competitor (Dawson & Ellis 1979; Creese *et al*. 2019). However, our results indicate that dietary overlap is also occurring between YFRWs and euros. As such, both the feral goat and euro have the potential to significantly inhibit YFRW feeding preferences and nutritional intake, especially during dry conditions.

Further Research:

This study used a plant genetic reference library which represents South Australian vascular flora at the generic level, but is not completely inclusive of all flora at the species level. This is the first time this approach using DNA hybridization capture with myBaits® has been utilised with DNA extracted from animal scat samples. However, one of the benefits of this technique is the formation of genetic libraries from extracted plant DNA, allowing for higher specificity in sequencing coverage and providing a higher detection rate of plant species. Although some of the species indicated in sequencing results were determined to not be present at either study site and were reclassified to a genus level, this is likely to be as a result of highly conserved chloroplast gene regions between taxa, coupled with the lack of a fully comprehensive genetic library, rather than sequencing errors. Therefore, further development of this genetic library would be greatly beneficial for future studies examining the diet of semi-arid wildlife species.

Additionally, continued dedicated surveying of the diet of all three sympatric species during differing environmental conditions is needed. This is due to plant abundance and diversity in semi-arid and arid regions being driven by sporadic and seasonal rainfall (Robertson *et al*. 2010; Prevéy *et al*. 2014; Liang *et al*. 2021) causing dramatic changes in vegetation in response to low or high rainfall periods. This will also become particularly prevalent as climate change alters vegetation characteristics.

Conclusions:

This study has examined the variability of YFRW dietary preferences and highlighted how dietary diversity and seasonal preferences are driven by rainfall. The majority of plant species detected in YFRW scat samples were herbaceous, and dietary diversity was greatest during dry times, in which more shrub species were also detected, indicating that the YFRWs diet broadens as preferential plant (i.e. herbs) availability decreases.

The plants detected in feral goat and euro scat samples overlapped with YFRW samples, which was especially prominent during periods of low resource abundance and low plant nutrient content. The feral goat is likely the biggest competitive threat to YFRWs during dry times. However, this study also indicated the potential for euros to have high competition rates with YFRWs, which would be exacerbated during periods of euro overabundance. During drought and drought recovery periods, management programs involving YFRWs should focus on increased feral goat control to alleviate resource competition levels. Management of overabundant euros during the initial phases of drought may also be beneficial for YFRWs.

The ability for YFRWs to adapt their diet has allowed them to persist in their harsh semi-arid environment, but as drought frequency and severity begins to increase with climate change, YFRWs may be challenged by a lack of resource availability for longer periods of time. Long-term monitoring of YFRW populations will be vital for assessing the environmental effects of climate change on YFRW population health. This will be an important tool for informing the YFRWs management and enable early detection and quick intervention for populations at risk.

Chapter Four

"The influence of season, habitat and diet on the faecal microbiome of the yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*)"



"*Some hundreds of skins are annually imported to London from Adelaide, their value ranging from one-and-fourpence each*"

– Lydekker 1894

Statement of Authorship

Title of Paper	The influence of season, habitat and diet on the faecal microbiome of the yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*)
Publication Status	☐ Published ☐ Accepted for Publication ☑ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Submitted to Austral Ecology.

Principal Author

Name of Principal Author (Candidate)	Lauren Werner		
Contribution to the Paper	Study design, field work, sample collection, laboratory preparation, statistical analyses, writing manuscript.		
Overall percentage (%)	85		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	14/10/2024

Co-Author Contributions

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

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

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Signature		Date	14/10/2024

The influence of season, habitat and diet on the faecal microbiome of the yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*)

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Abstract:

Knowledge of an animal's microbiome is becoming increasingly recognised as an important consideration in the conservation of threatened species, particularly in the face of wide-spread habitat loss, novel diseases, and changing climate and rainfall patterns. The yellow-footed rock-wallaby (YFRW; *Petrogale xanthopus xanthopus*) is a Vulnerable macropodid marsupial endemic to the semi-arid regions of South Australia and New South Wales (EPBC Classification). This study aimed to characterise the faecal microbial diversity and its relationship to diet and season/rainfall in two South Australian YFRW populations which are geographically separated and have differing habitat characteristics. Sequencing targeting the 16S rRNA gene revealed that location was the greatest driver of faecal microbial richness, evenness and composition ($p < 0.01$), with season ($p < 0.01$) and the interaction of *location x season* also statistically significant ($p < 0.01$). The main phyla identified throughout were the *Firmicutes* and *Bacteroidota*, representing over 80% of total ASV diversity. Diet varied between individual YFRWs, but *Acacia* species were commonly detected at each study site, although at opposing times of the year. There appeared to be an increase in the proportion of *Firmicutes* present when a high proportion of *Acacia* was consumed, although further sampling is required to confirm this. Further research and continued monitoring of these populations is required to understand the function of these microbes, and specifically, the role these microbes may play in an individual's resilience to dry times and drought, which will be especially pertinent in the semi-arid region where wildlife is at a heightened risk from climate change.

Introduction:

The gut microbiome, that is the community of microorganisms residing in the digestive tract, has been linked with the general health and wellbeing of humans and other animals (Haller 2018; Amato *et al*. 2021; Huang *et al*. 2022; Pannoni *et al*. 2022; Ribas *et al*. 2023). Deviations from a normal gut microbial composition can result in health issues such as nutritional deficiencies (Kau *et al*. 2011), decreased immunological function (Round & Mazmanian 2009), and abnormal physical development (Sommer & Bäckhed 2013). The importance of gut or faecal microbes in wildlife conservation has recently been recognised (Banerjee *et al*. 2020), with studies suggesting that the microbiome could act as an early indicator of ecosystem declines, aid in increasing species fecundity, and help improve

reintroduction success (Redford *et al*. 2012; West *et al*. 2019; Ribas *et al.* 2023). As a result, wildlife microbiome studies are increasing in popularity and are often associated with conservation priority species such as the critically endangered Indri (*Indri indri*; Correa *et al*. 2021), black-and-white ruffed lemur (*Varecia variegata;* Donohue *et al*. 2019), and the endangered Malayan tapir (*Tapirus indicus*; Arumugam *et al.* 2023).

In Australia however, the number of gut or faecal microbiome studies are still relatively few, with investigations conducted on only a small subset of native species such as the Tasmanian devil (*Sarcophilus harrisii*; Cheng *et al*. 2015), koala (*Phascolarctos cinereus*; Brice *et al*. 2019; Blyton *et al*. 2023; Eisenhofer *et al*. 2023), tammar wallaby (*Notamacropus eugenii*; Chhour *et al*. 2010) and the southern hairy-nosed wombat (SHNW; *Lasiorhinus latifrons*; Eisenhofer *et al*. 2021). Building the understanding of faecal microbial characteristics of Australian native species is integral for assessing how microbiome manipulation can be used to assist conservation programs. This is particularly pertinent as climate change is predicted to result in higher average temperatures, decreased annual rainfall, longer and more severe droughts, and more extreme climatic events (Department for Environment and Water 2022), forcing wildlife to adapt to new environmental conditions, move their current distribution, or risk significant population decline (Mills 2012; Davis *et al*. 2005; Höglund *et al*. 2019).

Habitat specialists are predicted to be at highest risk under progressive climate change (Klamt *et al*. 2011; Adams-Hosking *et al*. 2012; Gibson *et al*. 2018; Marshall *et al*. 2018). The yellow-footed rock-wallaby (YFRW; *Petrogale xanthopus xanthopus*), a semi-arid dwelling, medium-sized macropodid marsupial is one such species. The YFRW utilises foregut fermentation to extract short-chain fatty acids (SCFAs) from their herbivorous diet (Hume 1999; Armati *et al*. 2006). Currently, there are no direct studies on energy metabolism for YFRWs, but macropodids in general are thought to produce approximately 42% of their total digestible energy intake from fermentation in the foregut region (Armati *et al*. 2006). However, the YFRW has yet to have any microbiome examinations completed, leaving the microbial communities which acquire their energy requirements relatively unknown.

In South Australia, the YFRW is found in the Flinders, Gammon, Gawler and Olary Ranges in rocky habitats which they rely upon for shelter from environmental extremes and predation (Copley 1981; Short 1982; Copley 1983; Lim *et al*. 1987; Ruykys 2017; Michael & Lindenmayer 2018). This study aimed to examine the faecal microbiome of YFRWs from two geographically separated regions with differing habitat characteristics, within their current distribution in South Australia. As faecal microbiome is known to be associated with dietary intake (Chen *et al*. 2014; Maria Moise 2017), we also assessed how dietary composition interacts with microbial diversity and composition in YFRWs, a factor which has not been assessed in-depth in other faecal microbiome studies.

Understanding the interactions between habitat connectivity, diet, rainfall, and faecal microbiome may prove to be important information for managing the YFRW in the future, particularly as the impacts of climate change intensify.

Methods:

Ethical Statement:

This project has been approved by the University of Adelaide's Animal Ethics Committee (S-2020-097), and the Department of Environment and Water (Scientific Research Permit #Y26992-1).

Study Sites:

This study was conducted at Italowie Gap in the Vulkathunha-Gammon Ranges National Park, and Bimba Hill in the Bimbowrie Conservation Park, in South Australia. These locations represent the large variability in YFRW habitat in South Australia, and are geographically separated by approximately 180km (Figure 1).

Italowie Gap in the Vulkathunha-Gammon Ranges National Park (Figure 1; -30.558691, 139.164285) is approximately 500km north of Adelaide. Long-term data indicates that this area receives approximately 257mm of rainfall annually, however, across the 15 months of this study it received 470mm of rainfall. Rainfall in the three-months prior to each sampling period was recorded due to previously documented effects of rainfall during this period on vegetation quality and some animal health parameters (Willers *et al*. 2011) (Table 1; Figure 2; Bureau of Meteorology). YFRW habitat at Italowie Gap is characterised by steep escarpments with simple stratified sandstone, siltstone, and quartzite rock formations with few areas of high rock complexity, interspersed with loose earth and scree slopes. Due to this colony's location along an extensive range system (Balcanoona Range; see Appendix 1, Figure S1 pg. 212), this YFRW population is highly connected to other colonies, and as such, have greater access to a range of resources. The vegetation at Italowie Gap is mostly confined to the adjacent creek beds, dominated by river red gum trees (*Eucalyptus camaldulensis*) and a variety of shrubs (e.g. *Senna artemisioides*, *Acacia* species), and the slopes, where there are scatterings of rock fuchsia bush (*Eremophila freelingii*) and white cypress pine (*Callitris glaucophylla*). Following times of high rainfall, the hills are dominated by rock sida (*Sida petrophila*) and desert lantern-bush (*Abutilon leucopetalum*) with small herbaceous plants and grasses growing amongst the rocks.

Bimba Hill in the Bimbowrie Conservation Park, situated in the Olary Ranges of South Australia (Figure 1; -31.983341, 140.211817), is approximately 350km north-east of Adelaide and receives on average 203mm of rainfall annually. During the 15-month period of this study however, it received

311mm of rainfall (Table 1; Figure 2; Bureau of Meteorology, data from Bimbowrie weather station). YFRW habitat at this site consists of isolated monzogranite outcrops, comprised of flat rock sheets and large, complex boulder jumbles interspersed with earthen slopes. Due to Bimba Hill being separated from other colonies by relatively large, open distances (>5km, see Appendix 1, Figure S2 pg. 213), this YFRW colony is considered largely isolated and therefore, resource availability is more limited. The vegetation at Bimba Hill is dominated by rock fuchsia bush (*Eremophila freelingii*), with the occasional mulga (*Acacia aneura*) and dead finish (*Acacia tetragonophylla*). Following high rainfall, the slopes are dominated by rock sida (*Sida petrophila*) and *Solanum* species, with herbaceous plants and a variety of grasses growing between rocks and at the base of boulders.

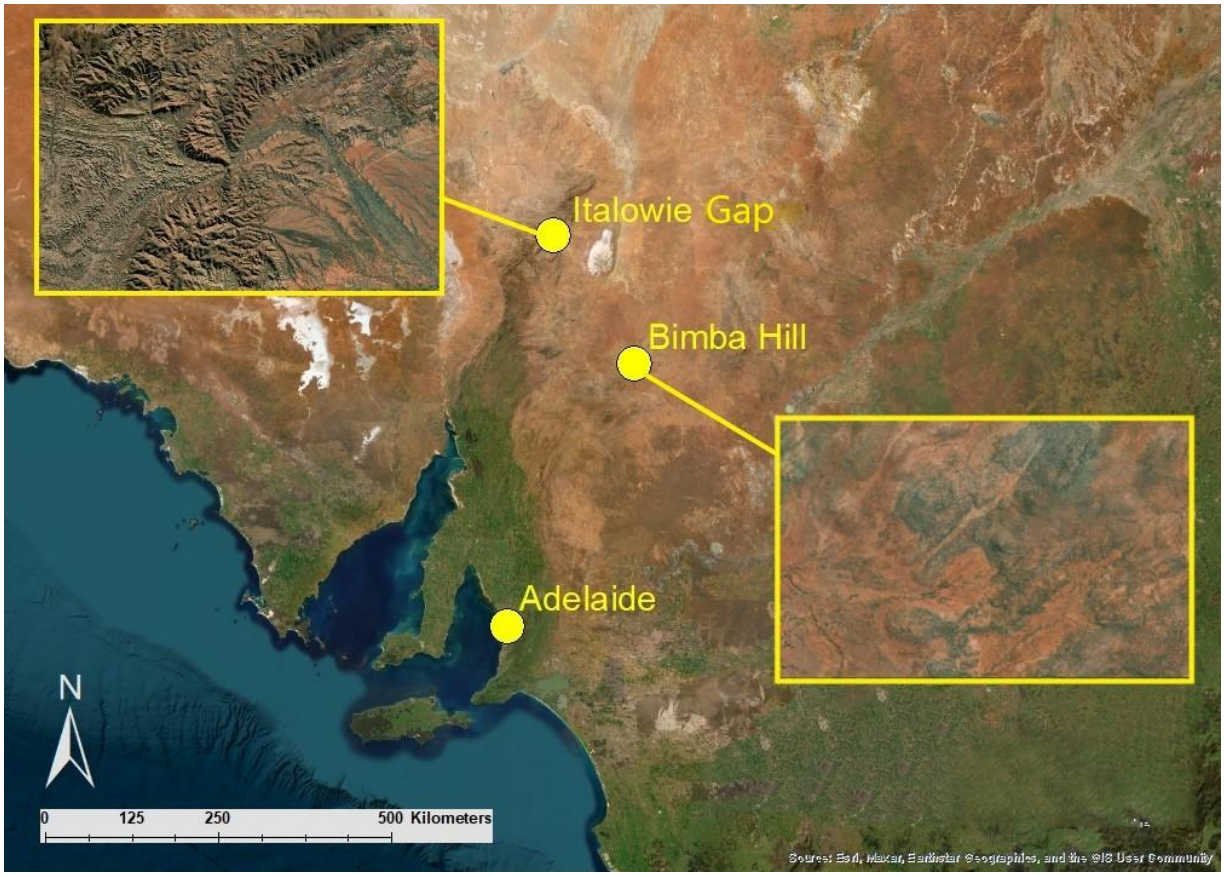


Figure 1: The location of the yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) study sites, Italowie Gap in the Gammon Ranges, and Bimba Hill in the Olary Ranges, in South Australia, Australia. Inset images show a closer aerial view of each study site.

Table 1: The amount of rainfall (mm) which was received in the three-month period prior to trapping and the collection of yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) scats from Italowie Gap and Bimba Hill, South Australia. Rainfall data presented for the three different trapping periods per site; autumn 2021, spring, and autumn 2022.

Italowie Gap			
	Autumn 2021	Spring 2021	Autumn 2022
Rainfall in preceding 3-month period to sampling (mm)	64.4	23.6	84.5
Bimba Hill			
	Autumn 2021	Spring 2021	Autumn 2022
Rainfall in preceding 3-month period to sampling (mm)	45.1	32.7	62.8

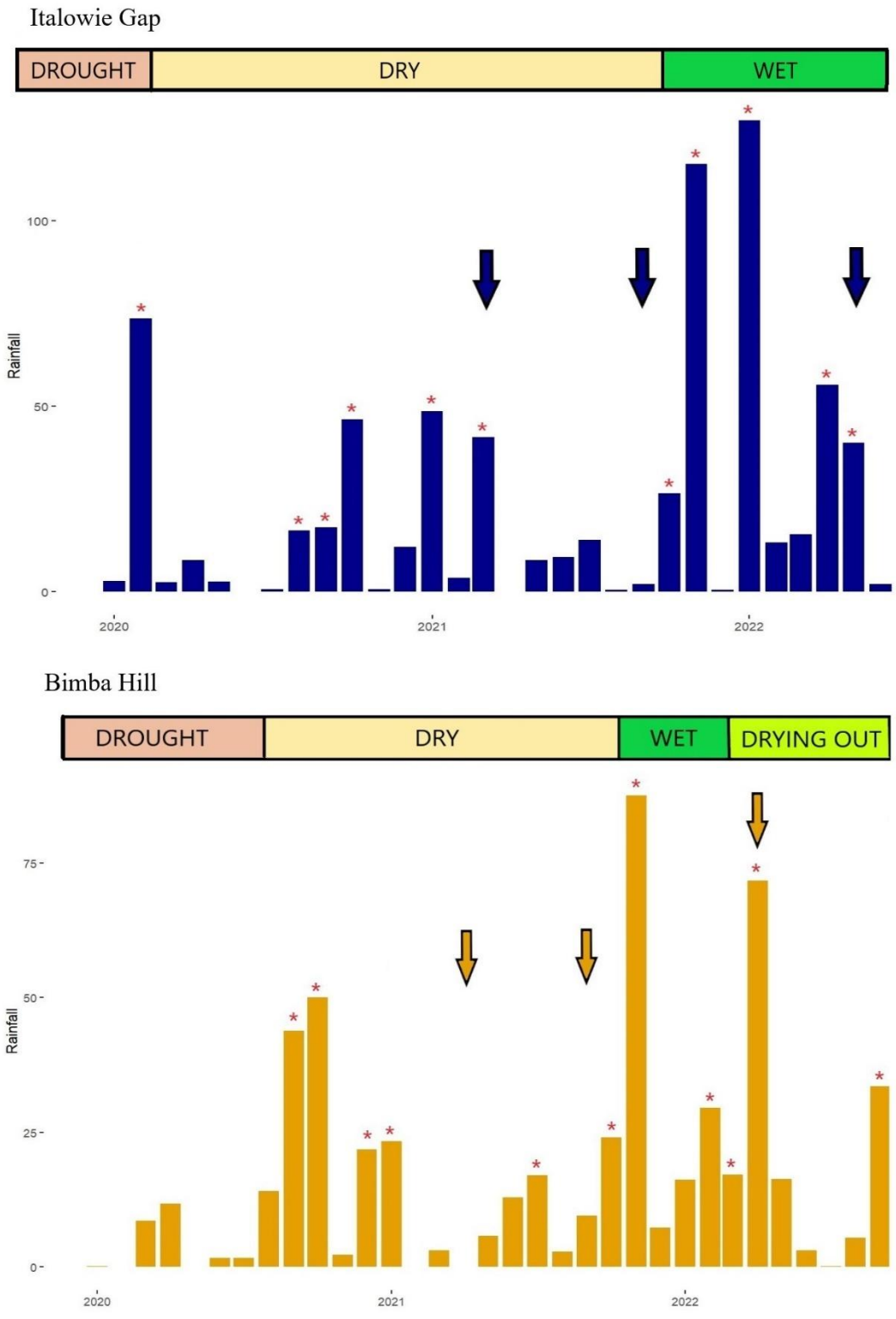


Figure 2: The total monthly rainfall (mm) received preceding and during the study period at Italowie Gap (*top*) and Bimba Hill (*bottom*), South Australia. Months of higher-than-average rainfall (*), conditions at each site (drought, dry, wet or drying out), and yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) trapping periods indicated (downward arrow). Rainfall data was obtained from the Bureau of Meteorology.

Sample Acquisition:

Faecal samples (scats) were collected as part of a larger YFRW trapping program during autumn 2021, spring 2021, and autumn 2022 (Figure 2). Trapping and sample collection methodology is described in the *Supplementary Chapter* of this thesis. Scats (microbiome n = 42, diet n = 24) were collected from each trapped YFRW by extracting fresh scats from the bottom of the trap. Only fresh scats were collected to minimise microbial die-off after deposition, and were collected by inverting a snap-lock bag over the collector's hand to avoid human contamination. As presented, matched faecal microbiome and diet samples were collected from trapped individuals, but this was not possible for all animals captured. For microbiome analysis, scats were opened, and an internal sample of the scat collected and stored in 90-100% ethanol in a 1.5ml Eppendorf tube, to fix microbial community composition. For diet analysis, scats were stored in individual plastic snap-lock bags. Scats in snap-lock bags and microbiome samples were kept frozen (-4ºC to -20ºC) until laboratory processing.

Laboratory Processing:

Faecal Microbiome:

Faecal microbiome samples (n = 42) were processed in a pre-PCR microbiology laboratory in 5%-sodium hypochlorite cleaned and UV irradiated hoods to avoid contamination of samples. Faecal samples were processed using the QIAGEN DNEasy PowerSoil kit following the manufacturer's protocol. Extracted samples were then kept frozen at -20ºC until further processing.

Extracted microbial samples were amplified by PCR targeting the 16S gene V4 region (Caporaso *et al*. 2011). PCRs were set up using a master mix of 19.2µl of dH_2O, 2.5µl 10x buffer, 1µl $MgSO_4$, 0.2µl dNTP mix (100mM), 0.5µl of forward primer (10µM), and 0.1µl of Platinum™ Taq high fidelity DNA polymerase (Invitrogen™) for each sample. Finally, 1µl of sample and 0.5µl of a uniquely barcoded reverse primer (10µM) were added to make up a final PCR volume of 25µl. PCR conditions were an initial denaturing step of 94ºC for 3min, followed by 35 cycles of 94ºC for 45sec, 50ºC for 60sec and 68ºC for 90sec, with a final adenylation step of 68ºC for 10min. PCR products were then assessed on a 3.5% agarose gel and visualised on a ChemiDoc imager (Bio-Rad Laboratories, Australia) for successful PCR amplification.

PCR products were then quantified using a Qubit 2.0 fluorometer (Thermo Fisher Scientific, Australia) at a 1:199 ratio of sample to Qubit working solution. Qubit results were then used to bulk samples in equimolar pools. DNA Extraction negative control samples were pooled separately because of their lower DNA concentration than positive samples. Each pool was cleaned using AxyPrep (Axygen) following the manufacturer's protocol and visualised using the Agilent TapeStation 4150 to assess the quality and quantity of products. Pools were then bulked together

equimolar and re-assessed on the TapeStation before being sent to the South Australian Genomics Centre (SAHMRI, South Australia) for sequencing on the Illumina MiSeq (v2) using a 2 x 150bp run.

Diet:

Individual scat samples (n = 24) were prepared for DNA extraction using a Labconco FreeZone 4.5L -50^{0}C freeze-drier (Labconco Corporation, USA) for 48-72 hours. Samples were then pulverised using a mortar and pestle to form a powder. Subsets of the pulverised samples were then processed using the Isolate II Plant DNA kit (Meridian Bioscience, USA) following the standard protocol and lysis buffer PA1. The eluted DNA was then quantified using a Quantus Fluorometer (Promega, Australia) before being stored frozen at -20ºC.

Genomic libraries compatible with Illumina sequencing were generated using the extracted DNA (n = 24) and the NEBNext® Ultra™ II FS DNA Library Prep Kit (New England Biolabs, Australia). In brief, DNA was fragmented enzymatically, end-repaired, a-tailed, and custom stubby Y-adaptors ligated to the size selected inserts. All preparation was performed using an Eppendorf epMotion 5075 (Eppendorf, USA) liquid handler. A detailed description of the methodology can be found in Waycott *et al*. (2021). Libraries were PCR amplified over 21 cycles and the resulting intermediate-stage library was then visualised on a 1.5% Agarose gel.

The library gel images were assessed visually for intensity to determine the relative fractions of each sample added into a pool. Pools were then purified with MagNA beads (Rohland & Reich 2012) at a 1:1x ratio and eluted in 35µL of 1x TE.

Target capture was performed using the OzBaits_Cp bait set (Waycott *et al*. 2021) on the library pools following the manufacturer's instructions (myBaits® targeted NGS capture; Manual version 4.01, Arbor Biosciences, USA), and hybridization conducted at 65ºC for 24 hours. The bait design targets approximately 50 regions of the chloroplast genome and can capture these regions for any vascular plant species (Waycott *et al*. 2021). After hybridization and clean-up, the resulting enriched libraries were finalised (made compatible with Illumina sequencing flow cell and sequencing primers) by performing a PCR for 18 cycles with indexed P5 and P7 fusion primers (Waycott *et al*. 2021). Following this PCR stage, the products were purified with MagNA beads at a 1:1 ratio.

The finalised libraries were then analysed on an Agilent TapeStation 4150 (Agilent Technologies, USA) with high-sensitivity tapes to assess molarity and fragment size distribution (region setting set to 300-700bp). All libraries were then pooled in equimolar concentration based on TapeStation results. The pooled library was then size selected on a Pippin Prep (Sage Science) using a 2% Agarose gel cassette with marker 'L' and range set to 300-600bp. The final library was again visualised on the Agilent TapeStation 4150 to ensure the quality and molarity of the sequencing products.

The final pooled library was then sent to the Australian Genome Research Facility, Melbourne, for sequencing using one lane of the Illumina NovaSeq X 10B flow cell for 2 x 150bp.

Sequence Preparation and Informative Analysis:

Faecal Microbiome:

Raw sequencing data was processed and analysed using QIIME2 (Bolyen *et al*. 2019). Reads were imported into QIIME2 and using the deblur plug-in (Amir *et al*. 2017) with a 150bp trim length, amplicon sequence variants (ASVs) were identified. The QIIME2 feature-classifier plug-in (naïve Bayesian approach) on the pre-trained SILVA (Quast *et al*. 2013) 132 V4 region classifier was then used to assign ASVs to taxonomic units (Bokulich *et al*. 2018). Classified sequences were then imported into *R* (R Core Team 2023) and filtered for chloroplast DNA sequences (9.5% present in total sequences), low abundance ASVs (threshold = 0.05%), and ASVs present in only a single sample (37.2% ASVs removed). The filtered sequences were then analysed using the packages *microbiome*, *phyloseq* (McMurdie & Holmes 2013) and *ggplot2* (Wickham 2016) to calculate and visualise Alpha diversity metrics, ASV richness and evenness (Pielou), and Beta diversity metrics (weighted and unweighted UniFrac distances; Lozupone & Knight 2005; Lozupone *et al*. 2007). A Venn diagram was also developed using the *VennDiagram* package (Chen & Boutros 2011).

For statistical analysis, firstly data was examined to assess normality using a Shapiro-Wilk test (*shapiro.test* function). Once normality was established, Alpha diversity statistical differences were calculated using an ANOVA with the *lme4* package (Bates *et al*. 2015). Statistical differences in Beta diversity metrics (microbial composition) were conducted using an adonis test with the *vegan* (Oksanen *et al*. 2022) package. The differences in faecal microbial composition between sampling times was examined using a principle coordinates analysis (PCoA).

Diet:

Sequencing of samples returned paired-end sequence reads that were transferred to the Phoenix HPC and demultiplexed using Axe (Murray and Borevitz 2018) by recognition of the internal barcodes imbedded in the ligated stubby Y-adaptors, and was followed by quality and adaptor trimming using FASTP (Chen 2023). Trimmed paired-end reads were imported into CLC Genomics Workbench v. 20.0.2 (Qiagen) and *de novo* assembled (settings: length and similarity fraction = 0.9, minimum contig length = 300). The contigs generated from the *de novo* assemblies were then imported into Geneious v 2022.0.12 (https://www.geneious.com). Each contig was then compared with a plant DNA reference database, which includes sequences of chloroplast gene regions from native and introduced

flora found across South Australia (https://data.environment.sa.gov.au/Content/Publications/vascular-plants-bdbsa-taxonomy.xlsx), with all flora represented to the generic level. To identify matches within the database, BLASTn was used with an expect value of 1e-20, and 10 hits for each matching contig were saved. In Geneious, the sequence classifier tool, 'Classify Sequences' (http://assets.geneious.com/documentation/geneious/Sequence_Classifier_Manual.pdf), was then used to compare the contigs with positive hits within the database of pooled BLASTn outputs obtained from the previous step. The Classify Sequences tool matches the query and database sequences using pairwise alignments and reports the identity for the proportion of overlap between the two sequences. The Classify Sequences tool uses a GenBank taxonomy string (https://www.ncbi.nlm.nih.gov/taxonomy/) attached to each database sequence to decide the appropriate levels of classification based upon user defined thresholds. Here, we used a 95% overlap identity threshold to record a match and a ≥98% identity overlap to classify a query sequence to genus. If there was a single match above this threshold, it was identified to species but for multiple near equal matches at ≥98% identity, the sequence was classified using the lowest common ancestor from the taxonomic string (e.g. two or more congeneric species with the same identity would be classified at the genus level, and species from different genera in the same family would be classified to a family level).

All plant species detected were then cross-referenced with the Australasian Virtual Herbarium (AVH; https://avh.chah.org.au) and the Atlas of Living Australia (ALA; https://www.ala.org.au) to assess the accuracy of sequencing results and determine their presence at either site. Where sequences were classified to a species level but were determined as absent from the site, and when multiple species from the same genus were present, they were each reclassified for ease of visualisation (represented as '*Genus* sp.').

The proportion of sequencing reads per sample was determined by calculating the proportion of each plant species in relation to the total number of sequencing reads:

$$(x \div Total\ Number\ of\ Sequencing\ Reads) \times 100$$

For ease of visualisation, species that had an abundance of <5% were grouped into their corresponding plant type, indicated in graphics as "other *plant type*". A full list of each plant species which was detected for each individual YFRW is included in Appendix 4 (pg. 227).

Results:

Faecal Microbiome:

The most commonly detected microbes across all YFRW faecal samples were from the phyla *Bacteroidota* and *Firmicutes* which constituted over 80% of the total ASVs detected in all samples (Figure 3; *Bacteriodota* – browns; *Firmicutes* – greens). The phyla *Verrucomicrobiota* and *Proteobacteria* were also commonly detected but in much lower proportions (Figure 3). Changes in the relative abundance of microbes was evident when examining the microbial composition at Bimba Hill, where *Bacteroidota* slightly decreased and *Firmicutes* increased between spring 2021 and autumn 2022, but appeared stable at Italowie Gap throughout (Figure 3).

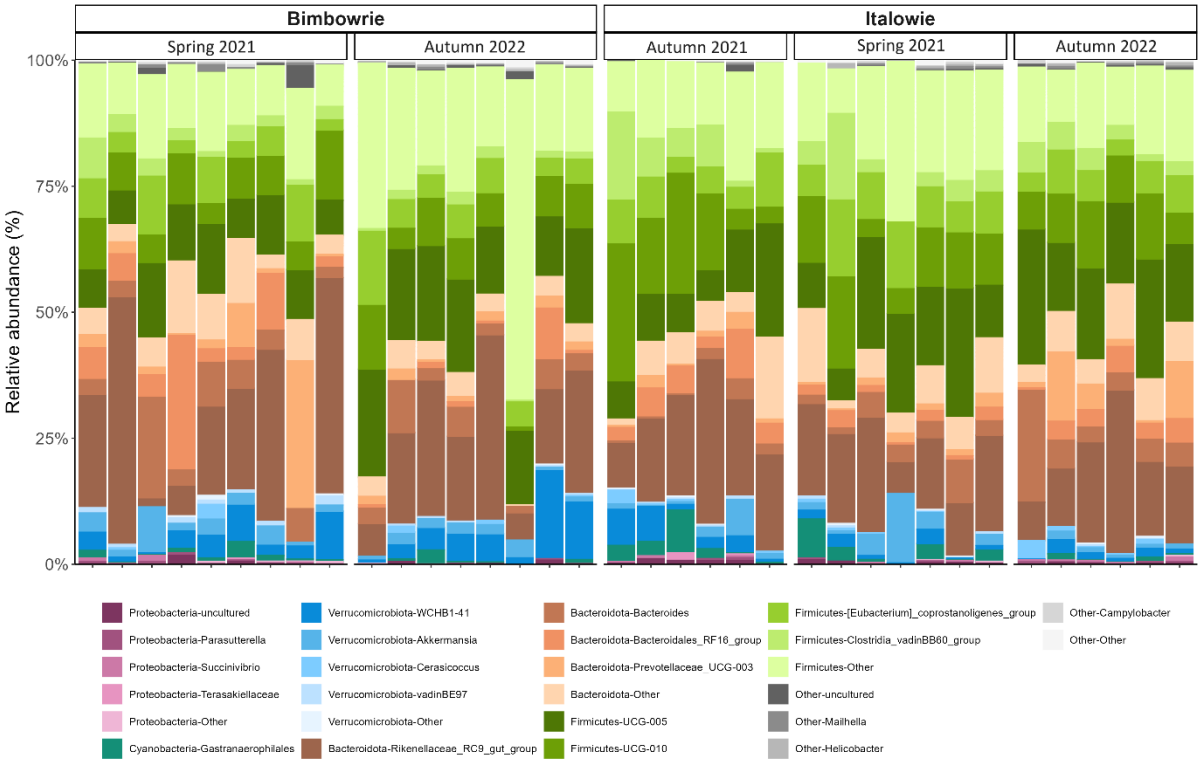


Figure 3: The relative abundance (%) of microbial communities detected in faecal samples from yellow-footed rock-wallabies (*Petrogale xanthopus xanthopus)* at two different study sites (Italowie Gap and Bimba Hill) across three sampling times: autumn 2021, spring 2021, and autumn 2022.

Site and Seasonal Differences:

Across all three sampling times, a total of 1,065 ASVs were detected for Italowie Gap and 816 ASVs for Bimba Hill. Of these, 659 ASVs (54%) were shared between both sites, where Italowie Gap had 406 distinct ASVs and Bimba Hill had 157 distinct ASVs (Figure 4).

Location had a significant effect on the Alpha diversity metrics of microbial richness (*ANOVA*; $p = 9.403 \times 10^{-7}$), and microbial evenness (*ANOVA*; $p = 0.01$), where microbial richness was significantly higher in YFRW faecal samples collected from Italowie Gap compared to Bimba Hill (Figure 5).

Microbial composition was also statistically different depending on location when using Unweighted UniFrac (*Adonis*; $p = 0.0001$) and Weighted UniFrac (*Adonis*; $p = 0.001$) distances.

Season had a significant effect on microbial richness at both sites (*ANOVA*; $p = 0.002$), and also significantly affected microbial composition (*Adonis*; $p = 0.0002$) when using Unweighted UniFrac distances. Interestingly, the interaction between site and season also significantly affected microbial richness (*ANOVA*; $p = 0.008$).

Microbial composition estimated using Unweighted UniFrac for Italowie Gap accounted for approximately 14% of variance but did not significantly differentiate between samples from spring 2021 and autumn 2022 (Figure 6). In contrast the PCoA at Bimba Hill accounted for approximately 19% of variance and displayed significant differences in microbial composition between seasons (Figure 6). The low levels of variation explained by these values indicates that other factors are contributing to changes in microbial composition. However, there does appear to be an affect of rainfall at Bimba Hill where microbial richness (Figure 5) and composition (Figure 6) have altered following high rainfall and an increase in vegetation abundance (Table 1; Figure 2).

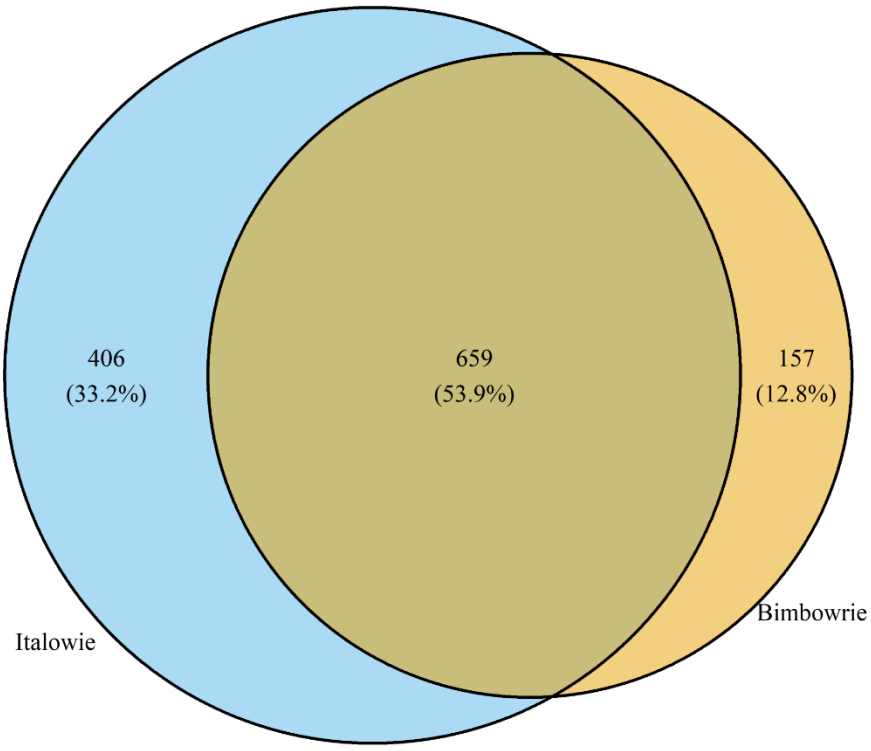


Figure 4: A Venn diagram showing the proportion of faecal microbial ASVs which are unique or shared between two yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) populations in the Vulkathunha-Gammon Ranges National Park (Italowie Gap - blue) and Bimbowrie Conservation Park (Bimba Hill - orange), South Australia.

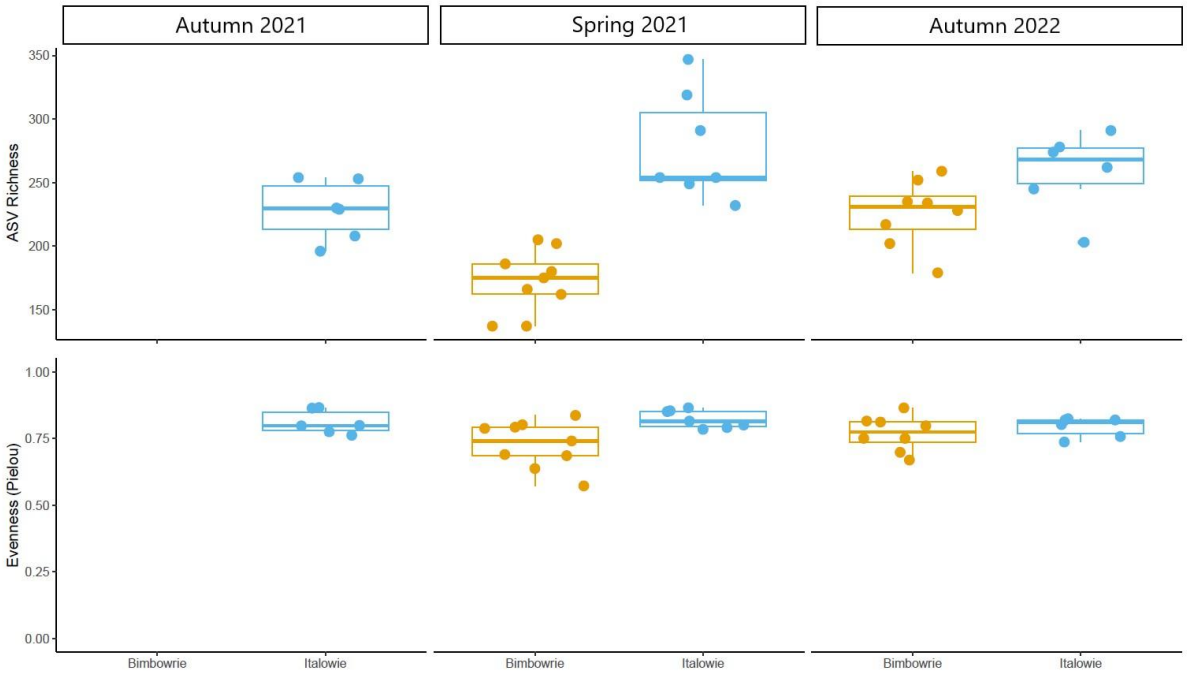


Figure 5: The faecal microbial Alpha diversity, ASV richness (*top*) and evenness (*bottom*), for yellow-footed rock-wallabies (*Petrogale xanthopus xanthopus*) at Italowie Gap (blue) and Bimba Hill (orange), South Australia, across three sampling times: autumn 2021, spring 2021, and autumn 2022.

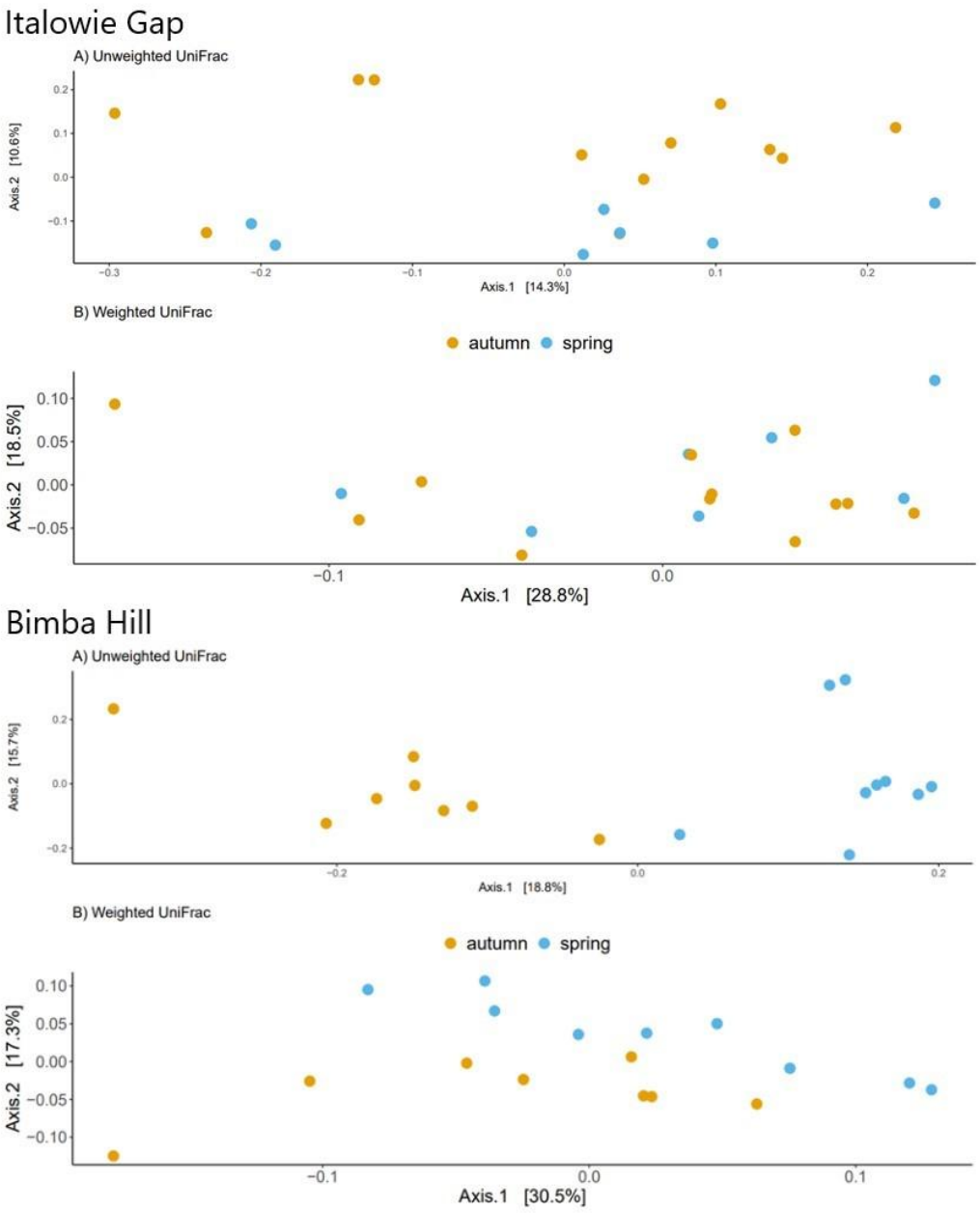


Figure 6: The Beta diversity of yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) faecal microbes from two different sites, Italowie Gap (*top*) and Bimba Hill (*bottom*)**,** sampled during spring 2021 (blue) and autumn 2022 (orange) using Unweighted UniFrac (*top*) and Weighted UniFrac (*bottom*) distances.

Intra-individual Variation:

Faecal microbial samples were collected from nine individuals across multiple sampling periods, allowing for variations within individuals to be examined. Intra-individual microbial composition at Bimba Hill appeared to be variable and included more variation than that observed in the Italowie Gap samples, where microbial composition was comparatively more stable between sampling periods (see Appendix 5, Figure S1 pg. 230). Individuals from Bimba Hill showed a slight trend towards having a lower relative abundance of *Bacteroidota* and higher abundance of *Firmicutes* species during spring 2021 compared to autumn 2022 (see Appendix 5, Figure S1 pg. 230), as was also observed when examining the population as a whole (Figure 3).

The only individual which showed a major variation in faecal microbial relative abundance was 'Pumpkin' at Bimba Hill (see Appendix 5, Figure S1 pg. 230), where there was a significant decrease in *Bacteroidota* species during the second sampling (autumn 2022) compared to the first sampling (spring 2021). Due to the lack of change in Pumpkin's microbial richness (Figure 7), this suggests that microbial composition was different between sampling periods, with '*Firmicutes – Other*' becoming more abundant in autumn 2022 (Figure 7; Figure 8).

Variation in microbial richness was also observed for other individuals across both study sites although they were not evident when examining relative abundance (Figure 7; see Appendix 5, Figure S1 pg. 230). The three individuals which showed high variation in ASV richness throughout the sampling periods were 'Eileen' (Italowie Gap), 'Pandora' (Bimba Hill) and 'Ringo' (Italowie Gap) (Figure 7).

Samples were clearly grouped by location when using Unweighted UniFrac distances along axis 1 and 2 (Figure 8). However, along axis 2 and 3 samples were grouped by individual variation (Figure 8). This further indicates that variation in microbial composition is driven by location.

No significant differences in microbial composition or diversity were detected between the sexes (see Appendix 5, Figure S1; Figure S2 pg. 231-232).

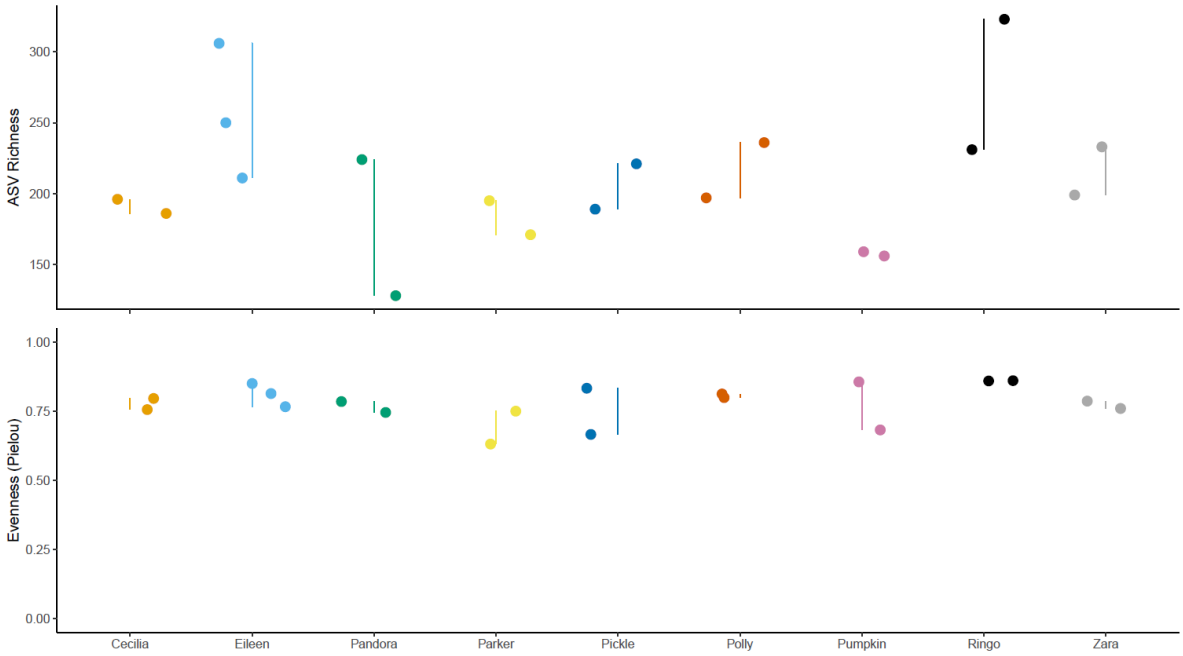


Figure 7: The differences in faecal microbial Alpha diversity within individual yellow-footed rock-wallabies (*Petrogale xanthopus xanthopus*) sampled across autumn 2021, spring 2021, and autumn 2022, in relation to microbial ASV richness (*top*) and evenness (*bottom*). Matching coloured points represent data from the same individual with the variation between sampling of that individual indicated with a vertical line.

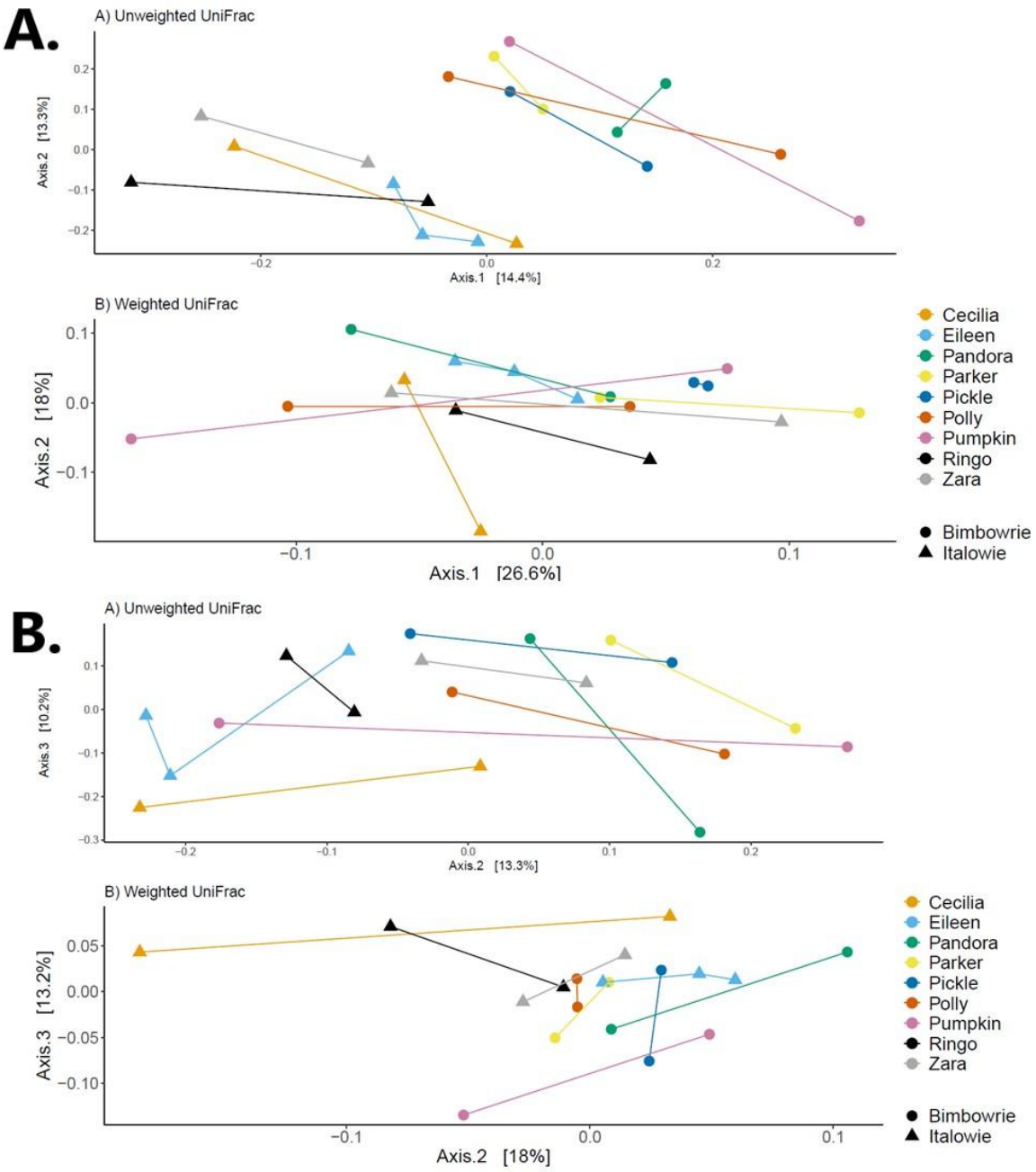


Figure 8: The differences in faecal microbial composition of yellow-footed rock-wallabies (*Petrogale xanthopus xanthopus*) which were sampled during autumn 2021, spring 2021, and autumn 2022 from Italowie Gap (Δ) or Bimba Hill (O) using Unweighted UniFrac (*top*) and Weighted UniFrac (*bottom*) distances. (a) Axis 1 vs 2, (b) Axis 2 vs 3. Each colour represents an individual, with differences in microbial composition between sampling indicated with a line.

Dietary Influences:

Italowie Gap:

Matched faecal microbiome and diet samples (n = 16) were collected from a total of 13 individuals across both sites to examine the interaction between these factors.

The average number of plant species detected across all individuals at each time point was nine in autumn 2021, eight in spring 2021, and nine in autumn 2022. Although the diet of individuals varied across the sampling period, *Acacia* species and *Portulaca oleracea* were detected regularly (Figure 9). In general, *Acacia* species were detected at higher proportions during spring 2021, whereas *P. oleracea* typically occurred at higher proportions during autumn sampling periods (Figure 9).

In the paired microbiome samples, the main phylum of microbiota detected were *Bacteroidota* and *Firmicutes*, with a small contribution from *Verrucomicrobiota*. During both autumn sampling periods (2021 and 2022), the proportion of *Firmicutes* seemed to decrease slightly in comparison to spring (2021) but this decline was not statistically significant. This appeared to be related to increased *Acacia* species detection rates, suggesting there may be a relationship between increased *Acacia* intake and increased *Firmicutes* presence (Figure 9), although the sample size is not great enough to support this observable trend statistically.

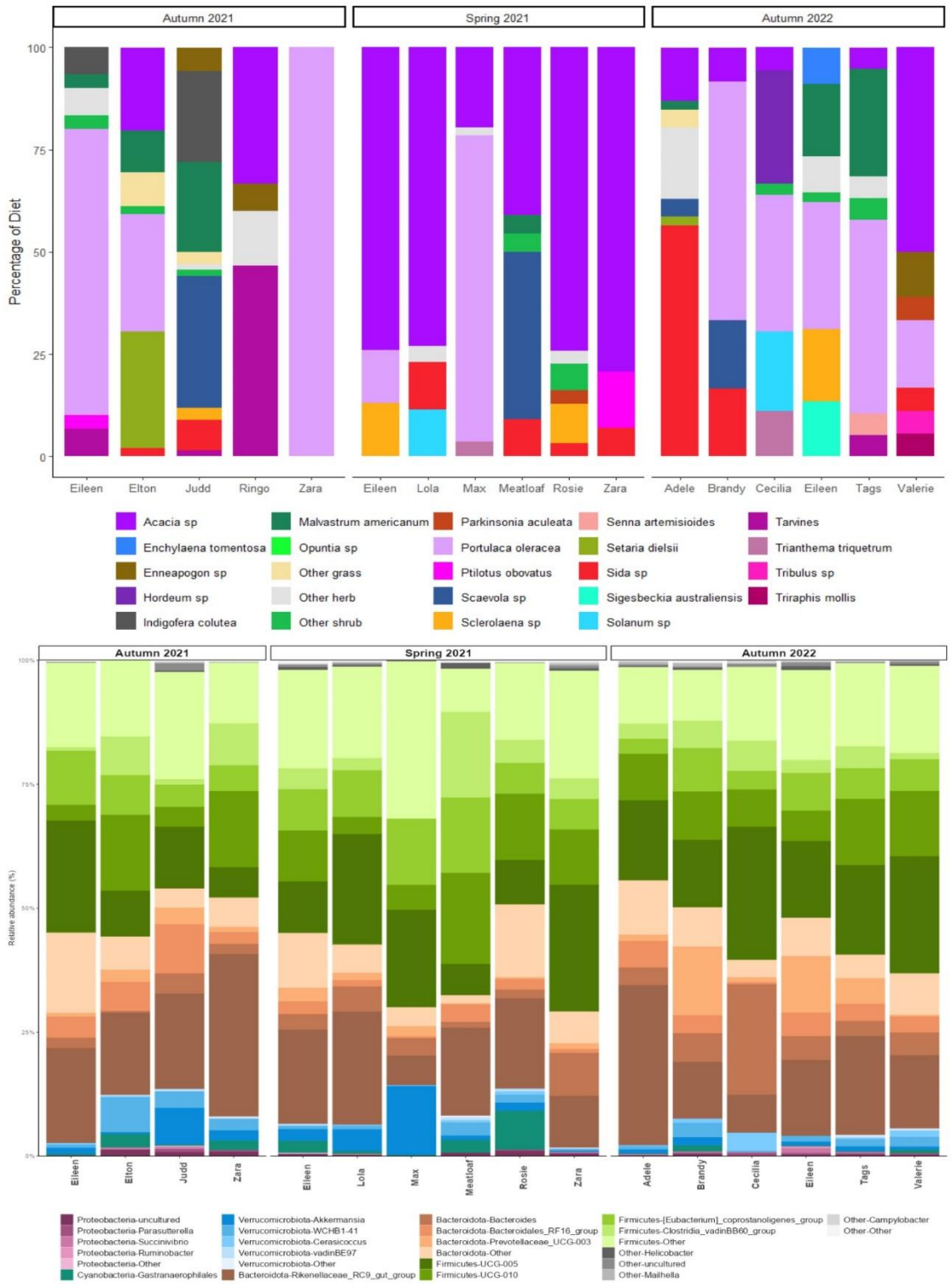


Figure 9: *Top*: The proportion (%) of genetic sequencing reads from plant species that were detected in yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) scats from Italowie Gap during autumn 2021, spring 2021 and autumn 2022. *Bottom*: The relative abundance (%) of faecal microbes detected in scats from the same yellow-footed rock-wallabies (*Petrogale xanthopus xanthopus*), collected from the same location and sampling periods as above.

Bimba Hill:

Matched faecal microbiome and diet samples (n = 8) were acquired from seven individuals at Bimba Hill, with one individual, 'Pumpkin', sampled during two time periods.

During spring 2021, there was an average of nine plant species detected in scat samples in four samples, compared to seven plant species detected from another four samples in autumn 2022. This suggests that there was an increase in dietary diversity during spring 2021.

The detection of individual plant species from YFRWs at Bimba Hill was highly variable during spring 2021, but *Calandrinia eremaea* and *Sclerolaena* species were common. However, during autumn 2022 sequencing reads were dominated primarily by *Acacia* and *Dysphania* species (Figure 10).

In general, the microbial composition of scats at Bimba Hill was similar to that observed at Italowie Gap where *Bacteroidota* and *Firmicutes* were the dominant phyla throughout sampling. However, the increased plant variability in diet samples during spring 2021 appeared to correspond to a higher abundance of *Bacteroidota* taxa, whereas the increase of *Acacia* species in autumn 2022 diet samples, appeared to coincide with an increase in *Firmicutes* microbes (Figure 10).

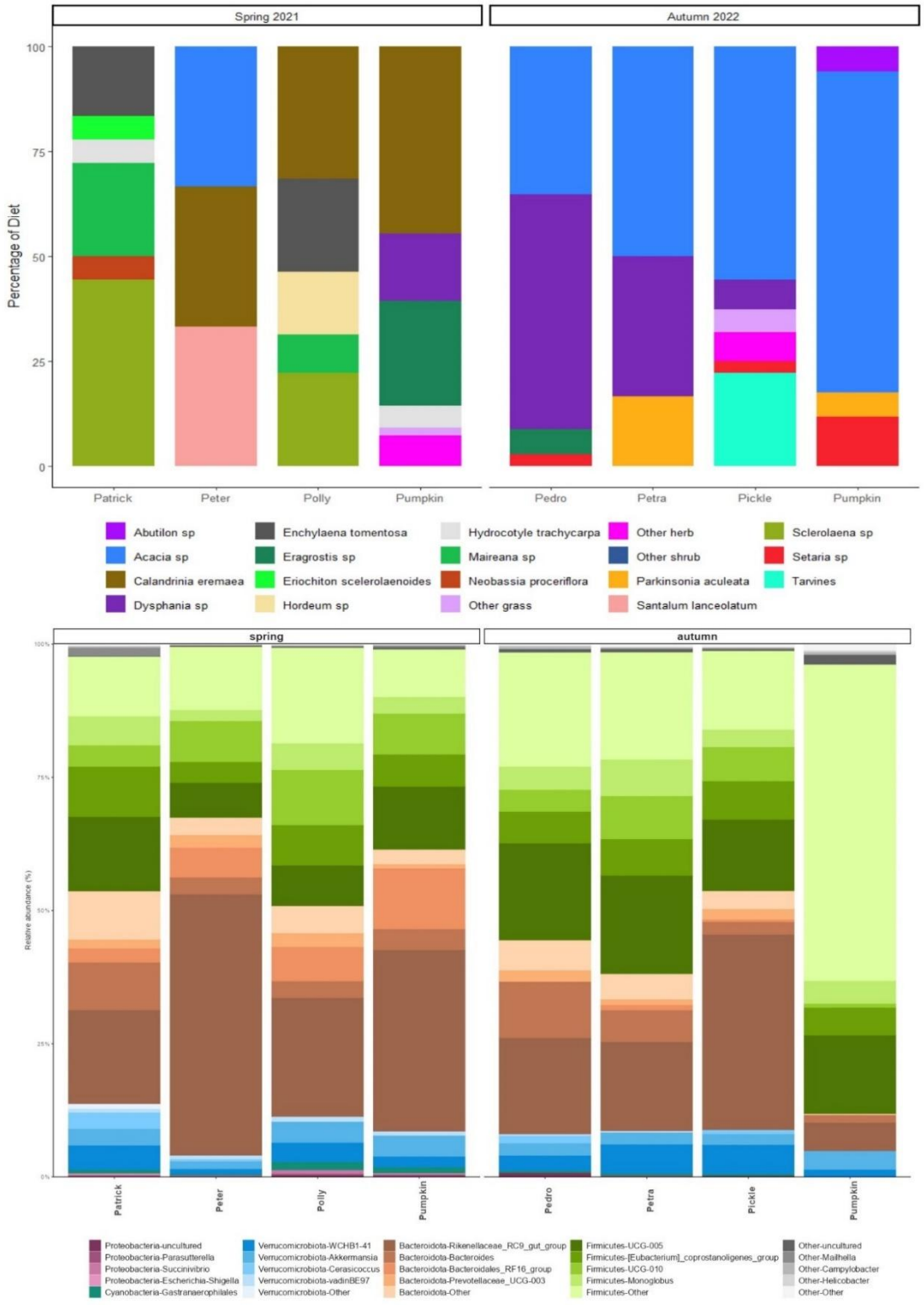


Figure 10: *Top*: The proportion (%) of genetic sequencing reads from plant species that were detected in yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) scats from Bimba Hill during spring 2021 and autumn 2022. *Bottom*: The relative abundance (%) of faecal microbes detected in scats from the same yellow-footed rock-wallabies (*Petrogale xanthopus xanthopus*), collected from the same location and sampling periods as above.

Discussion:

The importance of conserving the gut or faecal microbiome in wildlife has only recently been recognised (Banerjee *et al*. 2020), with few studies previously undertaken examining marsupials (Chhour *et al*. 2010; Cheng *et al*. 2015; Brice *et al*. 2019; Eisenhofer *et al*. 2021; Blyton *et al*. 2023; Eisenhofer *et al*. 2023). The faecal microbiome of the YFRW has not been characterised previously. Here we examined the faecal microbiome of this species across two geographically distinct populations, each with different habitat characteristics (Italowie Gap = connected, Bimba Hill = isolated). Due to their geographic separation, we also aimed to assess individual variation in microbial diversity and composition in response to season/rainfall, and how dietary composition might influence these parameters.

The dominant microbial phyla detected in all scat samples were *Firmicutes* and *Bacteroidota*, which is similar to that previously observed in other mammalian studies (Ley *et al*. 2008; Brice *et al*. 2019; Eisenhofer *et al*. 2021; Awosile *et al*. 2023). In the SHNW, another semi-arid dwelling marsupial, the faecal microbiome was consistently comprised of >70% *Firmicutes* and *Bacteroidota* species, but also included microbial species absent in YFRW samples (Eisenhofer *et al*. 2021). SHNWs however, utilise hindgut fermentation to access SCFAs, potentially explaining these differences.

This study found that most of the variation in microbiome diversity was explained by location, where microbial richness was higher at Italowie Gap compared to Bimba Hill, and microbial composition was also significantly different between sites. Habitat quality and type have previously been described as drivers of faecal microbial diversity in other species, including the black howler monkey (*Alouatta pigra*; Amato *et al*. 2013) and African elephants (*Loxodonta* species; Budd *et al*. 2020), and is often associated with differences in dietary intake.

Dietary differences between our study sites are likely driven by geographical variation in climate, rainfall, and soil type, altering vegetation community structure, are likely influencing the location-based variation in faecal microbiome observed in this study. In a study conducted on koalas (*Phascolarctos cinereus*; Brice *et al*. 2019), variation in faecal microbial composition was observed to be highly correlated with diet. While such a statistical correlation was not possible in the current study, and further research with a greater sample size is needed, a general trend was observed, where an increase in *Firmicutes* species occurred when *Acacia* species were detected in high proportions in diet samples. *Acacia* species, which are within the Family *Leguminosae*, utilise symbiotic bacteria within the root system to fix soil nitrogen, contributing to elevated protein accumulation within the plant tissue (Irlbeck & Hume 2003; Morton 2022). However, high levels of tannins are also present in the foliage which blocks microbial fermentation of plant protein, restricting the availability of nutrients (Gartner & Hurwood 1976; McSweeney *et al*. 2008). For this reason, *Acacia* species are not a food source preferred by many macropodids, and typically is only eaten during drought when

resource availability is low, where dead foliage with low tannin content is ingested (Irlbeck & Hume 2003). As it has been previously found that a high carbohydrate or protein diet can increase the proportion of *Firmicutes* species present in the faecal microbiome (De Filippo *et al*. 2010; Zhang *et al*. 2020), the increased proportion of *Firmicutes* in this study observed during autumn 2022 at Bimba Hill may be attributed to the higher proportion of *Acacia* detected in diet samples.

One individual ('Pumpkin') from Bimba Hill had a markedly higher proportion of *Firmicutes* present during their second sampling in autumn 2022, compared to sampling in spring 2021. This also corresponded to a major dietary shift where in spring 2021 a variety of herbaceous and grass species (i.e. *Calandrinia eremaea*, *Dysphania* species, *Eragrostis* species) were detected in the sample, but during autumn 2022, *Acacia* species were the dominant plants detected.

'Pumpkin' was an adult female YFRW in above average body condition and had a young in pouch during both sampling times. No obvious pathologies or underlying health conditions were observed during either sampling time. While a decrease in body weight, waist circumference, and body mass index has been correlated with an increase in *Bacteroidetes* in humans (Fava *et al*. 2013), Pumpkin experienced no notable weight change between samplings (<200g difference). As microbial richness for this individual did not change markedly, the shift in microbial composition indicates that there was a change in the dominant microbes present between sampling times. This was further supported by the high detection rate of *Acacia* species in diet samples at Italowie Gap in spring 2021 also showing a slight increase in *Firmicutes* during this sampling period. This suggests that geographical variations in rainfall which influences vegetation diversity and abundance, and the impact of habitat composition on vegetation structure, are more than likely influencing the dietary composition of YFRWs, which also appears to be driving variation in faecal microbial composition.

An additional potential cause of this microbial variation may be neutral allopatric speciation. This process reduces the ability of microbes to be shared amongst populations if there is geographic separation due to random genetic mutations of the microbial genome and co-speciation with their host occurs (Groussin *et al*. 2020). The two populations in this study are separated by a large geographic distance (~180km), and YFRWs are not known to migrate such large distances, with most migration events occurring over smaller distances (<13km; Potter *et al*. 2020; Smith *et al*. 2023a). As only approximately 54% of microbial ASVs are shared between populations, neutral allopatric speciation may well be influencing the microbial communities at these sites.

Conclusions:

The results of this study suggest that the YFRW faecal microbiome is highly influenced by their geographical location. YFRWs from Italowie Gap had higher faecal microbial diversity than was

observed at Bimba Hill, and microbial composition was markedly different between sites throughout. As only 54% of ASV were shared between sites, this suggests that microbial diversity and composition are mostly site-specific, likely influenced by geographical separation and local rainfall. This information may be important to consider for informing conservation efforts as there are underlying unique biological characteristics occurring within the species throughout its distribution. This may be particularly pertinent if future translocation programs were conducted between regions of YFRW habitation, or if the historic link between the Flinders and Olary Ranges populations were ever to be re-established. Now the YFRWs faecal microbiome has begun to be characterised, there is potential for faecal transplants to be developed for use as a conservation tool for this species, but the efficacy of faecal transplants and the spatial scale of microbial variation would need to be tested more thoroughly before implementation.

While diet appeared to have an effect on the YFRW faecal microbiome, a more in-depth investigation, including a higher sample size allowing for statistical analysis, would be needed to establish the correlation between faecal microbiome and diet. A trend between *Acacia* species and increased *Firmicutes* was observed (Italowie Gap in spring 2021, Bimba Hill in autumn 2022), and may be driven as a response to having a 'protein-rich' diet. This is worth further investigation, particularly if YFRWs are more reliant on *Acacia* species during dry/drought times. Additionally, how changes in microbial diversity and composition can affect YFRW health are unknown, and would require further monitoring and research.

This is particularly pertinent when, due to climate change, the frequency, severity and duration of droughts are predicted to increase, potentially altering, and decreasing plant diversity and abundance, and thus altering the YFRW diet. On-going monitoring of YFRW faecal microbiome, diet, and health parameters will assist in informing the future conservation management strategies for this species.

Data Availability:

Raw microbial DNA sequencing data are publicly available at NCBI as bio-project PRJNA1110055. Code for reproducing the microbiome analyses is available at https://github.com/EisenRa/2024_YFRW_faecal.

Chapter Five

"Habitat characteristics and the future distribution of the yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*)"



"*They leapt and clambered up among the steep sides of the cliffs in a manner quite incredible, and where it was perfectly impossible for any human to follow them*"

– Eyre 1841

Statement of Authorship

Title of Paper	Habitat characteristics and the future distribution of the yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*)
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☑ Unpublished and Unsubmitted work written in manuscript style
Publication Details	To be prepared for journal submission on PhD completion.

Principal Author

Name of Principal Author (Candidate)	Lauren Werner		
Contribution to the Paper	Study design, field surveys, computer model development, statistical analyses, writing manuscript.		
Overall percentage (%)	80		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	14/10/2024

Co-Author Contributions

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

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

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Signature		Date	22/10/2024

Habitat characteristics and the future distribution of the yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*)

Werner L.E., Kleemann S.L, Taggart D.A, Ostendorf B.

Abstract:

Predictive modelling of range contractions or expansions in response to climate change are becoming a vital component of wildlife conservation programs, and rely on our understanding of the species' distribution. This project used a two-tiered approach to develop a species distribution model for the yellow-footed rock-wallaby (YFRW; *Petrogale xanthopus xanthopus*) using scat surveys (a detailed, continuous density indicator) and regional animal presence-only data (Atlas of Living Australia). We initially used scat survey data collected from the Flinders/Gammon and Olary Ranges, South Australia, to examine the environmental characteristics most indicative of YFRW habitation. Significant habitat characteristics were then used to inform the input parameters for species distribution modelling (SDM) of the current distribution and predicted future distribution under climate change using regional species observation data from the Atlas of Living Australia. Mean annual temperature and terrain ruggedness were most strongly related to YFRW habitation, with the niche of YFRW habitation most evident at 18^{o}C and at terrain ruggedness values of 15 or greater (index values). However, significant terrain ruggedness differences were observed between regions, suggesting that habitat complexity varies considerably across the YFRWs distribution. Consequently, terrain ruggedness and climatic variables (mean annual temperature and annual precipitation) were used as explanatory variables in the SDM. The SDM showed constriction of the YFRWs distribution into highly suitable habitat with high connectivity, such as that in the northern Flinders/Gammon Ranges. Increased habitat suitability was also observed in the southern Flinders Ranges, a higher rainfall region. Peripheral populations (Gawler/Olary Ranges and New South Wales) were predicted to become highly contracted, restricting population growth and expansion in these regions. The area of suitable YFRW habitat is predicted to decline as climate change progresses, and a range of conservation management actions will be needed, especially in peripheral populations, to ensure the species' future viability. Additionally, continued monitoring of YFRW populations and detailed investigations into habitat use in different geographical regions will be vital to understand and manage the effects of future distribution shifts.

Introduction:

Modelling techniques used for predicting a species' distribution have considerable potential to guide management actions and resource allocation for threatened, conservation dependent, wildlife species. Globally, these techniques have been widely applied to conservation programs (Lawler *et al*. 2011; Domisch *et al*. 2019; Swan *et al*. 2021; Rathore & Sharma 2023) and have recently gained prominence in helping plan future conservation strategies in areas where the species' distribution is likely to be altered due to the environmental effects of climate change (Hill *et al*. 2001; Crozier 2003; Hitch & Leberg 2007; La Sorte & Thompson 2007; Guisan *et al*. 2013).

In Australia, climate change is predicted to cause increased average temperatures, lower annual rainfall, a higher frequency and duration of droughts, and more extreme climatic events (Head *et al*. 2014; State of the Climate 2022, www.bom.gov.au/state-of-the-climate/). These climatic changes threaten to displace sensitive wildlife species by altering their habitat characteristics and causing changes in behaviour patterns, forcing them out of their original distribution range (Walther *et al.* 2001; 2002; Parmesan 2006; Rej & Joyner 2018).

Some fauna and flora species in Australia, such as cane toads (*Bufo marinus*; Kearney *et al.* 2008), an endangered rainforest plant (*Triunia robusta*; Shimizu-Kimura *et al*. 2017), fruit flies (*Bactrocera* species; Sultana *et al*. 2020), and several lizard species (*Pogona* species; Rej & Joyner 2018) have already been suggested to be changing their distribution as climate change progresses. A species' ability to adapt to climatic and environmental changes has previously been linked to habitat quality, with some habitats conferring greater protection to changes than others (Smith *et al*. 2023b). As a consequence, habitat specialists, such as the koala (*Phascolarctos cinereus*; Adams-Hosking *et al*. 2012), the platypus (*Ornithorhynchus anatinus*; Klamt *et al*. 2011), the southern hairy-nosed wombat (*Lasiorhinus latifrons*; Marshall *et al*. 2018), and the mountain pygmy possum (*Burramys parvas*; Broome *et al*. 2013; Gibson *et al*. 2018) have been identified to be at risk of major range contractions due to climate change, predominantly due to shifts in vegetation diversity and temperature increases.

Another habitat specialist, and the focus of this study, is the yellow-footed rock-wallaby (YFRW; *Petrogale xanthopus xanthopus*). The YFRW is a semi-arid adapted, medium-sized macropodid marsupial currently classified as Vulnerable (EPBC classification; Woinarski *et al*. 2014; Baker & Gynther 2023). This species is found in South Australia and New South Wales, typically in and around complex rock formations, comprised of crevices, caves and rock-jumble niches, necessary for protection from predation and the harsh climatic extremes (Wilson *et al.* 1976; Copley 1981; Short 1982; Copley 1983; Lim *et al*. 1987; Ruykys 2017; Baker & Gynther 2023; Mallam *et al*. 2023). However, YFRW habitat characteristics vary markedly across the species' distribution, from extensive, steep, rocky gorges or ranges, comprised of highly connected habitat in the Flinders/Gammon Ranges, to much lower, flatter, isolated rocky outcrops with complex rock-jumbles

in the Olary Ranges (Copley 1981; 1983). Due to the variability in YFRW habitat, it is likely that the environmental attributes which correspond to YFRW presence differ across their distribution, however, this has not been investigated previously.

This study aimed to determine the environmental characteristics that correspond to YFRW presence to build a better understanding of their optimal habitat, and to identify currently inhabited areas that are threatened by climate change. This was conducted using a two-tiered model development process where, firstly, detailed scat survey data was used to identify the optimal environmental characteristics for use in species distribution modelling (SDM). Secondly, the informed species distribution model was used to examine the current YFRW distribution and assess how habitat in different geographical regions may be impacted by future climate change.

Methods:

Survey Locations and Methodology:

This study was conducted at two spatial scales: 1. detailed scat surveys at selected sites in the Flinders, Gammon, and Olary Ranges, South Australia, and, 2. broad-scale observations from YFRW presence data obtained from the Atlas of Living Australia (ALA; https://www.ala.org.au). Fine-scale YFRW scat surveys allowed for detailed data collection on both YFRW presence and absence, enabling investigation into the environmental characteristics that are indicative of species' habitation. Broad-scale ALA datasets consist of presence records which are inherently biased since species observations are more likely in accessible areas (e.g. roads, tracks) or highly-visible locations (e.g. high terrain in helicopter surveys). As such, absences must be inferred statistically, as a lack of presence observations does not necessarily mean that the species is absent from that area.

The broad-scale ALA presence data, the locations of our detailed scat surveys, and the area of distribution modelling are detailed in Figure 1, and the location of each geographical region referred to in this study are detailed in Figure 2 (scat survey) and Appendix 6 (SDM; see Figure S1 pg. 233).

The modelling process in this study involved six steps:

1. Preparation of informative environmental layers for input into models.
2. Preparation of fine-scale scat survey data.
3. Development of statistical models using regional scat survey data.
4. Preparation of broad-scale presence-only data from the Atlas of Living Australia.
5. Development of a species distribution model based on statistical modelling results using broad-scale presence data.
6. Future climate change analysis.

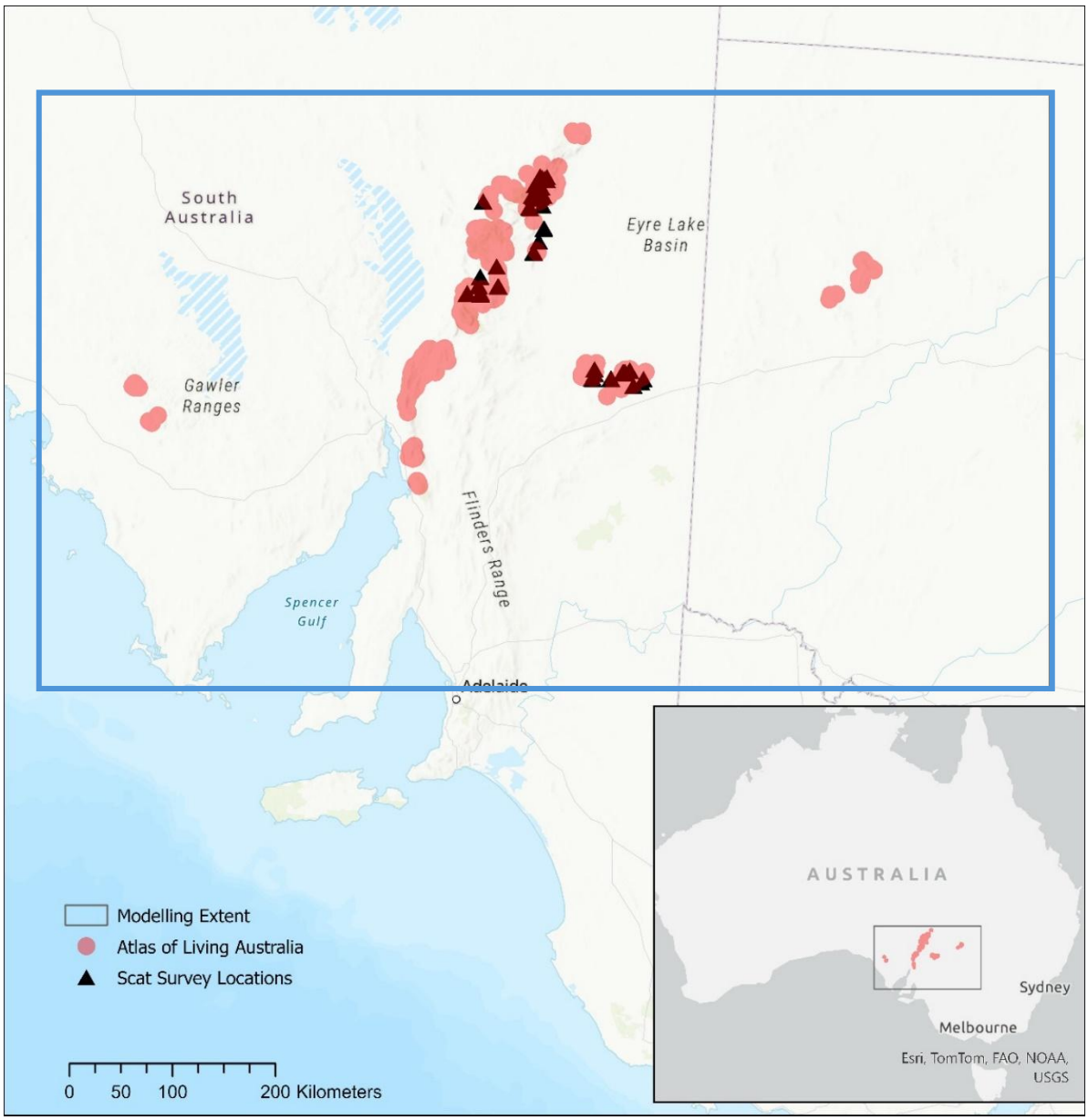


Figure 1: A map showing the distribution of yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) observations from the Atlas of Living Australia (ALA; red ○) in South Australia and New South Wales, and the location of scat surveys conducted for this study (▲) in South Australia. The predictive Maxent modelling extent of the current and future yellow-footed rock-wallaby distribution is indicated by a blue outline. *Inset*: A map of Australia indicating the extent of the modelling area (black outline box) used in this project and ALA presence data (red).

Preparation of Informative Environmental Layers for Input into Models:

Environmental layers were sourced for the modelling extent area, encompassing South Australia and New South Wales, Australia (Figure 1). A digital elevation model (DEM) was obtained from JAXA's (Japan Aerospace Exploration Agency) online Global Digital Surface Model (AW3D30) at 30 metre resolution. DEM tiles were mosaiced and projected into the 'GDA 1994 Geoscience Australia Lambert' coordinate system.

The JAXA DEM was used to compute slope, aspect, terrain ruggedness, and solar radiation (summer and winter). Summer solar radiation was calculated from December 2023 to February 2024, and winter solar radiation from June 2023 to August 2023. Both solar radiation layers were developed using the input parameters of 'uniform sky' with a diffuse proportion of 0.3 and transmittivity of 0.5. Climate layers were downloaded from WorldClim (www.worldclim.org) for historic/current climate data (Fick & Hijmans 2017) and for future climate predictions under the Coupled Model Intercomparison Projects 6 (CMIP6). WorldClim historic/current climate data is based on the average values of bioclimatic factors from 1970 to 2000. Annual mean temperature (BIO1) and annual precipitation (BIO12) were used as general climate indicators, and were provided at 30 metre (30sec) resolution for historic/current and future predictions.

Preparation of Fine-scale Scat Survey Data:

Scat surveys were conducted throughout the Flinders, Gammon and Olary Ranges, South Australia, during November and December 2022 (Figure 2). A total of 38 sites were surveyed: 20 in the Gammon and northern Flinders Ranges, nine in the central Flinders Ranges, and nine in the Olary Ranges (Figure 1; Figure 2). Surveying was conducted by two or three researchers per site and surveying effort ranged from 45 minutes at small sites with little sign of YFRW occupation, to 5 hours at extensive sites with high YFRW occupation. Time spent at each site also varied depending on habitat area and topography. A representation of surveying effort is displayed in Figure 2.

Scat surveys were conducted by walking random transects across an area of either known or unknown YFRW occupation which showed signs of supporting a colony (i.e. rocky, rock fissures/jumbles, steep cliffs). If a YFRW scat was encountered, it was GPS marked and the number of YFRW scats within a 1 x 1 metre area were counted and recorded. A minimum of five metres between records was maintained, regardless of scat presence or absence. Efforts were made to ensure that the full variety of habitat characteristics at each site were sampled from.

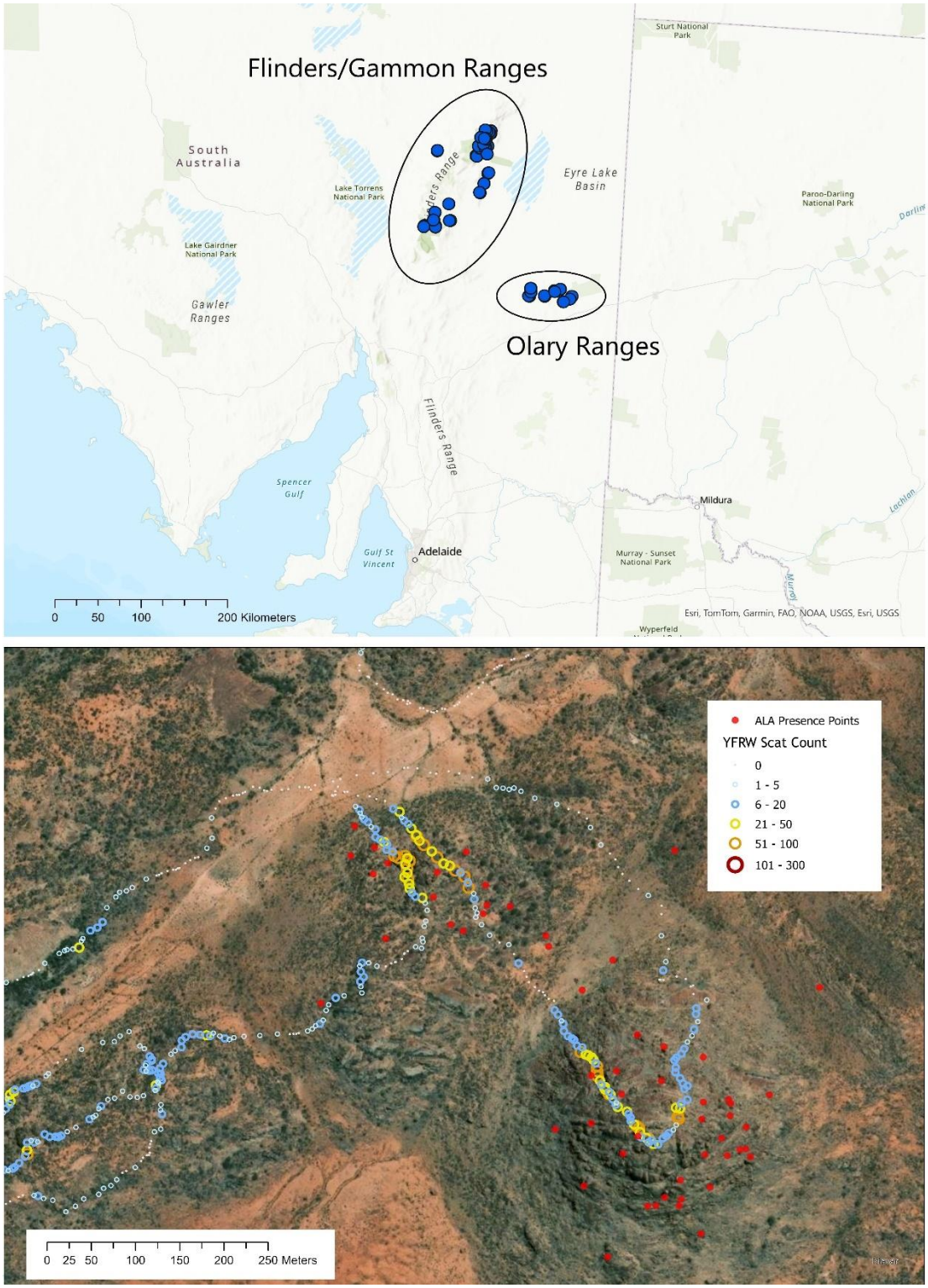


Figure 2: *Top:* The location of yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) scat surveys which are classified into 'Flinders/Gammon Ranges' and 'Olary Ranges' regions. *Bottom:* A representation of yellow-footed rock-wallaby scat surveying effort at a single site, with the number of scats observed at each point indicated (○, differing colour and size), and species observation points obtained from the Atlas of Living Australia (red ●) for the same area.

Identification of scat absences was done using track data from the GPS, where each track has presence and absence data combined. Because more time was spent traversing difficult terrain, track points have a higher density in these areas, requiring the development of customised filter processes. GPS track points were considered absence locations if they were >10 metres away from a scat presence point. Additionally, absence points were thinned out using an iterative process that randomly samples one quarter of absences, and randomly removes points that are within 2.5 metres of another, until <1% had a neighbouring point within 2.5 metres. Presence and absence data was then re-combined for analysis.

To minimise spatial autocorrelation, counts were consolidated to match the pixel size of the environmental layers, producing a single mean count of scats for each pixel (i.e. averaging scat counts of presences and absences within each 30 x 30m pixel). This resulted in a spatial dataset where each pixel that was traversed during surveying become a sample point for statistical modelling (Table 1), from here on referred to as 'samples'. The units of samples are in numbers of scats per m^2. At each sample location, we then extracted the DEM, ruggedness, slope, aspect, solar radiation, and climate values of the pixel.

After importing GPS data, there was 3,540 presence points and 10,228 absence points from the entire survey area. After consolidating scat counts to pixel size, there was 1,136 presence and 2,105 absence samples, resulting in 3,241 samples available for statistical modelling (Table 1). This corresponded to 565 presence and 1,398 absence samples from the Flinders/Gammon Ranges region, and 571 presence and 707 absence samples in the Olary Ranges region (Table 1). A detailed description of the raw number of scat counts in each region are provided (see Appendix 6, Table S1 pg. 234).

Table 1: Yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) scat survey data obtained from the Flinders/Gammon and Olary Ranges, South Australia. 'GPS total' relates to the number of raw data points acquired from GPS waypoints and tracks during surveying. 'Total samples' is the average scat count within a 30 x 30 metre pixel. A breakdown of the number of presence and absence samples for each study region (Flinders/Gammon Ranges and Olary Ranges) is also provided.

	GPS Total	*Total Samples*	*Flinders / Gammon Ranges Samples*	*Olary Ranges Samples*
Presence	3,540	1,136	565	571
Absence	10,228	2,105	1,398	707
Total Points Used	13,768	3,241	1,963	1,278

Development of Statistical Models Using Regional Scat Survey Data:

Several generalized additive models (GAM) were examined to evaluate the environmental factors associated with YFRW habitation. Because of high correlation between variables (see Appendix 6, Figure S2 pg. 236), we initially assessed models using a single environmental variable. We then added 'region' to this model to assess geographical differences in the YFRWs distribution, and to compare model performance based on the amount of deviance explained. The two variables which were deemed to have the highest model performance (i.e. deviance explained) were then used to create an interaction (tensor product) 'base model' for evaluating multivariate models. Multivariate models were constructed from the 'base model' with the addition of a single environmental variable. Multivariate models were also assessed with either the exclusion or inclusion of 'region' to test geographic differences across the YFRW distribution.

All GAMs were developed using the *mgcv* (Wood 2003; 2004; 2011; 2017; Wood *et al*. 2016) and *mgcVis* (Fasiolo *et al*. 2020) packages, where the logarithmic value of scat count samples was used as the response function of a smoothed (*s*) or tensor (*te*) product model using the 'REML' methodology and 'Tweedie' family. All default modelling parameters were used.

Preparation of Broad-scale Presence-only Data:

Presence data for the *P. x. xanthopus* species was acquired from the Atlas of Living Australia (ALA; https://www.ala.org.au; Data DOI: https://doi.org/10.26197/ala.bdbb0e4f-c1c9-4e8a-8456-55b36760c135). The original ALA dataset consisted of 8,690 species occurrence observations, which

encompassed the whole *P. xanthopus* distribution in Australia. The data was cleaned by removing data points that were not within the known YFRW distribution, observations on roads or within towns or homesteads, records >60 years old, and points generated from tissue collections. Records of the *P. x. celeris* subspecies in Queensland were also removed as this project was focussed on the *P. x. xanthopus* subspecies in South Australia and New South Wales. A total of 2,893 occurrence records were removed during cleaning, leaving 5,797 occurrence records.

For use in Maxent (Phillips *et al*. 2004; 2006) species distribution models (SDM), the ALA dataset was spatially thinned (using 'Delete Identical' geoprocessing tool in ArcGIS Pro) where points were randomly removed if within a one-kilometre radius from another point to reduce spatial autocorrelation, sampling bias and model overfitting. This process removed 5,335 points from the ALA dataset, resulting in 462 presence points available for use in the Maxent SDM.

Species Distribution Models with Broad-scale Presence-only Data:

A Maxent species distribution model for the current distribution of YFRWs was developed using ArcGIS Pro's 'Presence-only Prediction (MaxEnt)' tool. The cleaned and spatially thinned ALA species occurrence points were used as the input point feature, with the environmental variable of terrain ruggedness (informed by statistical analysis of scat survey samples) and climatic conditions (BIO1 and BIO12) as explanatory variables. We used linear, quadratic and product basis functions, a relative weight of 100 for presence to background points, the C-log-log link function, and 0.5 as the presence probability cut-off. Random cross-validation was used where data was separated into five groups where one group was used to test the performance of the model after training.

After the development of the current species distribution model, the 'Predict using Spatial Statistics Model File' tool in ArcGIS Pro created future prediction rasters, using terrain ruggedness and future climate scenarios as explanatory variables.

Future Climate Change Analysis:

The bioclimatic factors of BIO1 and BIO12 were used for future climate change predictions from the CMIP6's global circulation model (GCM) IPSL-CM6A-LR (Boucher *et al*. 2018). We used scenarios for shared socioeconomic pathways (SSP) 2-4.5 and SSP5-8.5 corresponding to moderate and high carbon emission scenarios, respectively, for the twenty-year period of 2081-2100.

Results:

Statistical Models:

The first series of GAMs were conducted with two models: a single smoothed (*s*) environmental parameter, and then the same model with 'region' added. For the first model with only a single environmental parameter included, model performance (i.e. deviance explained) was low to moderate, with 'ruggedness' explaining the highest deviance (26.6%) and aspect the least (2%) (Table 2). In the second model when 'region' was included, the performance of most models improved. This increase was most significant for ruggedness (+ 16.7% deviance explained). All test model showed that 'region' significantly influenced the model ($p = <0.001$; Table 2), suggesting that YFRW habitat characteristics differ between regions.

These results then informed the development of a 'base model'. As there was a high degree of correlation between ruggedness and slope (see Appendix 6, Figure S2 pg. 236), only one of these parameters was used in the 'base model'. Ruggedness was chosen for inclusion in the 'base model' as it explained the highest deviance. In addition, temperature and elevation were also found to be highly correlated (see Appendix 6, Figure S2 pg. 236). Temperature however, had a slightly higher deviance explained and was therefore selected for inclusion in the 'base model' (Table 2). Solar radiation parameters were disregarded for inclusion in the 'base model' due to their association with aspect, which continually produced low quality models (Table 2). However, solar radiation parameters and aspect were retained for the second stage of GAM model development due to their low correlation with the 'base model' parameters (ruggedness and temperature). Following the aforementioned steps, an interaction 'base model' (*te*[*temperature, ruggedness*]) was developed.

The same methodology utilised in the first series of GAM model testing was also used in this second series of testing, where two models were assessed: 1. the 'base model' with a single smoothed environmental parameter added, and, 2. the same model with 'region' added as a variable. The results of adding a single environmental variable to the 'base model' showed that model performance was only <1% deviance explained added, although, this was deemed statistically significant (aspect p <0.01, solar radiation, winter $p < 0.001$, solar radiation, summer $p < 0.05$; Table 3). When models had 'region' added, performance increased by an average of 5% deviance explained, significantly improving model performance by accounting for the influence of regional differences ($p < 0.001$; Table 3). However, as the addition of any single environmental variable did not markedly increase the performance of the 'base model' (Table 3), it was concluded that the 'base model' with the addition of 'region', *te*(*temperature, ruggedness, by = Region*), was in fact the model with the highest predictability for YFRW presence (model summary statistics included in Appendix 6, Table S2 pg. 237).

Table 2: Generalized additive model results examining the environmental parameters associated with yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) presence in two regions, the Flinders/Gammon Ranges and Olary Ranges, South Australia. 'Deviance explained' is from a model with a single smoothed environmental variable (i.e. *s*[*single environmental variable*]). 'Deviance explained + Region' is from the single parameter model with study region added as a parameter (i.e. *s*[*single environmental variable, by = Region*]). 'Regional difference' is the statistical effect that 'region' has on the model. *** = $p < 0.001$.

Environmental Parameter	Deviance Explained	Deviance Explained (+ 'Region')	Regional Difference
Temperature	17.7%	19.7%	***
Elevation	15.7%	19.8%	***
Ruggedness	26.6%	43.3%	***
Slope	25.9%	39%	***
Aspect	2%	3.4%	***
Solar radiation, winter	13.5%	23.4%	***
Solar radiation, summer	9.3%	18.8%	***

Table 3: Development of a multivariate generalized additive model from a base model (*te*[*temperature, ruggedness*]). 'Deviance explained' is from the base model combined with a single smoothed environmental variable, creating a test model (i.e. [*base*] + *s*[*single environmental variable*]). 'Deviance explained + Region' (Regions = Flinders/Gammon Ranges and Olary Ranges) is from the test model with study region added as a parameter (i.e. [*base, by = Region*] + *s*[*single environmental variable, by = Region*]). 'Regional difference' is the statistical effect that region has on the model. ANOVA *p*-values show a significant difference between the base model and the test model (without 'region' added). * = $p < 0.05$. ** = $p < 0.01$. *** = $p < 0.001$.

		Deviance Explained	Deviance Explained (+ 'Region')	Regional Difference	ANOVA *p-value*
Base Model: te(temperature, ruggedness)		44.2%	49.3%	***	
	+ Aspect	44.5%	49.9%	***	**
	+ Solar radiation, winter	44.8%	49.7%	***	***
	+ Solar radiation, summer	44.9%	49.6%	***	*

By examining the effects plots produced from the model *te*(*temperature, ruggedness, by = Region*), regional differences associated with YFRW habitation were able to be visualised. The presence of YFRWs in both regions were commonly associated with a temperature range of ~17.5-19.5ºC (Figure 3). However, the major difference between the study regions was associated with terrain ruggedness, where YFRW presence in the Flinders/Gammon Ranges was in habitat with ruggedness values ranging from 20-80, whereas in the Olary Ranges, habitat ruggedness values ranged from 0-60 (Figure 3). When both regions were visualised in a single effects plot, it highlighted that suitable YFRW habitat is often in a mean annual temperature zone of 18ºC, and with ruggedness values of 15 or greater (Figure 3).

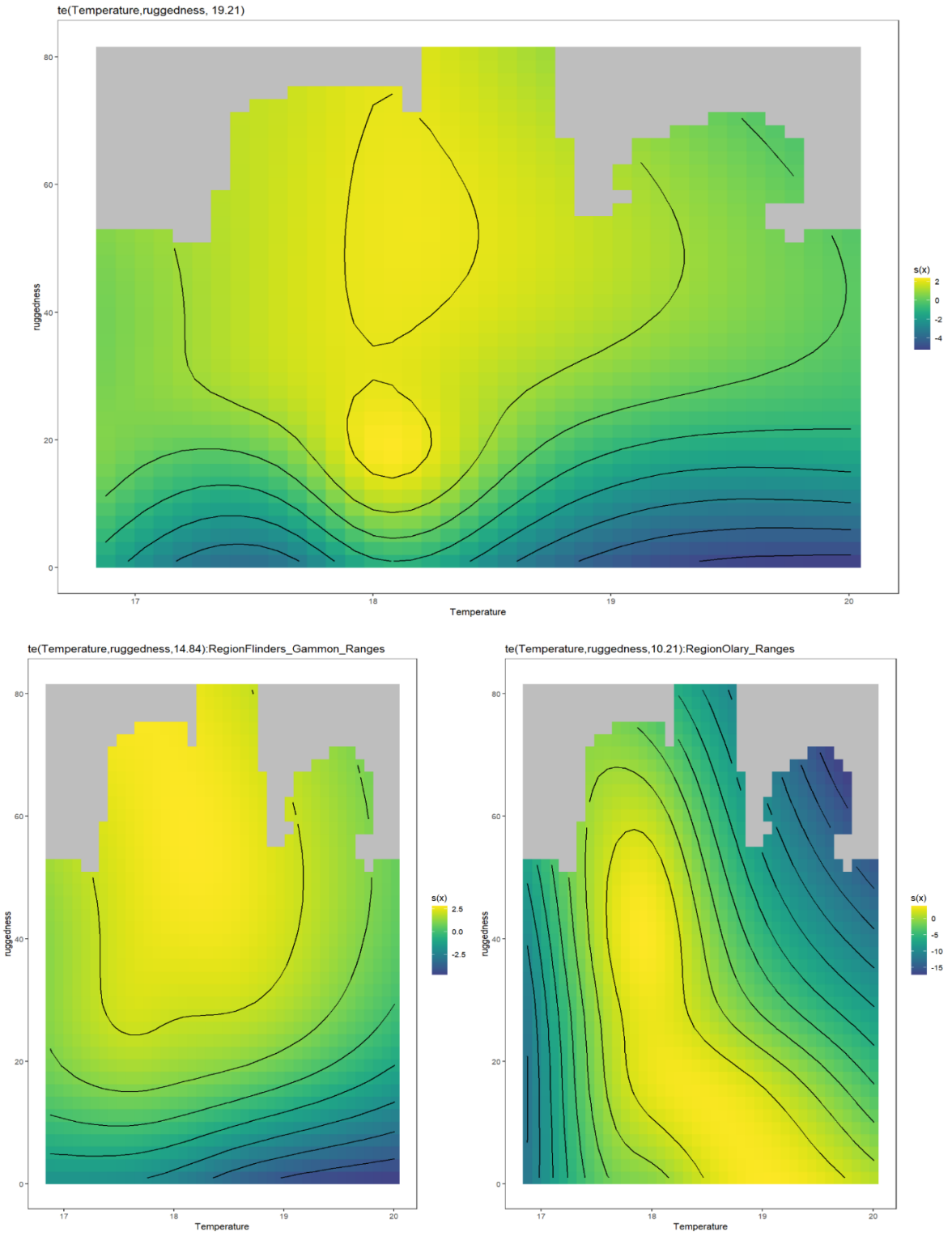


Figure 3: The effects plots of yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) scat presence in relation to mean annual temperature ('Temperature') and terrain ruggedness ('ruggedness') in the Flinders/Gammon Ranges and Olary Ranges regions of South Australia, where a high probability of scat presence is depicted in yellow, and low probability depicted in blue. *Top*: Data from both regions combined. *Bottom left*: Only Flinders/Gammon Ranges data. *Bottom right*: Only Olary Ranges data.

Species Distribution Models:

Findings from the GAM development stage, indicated that only terrain ruggedness should be included as an environmental parameter in the SDM model development stage, with mean annual temperature (BIO1) and annual precipitation (BIO12) used as climate variables. The SDM receiver operating characteristic (ROC) curve of the model produced an area under the ROC curve (AUC) value of 0.9730, indicating a good level of model coverage, and the omission rate was 0.3810 using a 0.5 probability of presence cut-off value (see Appendix 6, Figure S3 pg. 238). The response of ruggedness in the SDM was similar to that seen in the GAM results (see Figure 3) where YFRW presence was commonly associated with ruggedness values of 30-80 (Figure 4). The response of climatic variables showed that probability of YFRW presence was highest in areas with mean annual temperatures (BIO1) of 20-21^{o}C, and annual precipitation (BIO12) between 200-350mm of rainfall (Figure 4).

The YFRW SDM suggested that overall, areas of YFRW habitation are predicted to contract by 2080-2100. This was observed in all regions currently occupied by YFRWs (Figure 5 to Figure 8). SDM results also suggested that the northern Flinders and Gammon Ranges have the biggest area of suitable habitat present with high levels of habitat connectivity. This area is covered by the 'Operation Bounceback' fox baiting program (Figure 6). In comparison, the Olary Ranges and New South Wales populations (both fox controlled) have the least area of suitable habitat with no connectivity between potential habitat sites (Figure 6; Figure 8).

Across all regions of YFRW occupation, areas currently associated with a high probability of YFRW presence remain suitable habitat in the future under both moderate (SSP 2-4.5) and high (SSP 5-8.5) future carbon emission scenarios. However, the areas of highly suitable habitat (75-100% probability of presence) are predicted to increase in the southern Flinders Ranges (Figure 6). SDM results also identified that there are areas which are suitable for YFRW habitation in most regions which are currently not covered by the 'Operation Bounceback' footprint (i.e. not currently fox baited).

The greatest effect on the YFRWs predicted future distribution is in areas of low (25-49% probability of presence) and moderate habitat suitability (50-74% probability of presence). Areas of low or moderate habitat suitability in all regions of YFRW distribution are predicted to undergo substantial contractions or become unsuitable (Figure 5 to Figure 8). Both climate change emission scenarios (SSP 2-4.5 and SSP 5-8.5) predicted similar outcomes across the YFRWs distribution, however, habitat contractions were more severe under SSP 5-8.5 scenario.

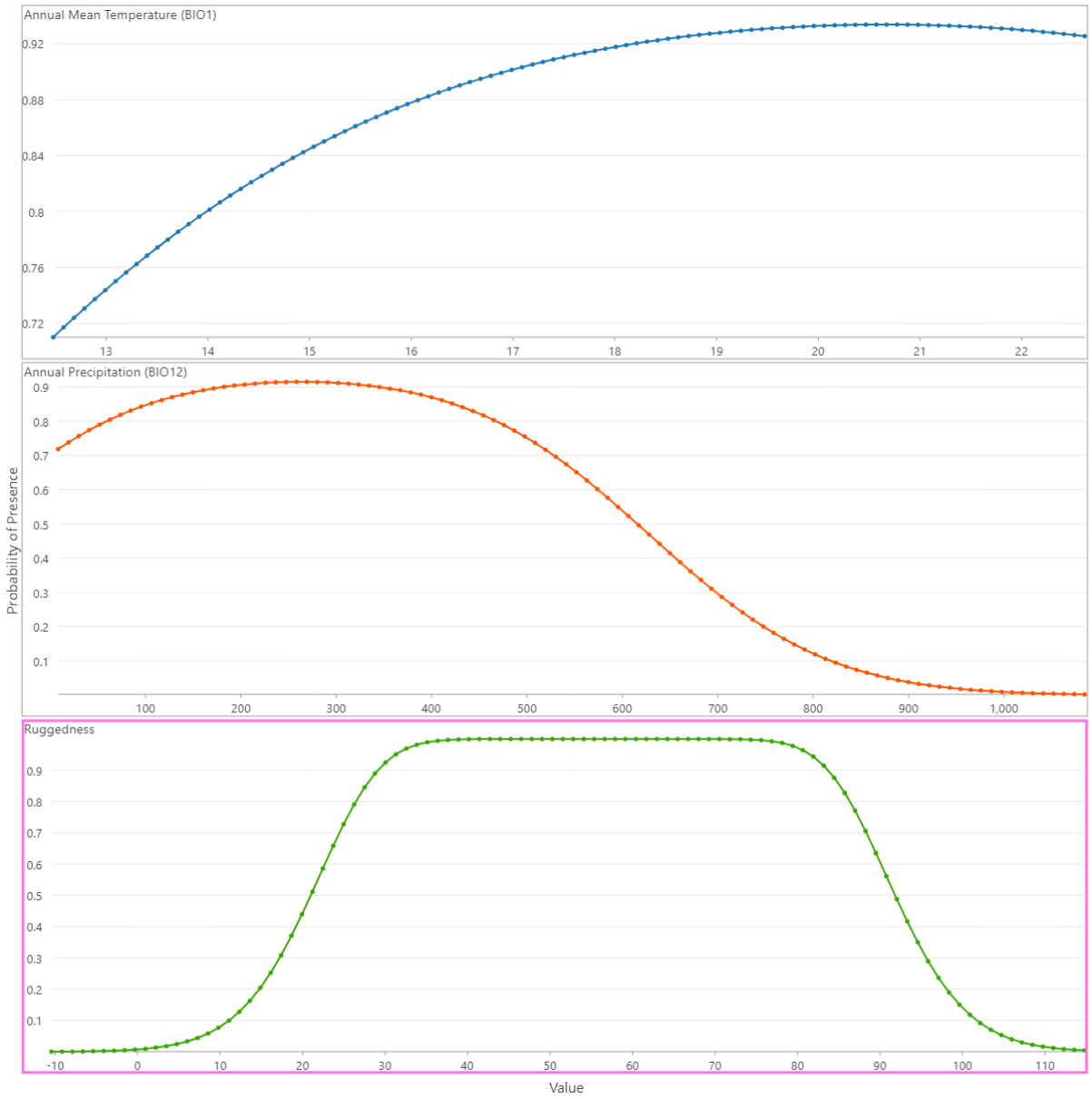


Figure 4: Probability of yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) presence in South Australia and New South Wales, Australia, as described by the partial response of variables output obtained from Maxent modelling. Variables used in the model were annual mean temperature (BIO1) (°C), annual precipitation (BIO12) (mm), and ruggedness index.

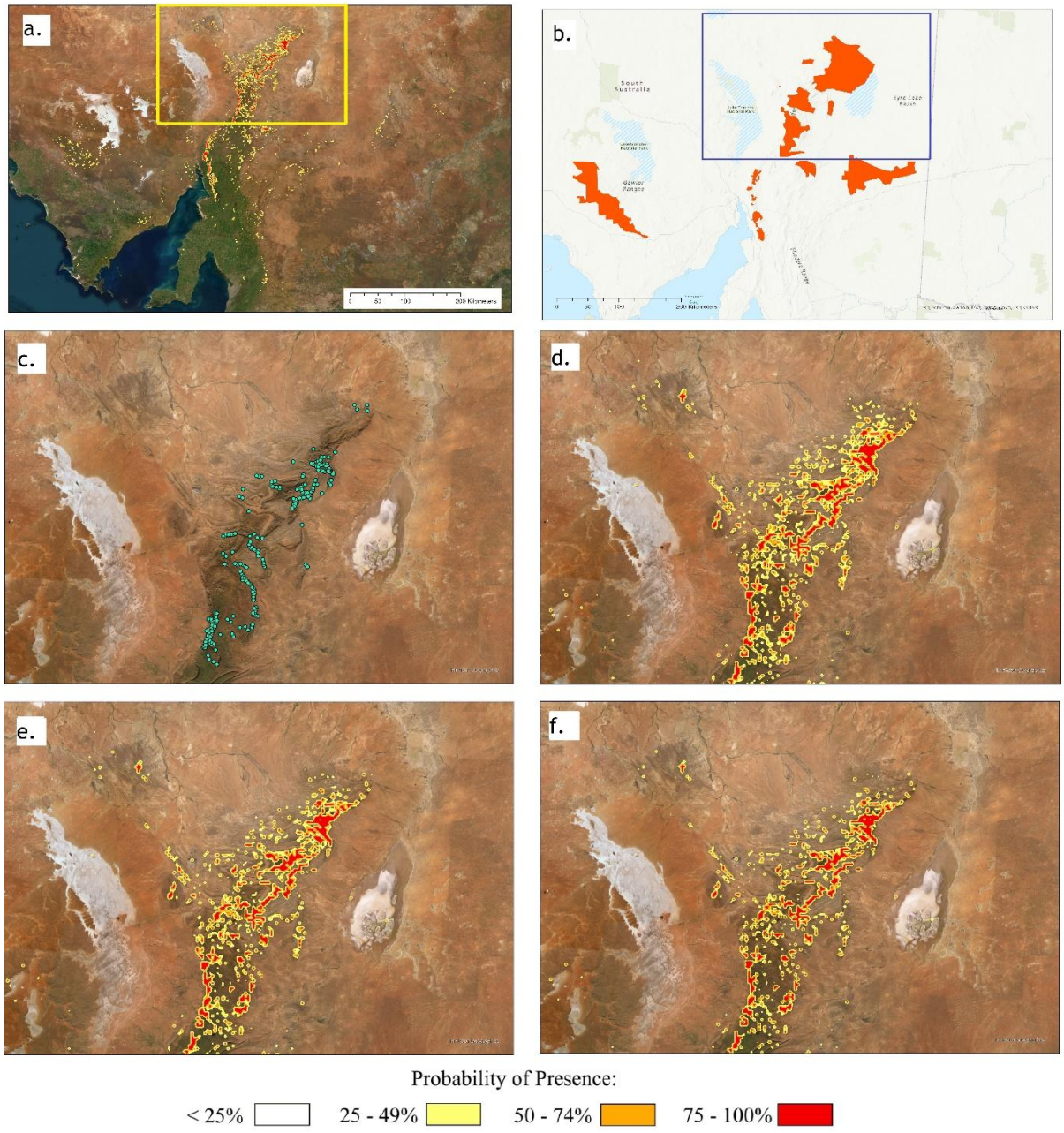


Figure 5: Maxent model outputs developed in ArcGIS Pro for yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) populations in the northern Flinders and Gammon Ranges, South Australia. *a.* The full extent of Maxent modelling with the extent of subset figures in yellow. *b.* The footprint of the 'Operation Bounceback' fox baiting program in South Australia (red), with extent of subset figures indicated (blue box). *c.* The Atlas of Living Australia observation data for yellow-footed rock-wallabies in the region (green). *d.* The current distribution model (as of 2024). *e.* The species' predicted future distribution model using IPSL-CM6A-LR shared socioeconomic pathways 2-4.5 in 2081-2100. *f.* The species' predicted future distribution using IPSL-CM6A-LR shared socioeconomic pathways 5-8.5 in 2081-2100.

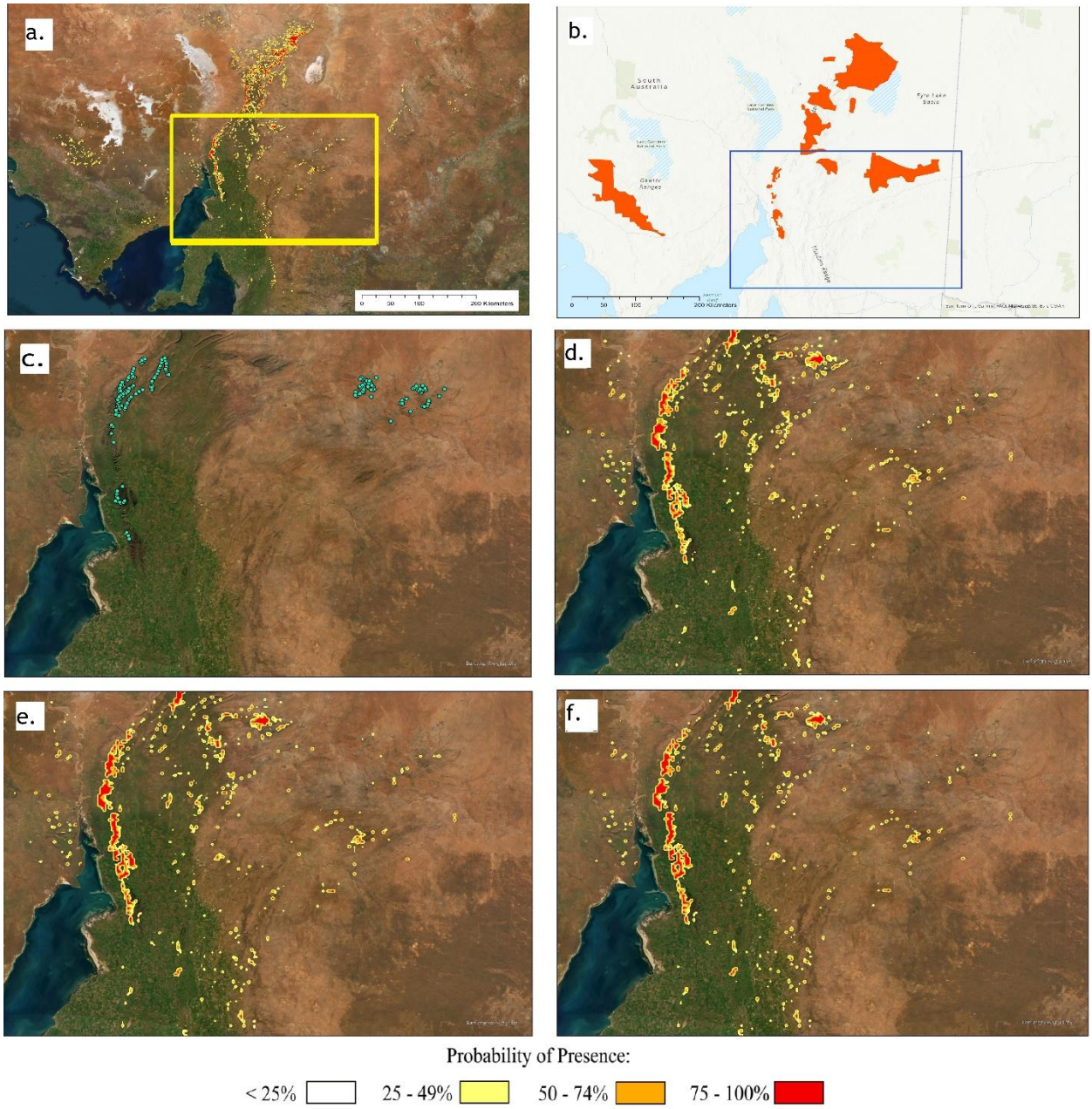


Figure 6: Maxent model outputs developed in ArcGIS Pro for yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) populations in the central/lower Flinders and Olary Ranges, South Australia. *a.* The full extent of Maxent modelling with the extent of subset figures in yellow. *b.* The footprint of the 'Operation Bounceback' fox baiting program in South Australia (red), with extent of subset figures indicated (blue box). *c.* The Atlas of Living Australia observation data for yellow-footed rock-wallabies in the region (green). *d.* The current distribution model (as of 2024). *e.* The species' predicted future distribution model using IPSL-CM6A-LR shared socioeconomic pathways 2-4.5 in 2081-2100. *f.* The species' predicted future distribution using IPSL-CM6A-LR shared socioeconomic pathways 5-8.5 in 2081-2100.

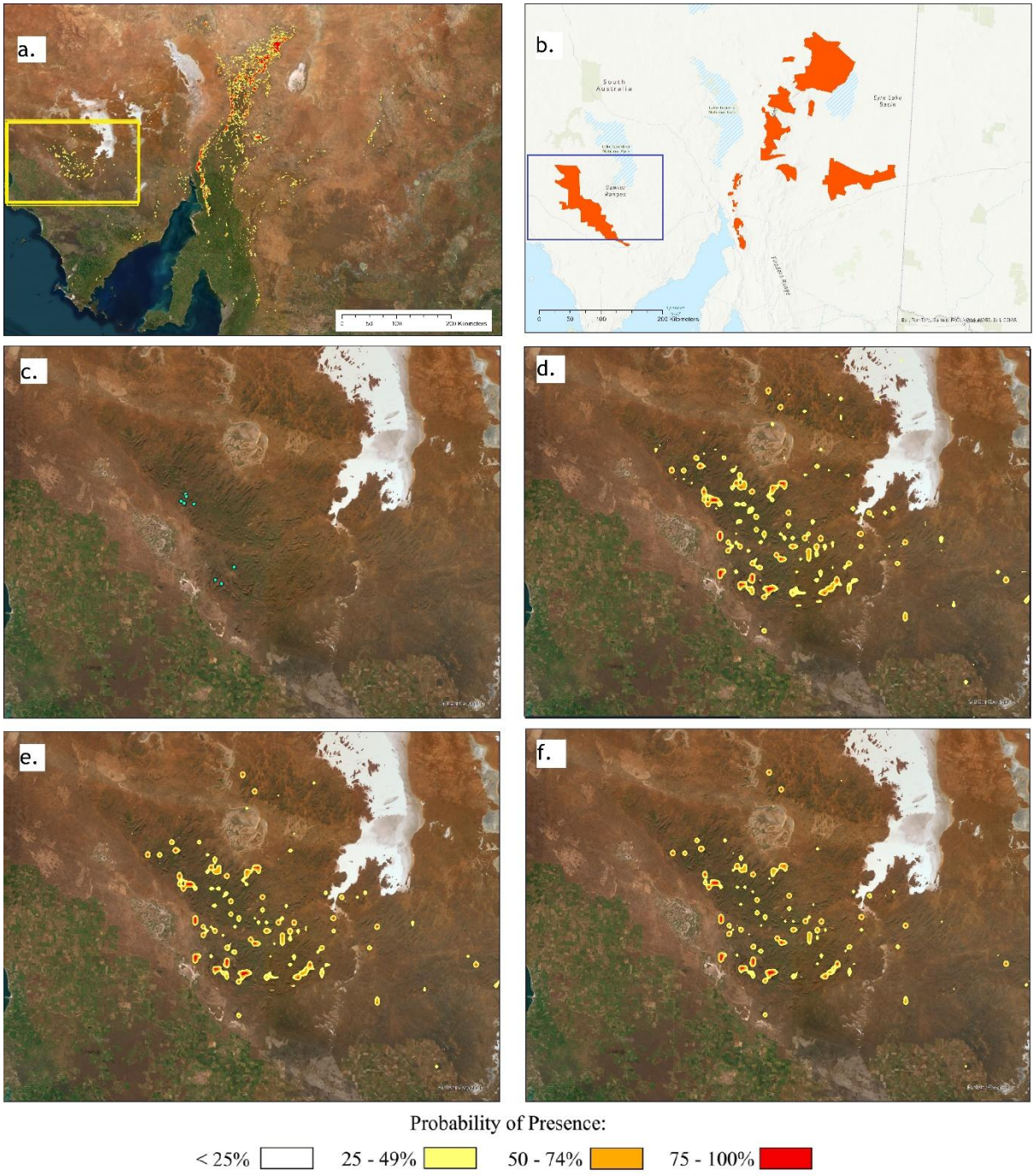


Figure 7: Maxent model outputs developed in ArcGIS Pro for yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) populations in the Gawler Ranges, South Australia. *a.* The full extent of Maxent modelling with the extent of subset figures in yellow. *b.* The footprint of the 'Operation Bounceback' fox baiting program in South Australia (red), with extent of subset figures indicated (blue box). *c.* The Atlas of Living Australia observation data for yellow-footed rock-wallabies in the region (green). *d.* The current distribution model (as of 2024). *e.* The species' predicted future distribution model using IPSL-CM6A-LR shared socioeconomic pathways 2-4.5 in 2081-2100. *f.* The species' predicted future distribution using IPSL-CM6A-LR shared socioeconomic pathways 5-8.5 in 2081-2100.

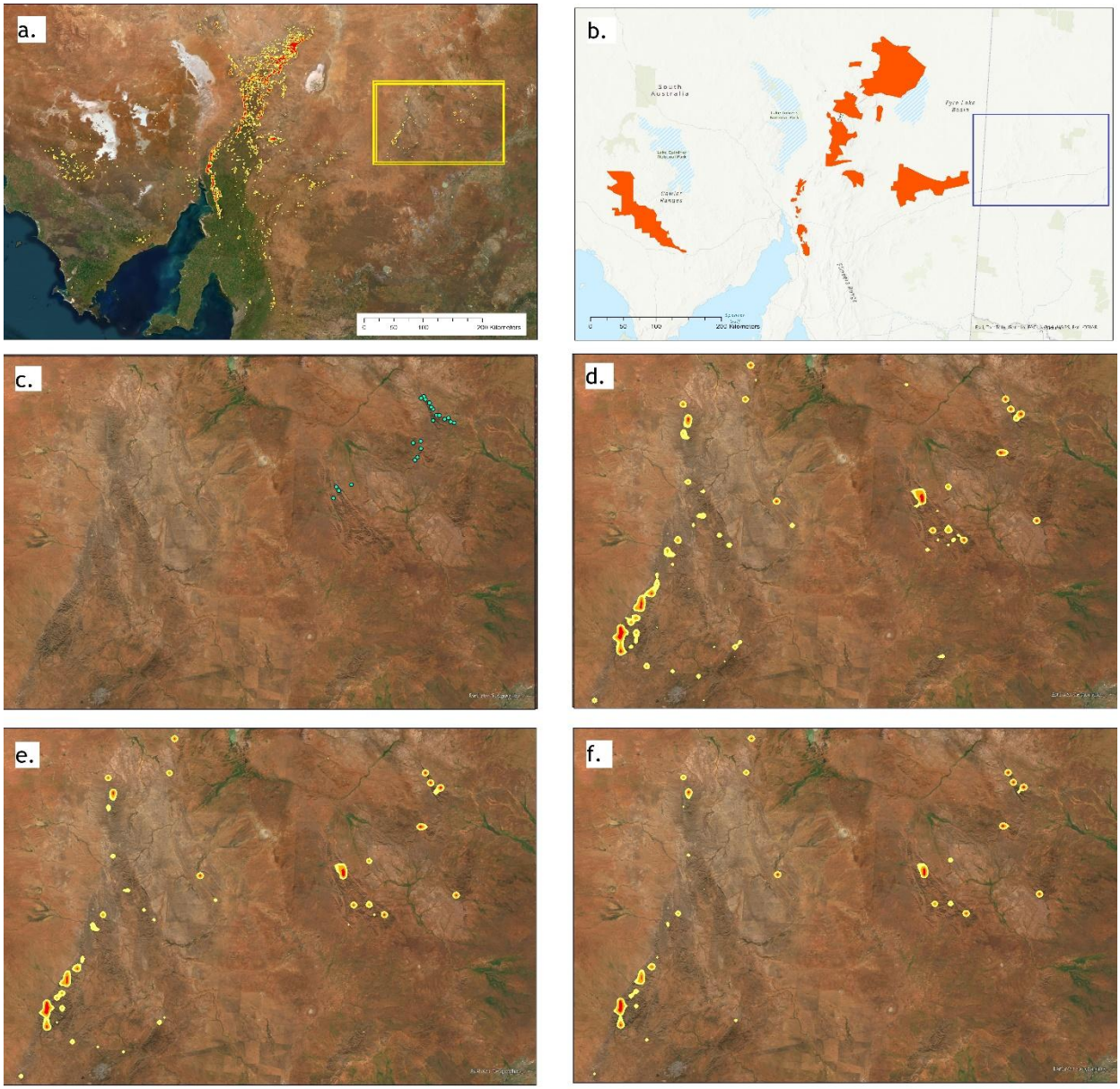


Figure 8: Maxent model outputs developed in ArcGIS Pro for yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) populations in western New South Wales, Australia. *a.* The full extent of Maxent modelling with the extent of subset figures in yellow. *b.* The footprint of the 'Operation Bounceback' fox baiting program in South Australia (red), with extent of subset figures indicated (blue box). *c.* The Atlas of Living Australia observation data for yellow-footed rock-wallabies in the region (green). *d.* The current distribution model (as of 2024). *e.* The species' predicted future distribution model using IPSL-CM6A-LR shared socioeconomic pathways 2-4.5 in 2081-2100. *f.* The species' predicted future distribution using IPSL-CM6A-LR shared socioeconomic pathways 5-8.5 in 2081-2100.

Discussion:

The Australian climate is expected to change in the coming decades in association with climate change, bringing increases in average temperatures, altered rainfall patterns, and increases in drought frequency, severity, and duration (Head *et al*. 2014; Department for Environment and Water 2022; State of the Climate 2022, www.bom.gov.au/state-of-the-climate/). For *Petrogale* species, a highly habitat-dependent genus (including several threatened species), previous modelling projects have focussed on determining habitat preferences and potentially suitable habitat for reintroductions (Short 1982; Murray *et al*. 2008; Smith & Allen 2021; Thurtell *et al*. 2022). These models however, have not predicted distribution changes as a consequence of climate change.

The scat survey data collected in this study enabled for the examination of the relationships between environmental variables and YFRW presence across two regions of their distribution, each with considerably different characteristics. Environmental variables were developed and assessed from a 30-metre resolution DEM. This allowed for broad assessments of these parameters over relatively large areas in the YFRWs range, as opposed to the assessment of fine-scale characteristics within individual YFRW colonies, such as the presence of rock crevices or fissures. Therefore, we suggest that the statistical modelling results in this study be used for observing environmental patterns associated with YFRW presence, rather than being predictive.

Ruggedness, a measure of habitat complexity, was the most informative environmental factor associated with YFRW presence throughout statistical testing. However, ruggedness differed significantly between the study regions, where YFRW presence in the Flinders/Gammon Ranges was associated with higher ruggedness values than those observed in the Olary Ranges. This may be indicative of the difference in habitat characteristics between the regions, as habitat in the Flinders/Gammon Ranges is largely comprised of steep, sandstone, mountainous ranges, whereas in the Olary Ranges, habitat is comprised of low-lying, isolated, granite, rocky outcrops. As there is a known relationship between elevation, terrain ruggedness and high erosion rates (Shit 2022; Yang *et al*. 2024), the soft sandstone at high elevation in the Flinders/Gammon Ranges region may be creating highly rugged habitat. Overall, our results suggest that there are general YFRW habitat characteristics (i.e. terrain ruggedness and mean annual temperature range) which are consistent across each geographical region of occupation. However, the significant regional differences observed throughout this study indicate that these characteristics vary, especially terrain ruggedness, in each region, suggesting that YFRW may utilise specific habitat features for their persistence differently depending on their location. Further investigation into how YFRW habitat is utilised across their highly heterogenous distribution is needed to produce a model which accounts for regional habitat-use adaptations.

Lim & Giles (1987) developed a scoring system for determining suitable YFRW habitat based on a range of habitat characteristics (Lim & Giles 1987). Their findings revealed that the most important habitat characteristics associated with New South Wales YFRW colonies were topographic complexity, access to water, presence of large smooth surfaced boulders, and shelter from direct sunlight. Topographic complexity in the Lim and Giles (1987) study was determined as an index calculated from the presence of caves, gullies, outcrops, terraces, steep cliffs, and boulder characteristics. This scoring system was also implemented more recently by Smith and Allen (2021) in south-western Queensland populations, who found that the most significant predictors of YFRW presence in these populations were steep slope, high elevation, cliff presence, large boulders, a lack of goat spoor, and some vegetation parameters. Our results would suggest that Smith and Allen's (2021) findings of 'steep slope' is likely due to the high correlation between habitat ruggedness and slope, and therefore this result is a measure of habitat ruggedness rather than of slope itself.

Previous studies examining the preferred habitat of brush-tailed rock-wallabies (BTRW; *Petrogale penicillata*) have returned varying results (Short 1982; Murray *et al*. 2008; Thurtell *et al*. 2022). A study by Short (1982) found that cliffs, ledges, and a northerly aspect were associated with BTRW presence. Whereas Thurtell *et al*. (2022) suggested that vegetation and distance to water were the greatest influencing factors for BTRW presence, although elevation and slope were also identified as important factors. Another study by Murray *et al*. (2008), found that habitat complexity and slope were the most important features associated with BTRW presence, and as with Smith and Allen (2021), is likely as a result of the correlation between habitat complexity and slope. In support of the findings from this current study, previous BTRW habitat studies have often identified habitat complexity as the one of the most important factors associated with rock-wallaby presence. Additionally, this suggests that the use of a terrain ruggedness index, such as that used in this current study, is likely a reliable input for all future rock-wallaby species modelling projects.

The SDM response curves supported the scat survey GAM results, especially from the Flinders/Gammon Ranges region, where areas with moderate to high ruggedness index values (30-80) resulted in the highest probability of YFRW presence. This suggests that the public observational ALA dataset was comparative with data collected from random scat surveys in this study, and that this broad-scale dataset was useful for producing a SDM of high ecological relevance.

The SDM model showed that the northern Flinders/Gammon Ranges region had the greatest area of suitable YFRW habitat with high connectivity. The Flinders and Gammon Ranges regions represent habitat which historically supported abundant populations of YFRWs (Fountain 1907; Copley 1981; 1983; Lim *et al*. 1987). Additionally, results also suggested that there are areas currently suitable for YFRW habitation in the region that historically linked the Flinders and Olary Ranges, which are

without known populations of YFRWs present today. This historic YFRW corridor is not currently included in the fox control footprint of 'Operation Bounceback'. Therefore, it is highly likely that fox predation, previously shown to restrict YFRW populations (Sharp *et al*. 2014), is a significant factor contributing to the absence of YFRWs in this corridor region today.

The SDM results for both SSP emission scenarios predicted that YFRWs will become more reliant on highly suitable habitat in all regions, with lower quality habitat becoming unsuitable for habitation as climate change progresses. The negative effects of low-quality habitat becoming unsuitable is likely to be greatest in regions with low habitat connectivity, such as the Gawler/Olary Ranges or New South Wales populations, where areas of suitable habitat are already limited. *Petrogale* species are known to favour areas which have caves and rock crevices which provide shelter from environmental extremes (Ruykys 2017; Baker & Gynther 2023; Mallam *et al*. 2023). These same rock structures also create areas within the rocky structures where microclimates facilitate the conservation of moisture, growth of vegetation and accumulation of water (Wilson *et al*. 1976; Main 1997). It is likely that high connectivity habitat will offer a greater potential to encounter these resources and as such, will become more favoured by YFRWs as climate change progresses. This trend has also been noted in the greater glider (*Petauroides volans*), where historically suitable habitat has become more arid, driving gliders to high elevation habitats where increased moisture has improved habitat quality and with it, higher glider densities (Wagner *et al*. 2020).

The southern Flinders Ranges region, where the current SDM shows mostly low to moderate habitat suitability, is the only region where areas of habitat are predicted to become more suitable for YFRW habitation. This is likely due to shifts in temperature and annual rainfall, where it is predicted that YFRWs will favour higher rainfall isohyets. This has been observed in other wildlife species where predicted distribution shifts occur towards the earth's poles as climate change progresses to account for changes in rainfall and temperature (Parmesan 2006; Stephens *et al*. 2018; Dahms & Killen 2023). A study on the semi-arid southern hairy-nosed wombat found that this species was also highly susceptible to changes in climatic variables with predictions indicating a substantial shift in its distribution away from the areas currently occupied as climate change progresses (Marshall *et al*. 2018). The results of the SDM suggest that areas of high habitat suitability, habitat complexity and connectivity will likely sustain YFRW populations, but in areas with low to moderate habitat suitability, climatic conditions will become a limiting factor on population viability. The predicted increase in abundance in the currently lowly occupied southern regions of the YFRW distribution may create future management issues associated with the control of foxes, and exotic and overabundant native herbivores and may require additional conservation actions to be implemented (i.e. expanding 'Operation Bounceback').

In isolated YFRW populations, such as those in the Gawler and Olary Ranges or New South Wales regions, future predictions suggest that as climate change progresses, the area of available YFRW habitat will decrease due to contractions of currently suitable habitat. These changes are predicted to be more severe under the SSP5-8.5 emission scenario than the SSP2-4.5. However, as both scenarios show a significant decrease in suitable habitat, it suggests that even with moderate climate change, these restricted populations are at significant risk of declines or local extinction. Therefore, as the future status of these populations is uncertain, conservation management in these regions will be vital for ensuring their future viability, and drastic measures may be needed to secure the unique genetic profiles found in these regions.

Conclusions:

By using a two-tiered model development approach, it allowed us to identify environmental parameters which were highly correlated and could influence model performance, and informed a SDM of high ecological relevance. Throughout the first-tier of model testing, terrain ruggedness was found to be most indicative of YFRW presence across the Flinders/Gammon and Olary Ranges regions. However, significant regional differences indicated that habitat characteristics differ across the YFRWs distribution. Additionally, these results revealed that some commonly used modelling parameters (i.e. elevation, slope, aspect) can be highly correlated with other environmental or climatic variables, and therefore future modelling projects are recommended to implement a similar model testing phase prior to SDM development.

Under future climate change scenarios, the YFRW distribution is predicted to contract to areas of highly suitable habitat with high connectivity (northern Flinders/Gammon Ranges), and regions with higher rainfall (southern Flinders Ranges). Currently, YFRWs in the Flinders and Gammon Ranges are not directly threatened by climate change. However, it was observed that areas of low to moderate habitat quality are likely to become markedly contracted or become unsuitable for habitation throughout the YFRWs distribution. Due to the decline in low quality habitat, isolated YFRW populations (Gawler/Olary Ranges and New South Wales) are predicted to be at highest risk of declines and local extinction as climate change progresses. Consequently, management strategies will need to adapt to address the shifts in the species' distribution, mitigate potential population declines, and facilitate connectivity between colonies to increase their adaptability.

Further investigation into important habitat characteristics which facilitate YFRW survival in different geographical regions would be beneficial for identifying environmental layers which need to be developed for fine-scale, in-depth, predictive modelling. This would allow models to account for

environmental heterogeneity across the YFRWs distribution, and would provide information on the habitat features which are currently supporting YFRW colonies and the critical resources they rely upon to survive climatic extremes. Additionally, with on-going scat survey monitoring, tracking of YFRW colony behavioural or habitat-use changes in response to climate change would be possible.

Due to the YFRWs highly specific habitat, their ability to adapt to climate change is restricted, and as such, isolated colonies will be reliant on conservation management actions to mitigate the negative effects of anthropogenic climate change to ensure their persistence in the future.

General Discussion

"Key Findings and Conservation Implications for the Yellow-footed Rock-wallaby (*Petrogale xanthopus xanthopus*)"



"*Petrogale xanthopus is a fitting example of an animal which needs sanctuary for its preservation and more stringent legislative efforts to check its slaughter*"

– Prof. Wood Jones 1924

General Discussion: Key Findings and Conservation Implications for the Yellow-footed Rock-wallaby (*Petrogale xanthopus xanthopus*)

Werner L.E

Introduction:

The yellow-footed rock-wallaby (YFRW; *Petrogale xanthopus xanthopus*), is a semi-arid dwelling, nationally Vulnerable species (Woinarski *et al*. 2014; Baker & Gynther 2023) threatened by predation from red foxes (*Vulpes vulpes*) and competition from exotic and over-abundant native herbivores (Dawson & Ellis 1979; Sharp *et al*. 2014; Lethbridge *et al.* 2019). This thesis examined the influence of habitat connectivity on various aspects of YFRW ecology to guide future species management across their distribution.

Aspects of the ecology of two populations with differing habitat connectivity were studied to assess parameters associated with colony health and fitness. Italowie Gap (highly connected habitat) and Bimba Hill (isolated, complex habitat) represent the extents of YFRW habitat variability in South Australia. The aim of this research was to identify issues that may arise in YFRW populations as rainfall declines and drought risks increase in association with climate change, and how habitat connectivity may aid in increasing population viability.

The data gathered here has improved our understanding of the impacts of habitat connectivity on YFRW ecology, and provides a useful baseline which can be used to inform habitat-specific species management plans and conservation actions broadly across their distribution.

Here the key findings from each thesis chapter are summarised to characterise the effects of habitat connectivity on YFRW ecology. The potential impact of climate change on YFRW populations is also discussed, with required future research and proposed conservation actions identified.

Key Findings:

Currently the management of YFRWs in South Australia is limited to broad-scale red fox and feral goat (*Capra hircus*) control twice annually, with occasional aerial survey counts (last conducted in 2018; Lethbridge *et al*. 2019). In-depth examination of YFRW populations has not been conducted since the monitoring of two reintroduced (Lapidge 2001) and one wild colony in the southern Flinders Ranges (Lim 1987; Lim *et al*. 1987), over 20+ years ago. A mark-recapture study was also undertaken some years ago (Lethbridge *et al*. 2010). The colonies investigated in this project have not been extensively trapped or examined previously, and their population status was previously unknown. This

project has provided a unique opportunity to compare YFRW populations with different habitat connectivity to build our knowledge of the ecological characteristics of the species. A summary of the key differences observed between the two study sites are presented in Table 1, and will be discussed in more detail below.

Table 1: Summary of the thesis results associated with yellow-footed rock-wallabies (*Petrogale xanthopus xanthopus*) at the study sites in connected (Italowie Gap) and isolated (Bimba Hill) habitat in South Australia.

	Italowie Gap (Connected Habitat)	**Bimba Hill (Isolated Habitat)**
Body Condition	Good, stable (Mean BCI = 70.71)	Average, stable (Mean BCI = 63.41)
Breeding	Strong autumn peak, continuous	No significant peak, continuous
Blood Biochemistry	Environmental condition dependent and within normal range for species	Environmental condition dependent and within normal range for species
Genetic Diversity (Observed heterozygosity: H_O ± S.D, Allelic Richness = A_R)	Moderate H_O = 0.226 (±0.2), A_R = 1.653)	Low (H_O = 0.182 (±0.22), A_R = 1.463)
Inbreeding (F_{IS})	Low (F_{IS} = 0.006)	Low (F_{IS} = -0.44)
Kinship within Colony	Moderate (Total average kinship = 0.15 ±0.08)	Low (Total average kinship = 0.1 ±0.07)
Diet (During dry times)	Diverse Dominant species: *Acacia* species, *Portulaca oleracea*	Diverse Dominant species: *Portulaca oleracea*, *Sclerolaena* species
Diet (During abundant resources)	Diverse Dominant species: *Abutilon* species, *Malvastrum* species	Diverse Dominant species: Tarvines, *Solanum* species, *Abutilon* species
Sympatric Herbivore Diet Overlap	High (when vegetation drying out) In particular: *Acacia* species, *Malvastrum* species, *Sigesbeckia australiensis*	High (during dry period) In particular: *Portulaca oleracea*, *Acacia* species
Faecal Microbiome Diversity	Moderate (Mean microbial richness = ~250)	Low (Mean microbial richness = ~205)
Habitat Characteristics of YFRWs	Moderate-high terrain ruggedness (20-80), mean temperature (17.5-19^{o}C)	Low-moderate terrain ruggedness (0-60), mean temperature (17.5-19.5^{o}C)
Climate change risk based on SDM model predictions	Low	High

Three YFRW population studies have been conducted previously (Lim 1987; Sharp 1997a, Lapidge 2001) but none have examined differences in population demographics and ecology associated with habitat connectivity and rainfall. Throughout trapping, few sub-adult YFRWs were captured at Italowie Gap (n = 4), compared to Bimba Hill where sub-adults were captured frequently (n = 15), suggesting that habitat connectivity may be facilitating the migration of young YFRWs to other colonies. The effects of high summer rainfall received during this study were evident at Italowie Gap where adult YFRWs had an increased body condition compared to the previous trapping period, and blood urea and creatinine levels changed in response to consuming high water but low protein content feed. These same effects were not evident at Bimba Hill. Birth rate peaked at Italowie Gap in autumn coinciding with the period following the months of historically high rainfall. Whereas at Bimba Hill, a region with consistent year-round rainfall, no significant seasonal increase in birth rates was observed. Observations about the number of sub-adults captured, the timing of breeding, and the rapid recovery of body condition following rainfall, suggest that habitat does play a role in some aspects of YFRW population dynamics and ecology. Furthermore, this investigation highlighted the YFRWs reliance on seasonal rainfall, which is likely to become less reliable as climate change progresses, for maintaining a good level of health. Further long-term monitoring of these populations is needed to determine the on-going effect of habitat connectivity and variable rainfall patterns on YFRW population health and fitness, and allow inferences to be made about conservation efforts required at other YFRW populations in the region.

Assessment of the genetic diversity, inbreeding and kinship of YFRW colonies has previously been conducted (Potter *et al*. 2020; Smith *et al*. 2023a). These previous studies indicated that YFRW colonies were maintaining moderate to high levels of genetic diversity and low inbreeding values. Smith *et al*. (2023a) examined the effect of habitat connectivity on the genetic diversity of YFRWs and found that a colony in connected habitat had higher genetic diversity than those with limited habitat connectivity. The results presented in chapter two of this thesis support these findings, showing that the genetic diversity of YFRWs at Italowie Gap was significantly higher than at Bimba Hill (Italowie Gap; $H_O = 0.226$, $uH_E = 0.226$, $A_R = 1.653$; Bimba Hill; $H_O = 0.182$, $uH_E = 0.172$, $A_R = 1.463$). The results also revealed that the Bimba Hill YFRWs had similar genetic diversity values to those of a captive population (Gorge Wildlife Park; $H_O = 0.185$, $uH_E = 0.179$, $A_R = 1.478$). Although, it was observed that inbreeding was not a concern currently in the colonies examined here (F_{IS}; Bimba Hill = -0.44, Italowie Gap = 0.006, Gorge Wildlife Park = -0.02), in opposition to our hypothesis, kinship was significantly higher at Italowie Gap compared to the Bimba Hill colony (Italowie Gap = 0.152, Bimba Hill = 0.104). The opposing kinship values observed may suggest that adaptive population behaviours may be are occurring, such as decreased female philopatry, or a greater admixture of breeding groups in the Bimba Hill colony, which are not occurring commonly at Italowie Gap. However, the low level of genetic diversity at Bimba Hill may suggest that long-term

population genetic stability is unlikely if abundance declines, and human-mediated admixture may be necessary to maintain population viability. In contrast, habitat connectivity in the Italowie Gap region appears to have facilitated gene flow via migration supporting moderate genetic diversity and low inbreeding values.

The dietary preferences of YFRWs have been examined four times previously; once in a reintroduced population (Lapidge 2000), twice in the southern Flinders Ranges (Copley & Robinson 1983; Lim *et al*. 1987), and once in western New South Wales (Dawson & Ellis 1979). All four previous studies used micro-histological examination of scats to identify plant fragments, a technique which has now been superseded by modern, more robust genetic techniques.

The dietary information obtained in this study (chapter three) was obtained using a targeted genetic sequencing technique (myBaits® and Next Generation Sequencing of plant DNA) to identify plant species present in YFRW, feral goat and euro scat samples. The plant species detected in YFRW scat samples varied between study sites, reflecting geographical differences in plant communities. Herbaceous plant species were detected highly at both sites during good environmental conditions (following high rainfall), and during dry environment conditions dietary diversity and shrub intake increased. This indicates that herbaceous plant species were the preferred food source when available, but when unavailable due to depletion and dry conditions, dietary diversity and shrub intake is increased. This is similar to the findings of previous studies (Dawson & Ellis 1979; Copley & Robinson 1983; Lim *et al*. 1987).

Dietary competition with feral goats and euros was found to be high during either a dry period (Bimba Hill) or when vegetation water content was decreasing (Italowie Gap), similar to findings outlined previously by Dawson & Ellis (1979). These results suggest that the YFRW is most vulnerable to resource competition from goats and euros during dry periods, or when vegetation is drying out after a pulse of plant abundance. Additionally, the dietary overlap between YFRWs and euros was observed for the first time, which had not been characterised in the previous study by Dawson & Ellis (1979). Overall, the results from this chapter revealed that the dietary differences observed between YFRWs at each site is likely attributed to abiotic habitat factors (i.e. soil, rock structure) and regional rainfall, influencing the plant species which grow locally (Copley & Robinson 1983).

There has been no previous investigation of the YFRW microbiome, although similar studies have been undertaken in some other Australian marsupials (*Notamacropus eugenii*, Chhour *et al*. 2010; *Sarcophilus harrissii*, Cheng *et al*. 2015; *Phascolarctos cinereus*, Brice *et al*. 2019; *Lasiorhinus latifrons*, Eisenhofer *et al*. 2021). The role of the microbiome is emerging as an important factor in species conservation programs (Banerjee *et al*. 2020) due to its role in maintaining animal health (Trevelline *et al*. 2019). As such, characterising the faecal microbiome of the YFRW was considered important baseline information for future conservation efforts. Results revealed that the YFRW

microbiome is similar to that of other marsupial species, with the majority of microbial species being from the *Firmicutes* and *Bacteriodota* phyla. Microbial richness and composition varied significantly between study sites and seasons, where Italowie Gap YFRWs had significantly higher microbial richness and differing microbial composition comparatively to Bimba Hill animals throughout. Additionally, increased microbial richness appeared to be associated with times of high resource availability.

The variability in faecal microbial richness and composition at each study site also appeared to be linked to dietary composition. At both sites, when *Acacia* species were ingested, *Firmicutes* microbial species were more prevalent, which also coincided with times of low resource availability following low rainfall. This suggests that as climate change progresses and vegetation diversity and rainfall decreases, increased ingestion of *Acacia* species is likely, and therefore *Firmicutes* microbial species may become even more prevalent in the YFRW faecal microbiome. Further research on this and the functionality of faecal microbes, could present future opportunities to alter YFRW, or other threatened marsupial species microbiomes, to enhance their adaptive potential or even their tolerance of drier environmental conditions. As such, the differences in faecal microbiome observed between sites are likely driven by local environmental factors (e.g. rainfall causing plant growth, vegetation structure, soil type, rock structure), in support of the findings also observed in chapter three.

The final chapter of this thesis used scat survey data, environmental GIS parameters, and statistical models to examine the habitat characteristics associated with YFRW presence, to inform the development of a current and future species distribution model (SDM) in South Australia and New South Wales. Habitat characteristics associated with YFRW presence have been examined previously in south-western Queensland (Smith & Allen 2021) and in western New South Wales (Lim & Giles 1987), and were based on a habitat scoring system informed by on-ground surveying, rather than the examination of spatial GIS data. Through a series of generalized additive model (GAM) testing, the characteristics most associated with YFRW presence were terrain ruggedness (a measure of habitat complexity) and mean annual temperature. In support of our findings, previous studies found that habitat complexity is an important factor in YFRW (Lim & Giles 1987) and brush-tailed rock-wallaby (*Petrogale penicillata;* Short 1982; Murray *et al.* 2008; Thurtell *et al.* 2021) habitation. Additionally, the results indicated that some environmental variables commonly used in modelling projects are highly correlated, suggesting that future rock-wallaby modelling should utilise a variable testing phase before commencing SDM development.

Informed by statistical GAM modelling of fine-scale survey data, a SDM was developed using terrain ruggedness as the sole environmental variable and two climatic variables (mean annual temperature and annual precipitation). The current SDM for the YFRW revealed areas of suitable habitat encompassing the known current and historic habitation range of the species, with areas not known to

support YFRWs also identified. Examination of two climate change carbon emission scenarios (SSP 2-4.5 and SSP5-8.5) revealed that the YFRW distribution will contract towards regions of high-quality, connected habitat, such as that in the northern Flinders and Gammon Ranges, by 2080-2100. Additionally, areas of connected habitat in the southern Flinders Ranges will become more suitable for YFRW habitation. The future SDM also showed that smaller, peripheral populations (Gawler/Olary Ranges and New South Wales) are likely to experience significant range contractions, with the predominantly low to moderate quality habitat present, becoming unsuitable for habitation. These findings suggest that there are likely to be future broad-scale management issues across the YFRWs distribution induced by climate change, possibly resulting in population declines or local extinction. Critically, the future SDM indicated that management in the Gawler/Olary Ranges and New South Wales will be vital to conserve these populations as climate change impacts are likely to be most severe in these regions.

Current Population Status, Future Research and Management Directions:

The results from this project indicate that there are some unique ecological characteristics occurring in each of the study colonies, which suggest that YFRW management should be tailored depending on their level of habitat connectivity.

Connected Population Management Strategies:

YFRW colonies in connected habitat, such as that at Italowie Gap, are likely to experience some impacts of future climate change induced by increased drought frequency and duration, and general changes in weather patterns (Head *et al*. 2014; Department of Environment and Water 2022). The Bureau of Meteorology (www.bom.gov.au) predicts that summer rainfall which currently underpins YFRW breeding and maintenance of body condition is likely to become more unreliable. Therefore, in years of below average summer rainfall and during droughts, targeted water supplementation and increased feral goat and euro (if overabundant) control at key colonies during peak periods whilst juveniles are being weaned (approximately October to December) is recommended. Water supplementation has been shown previously as a successful short-term strategy to increase recruitment in black-footed rock-wallabies (*Petrogale lateralis lateralis*) during dry times and drought (West *et al*. 2017), and may also be a viable option for maintaining YFRW recruitment rates. This strategy would help facilitate increased juvenile survival and recruitment, and reduce water and feed related stressors on lactating female YFRWs.

Genetic diversity results indicated that there is no current need for genetic intervention at Italowie Gap, and suggests that habitat connectivity is aiding in maintaining population genetic diversity.

However, expanded genetic assessments are needed to evaluate the risk level and rate of genetic changes in connected YFRW populations in the Gammon Ranges region. Scientific understanding of the migration dynamics of YFRWs in highly connected habitat is limited, especially in regards to the timing of migration events and management of breeding groups. Therefore, it is recommended that in-depth investigations into YFRW migration dynamics using GPS collars, be used as a means of measuring the environmental conditions which influence and trigger migration events. The use of GPS collars has been used successfully to study movement patterns in other marsupial species (Carthew *et al*. 2015; Yockney *et al*. 2015; Munn *et al*. 2016; Arkinstall *et al*. 2024).

Microbial richness and diversity have been associated previously with host genetic diversity (Ørsted *et al.* 2022). The results of this project further support this, where Italowie Gap YFRWs had better genetic diversity and faecal microbial diversity than was observed at Bimba Hill. The greater faecal microbial diversity at Italowie Gap may allow the animals to adjust their diet and have enhanced extraction of nutrients from poor-quality feed. Greater microbial diversity has also been linked to increased immune function and pathogen resistance in other species (Salzman 2011; Hooper *et al*. 2012). If this is true for YFRWs, it can be assumed that the maintenance of genetic diversity is also mediating faecal microbial diversity and immune health. As faecal microbial diversity and composition are influenced by diet, examination of more YFRW colonies surrounding Italowie Gap in degraded and intact vegetation habitats during a range of environmental conditions would help identify the most influential factors associated with microbiome maintenance in YFRWs in this region. Additionally, the scientific understanding of microbe functionality and the health effects of microbial compositional changes for YFRWs is lacking, and as such, further research is required to determine how the microbiome can be manipulated to improve animal health. This information, when available, could lead to the development of microbiome transplants as a tool for improving the fitness of YFRW populations as vegetation diversity and abundance changes in response to climate change.

Thesis results suggest that currently *Acacia* species are an important source of nutrition for YFRWs during dry conditions, however if increased ingestion occurs throughout the year, it may lead to negative health implications. The ingestion of *Acacia* has been documented to cause gastrointestinal morphological abnormalities, and decrease feed intake and liveweight of sheep (*Ovis aries*) (Robins & Brooker 2005). However, the impact of *Acacia* ingestion for YFRWs is unknown, and as this species has likely evolved ingesting this plant, there may be no toxic affect. Regardless, broad-scale diet studies and vegetation surveying at YFRW colonies in the Gammon Ranges region would be required to begin tracking the ingestion rate of *Acacia*, and identify which colonies which are utilising these plants as a food source in dry times. Colonies relying on *Acacia* species as a main food source may require on-going population health monitoring to detect pathologies associated with *Acacia* intake, and would provide evidence on whether this plant is also toxic to YFRWs.

Although habitat connectivity provides some buffer against climate change by facilitating migration and increasing resource availability (Rudnik *et al.* 2012; Kutschera *et al*. 2016), the temperature and rainfall changes predicted in the semi-arid region are likely to have biological and physiological implications for YFRW colonies, regardless of habitat connectivity. On-going monitoring of sites like Italowie Gap to track population ecological changes will provide a great opportunity to monitor and inform current or future management strategies for the species.

Isolated Population Management Strategies:

The abundance of YFRWs in the Olary Ranges (Lethbridge *et al*. 2019) and the reasonable general health observed in this project suggest that there are no current conservation management issues which need immediate attention at the Bimba Hill site. However, some concerning ecological characteristics were identified which will likely result in future management issues, and may indeed be current issues in smaller, even more isolated Olary Ranges YFRW colonies. Results from this study suggest that isolated YFRW colonies are at significant risk in the future due to a current lack of genetic diversity and microbiome richness.

Currently, recruitment rates appear stable at Bimba Hill and no distinct peak in birth rates was observed. Regular monthly rainfall throughout the year in the Olary Ranges appears to be supporting consistent recruitment, with no need for immediate conservation actions to be implemented. However, if the predicted climate change induced decrease in annual rainfall occurs, water supplementation and feral goat and euro (if overabundant) control in this region should be considered during summer and spring to support weaning young and lactating females. On-going monitoring is key to detecting changes in juvenile recruitment rates and will underpin conservation management actions here.

The higher number of males captured and frequency of encountering sub-adults suggests that migration into and out of the Bimba Hill YFRW colony is limited. Male-biased dispersal is generally favoured in *Petrogale* species, such as the short-eared rock-wallaby (*Petrogale brachyotis*; Potter *et al*. 2012) and the brush-tailed rock-wallaby (Hazlitt *et al*. 2004; 2006). However, if male-biased dispersal is being restricted at Bimba Hill through habitat isolation, this is likely contributing to the lack of genetic diversity, and subsequent low faecal microbial richness observed. Concerningly, the Bimba Hill colony, which is one of the largest and most abundant YFRW colonies in Bimbowrie Conservation Park, was genetically similar to a captive YFRW population which has undergone limited population management since its establishment in the late 1800's/early 1900's. Therefore, if large colonies which are capable of maintaining breeding group admixture are currently experiencing low genetic diversity, smaller, more isolated YFRW colonies in the Olary Ranges may have much lower genetic and microbial diversity parameters. Although, Smith *et al*. (2023a) suggests that migration does indeed occur between isolated YFRW habitats. As such, further research examining

migration dynamics of colonies varying in size in the Olary Ranges is needed to examine the factors associated with migration and genetic maintenance in this region.

For small, highly isolated Olary Ranges YFRW colonies at high risk of climate change, translocations into larger, more stable colonies may be beneficial. This would act to limit losses of genetic diversity for the species as a whole, and capture unique genetic profiles which may be beneficial for the recipient colony. To do this without disrupting the colony's established social structure (Jarman & Bayne 1997; Sharp 1997a; Hazlitt *et al*. 2006), further developing successful methods such as cross-fostering (Taggart *et al*. 2005; 2010; Kwek *et al*. 2009) to introduce new genetics safely into YFRW colonies is recommended. However, more extensive genetic sampling and testing would be needed across the Olary Ranges YFRW colonies to inform these genetic management strategies.

Additionally, these genetic management actions may act to address low faecal microbial richness (as per Ørsted *et al.* 2022) as was detected at Bimba Hill, and is likely to be a widespread issue across Olary Ranges colonies. The health implications of having low microbial richness are currently unknown for YFRWs, but in humans it is often indicative of a poor-quality diet causing malnutrition, which subsequently results in low gut microbial diversity and richness (Iddrisu *et al*. 2021). If this is also true for YFRWs, a decrease in food quality and availability due to decreased annual rainfall with climate change, and additional decreases in genetic diversity due to population declines, would be expected to further reduce faecal microbial richness. The established link between faecal microbiome and diet (Chen *et al*. 2014; Maria Moise 2017) would suggest that during dry environmental conditions when competition between YFRWs, feral goats and euros is greatest, control of these species may free up access to better quality feed, and aid in maintaining current levels of faecal microbial diversity. Our knowledge around the role of microbes and the implications of changing faecal microbiomes in YFRWs is lacking, and as such, further research is required in these areas. This research is particularly pertinent as faecal microbiome transplants from YFRWs in the more arid northern Flinders/Gammon Ranges may prove a useful tool for boosting climate resilience in YFRWs currently inhabiting higher rainfall zones.

The Species' Future Distribution and Management Actions:

SDM results suggest the future distribution of YFRW populations in South Australia and New South Wales will become contracted under moderate or high carbon emission scenarios. The only region where suitable habitat is predicted to increase is in the southern Flinders Ranges, suggesting that the area of YFRW occupation and population abundance in this region is likely to increase. It is therefore recommended that YFRW management plans for the southern Flinders Ranges be designed prospectively in anticipation of this shift occurring. Management plans should examine the feasibility of expanding pest control programs, such as 'Operation Bounceback', beyond the current southern

boundary to address competition for food, water or shelter resources from exotic and native herbivores. This will become a vital conservation action during dry times or drought to ensure YFRW persistence. As a tool for informing the timing of management actions, on-going plant diversity and biomass surveys should be considered for the southern Flinders Ranges to detect times of resource depletion.

These on-going plant surveys are also pertinent in the northern Flinders and Gammon Ranges regions where YFRW density will likely increase. These northern regions are at high risk of resource depletion due the unreliability of summer rainfall, which is likely to become more erratic under climate change. Vegetation surveys could be used to inform if food and water supplementation actions are required, and to build an understanding of the optimal timing of these actions as a tool to increase colony resilience until environmental conditions improve.

Most concerningly, smaller, peripheral YFRW populations (Gawler/Olary Ranges and New South Wales) are predicted to incur substantial decreases of suitable habitat, making them more susceptible to population declines or extinction events. Therefore, it is recommended that these YFRW populations be the focus of species management plans to mitigate the loss of these genetically valuable populations. As YFRWs in New South Wales are already listed at a state-level as Endangered (Threatened Species Conservation Act 1995), further declines in population abundance associated with climate change could render these colonies unviable and at risk of regional extinction.

The varying degrees of climate change risk identified by the SDM suggest that geographically distinct populations (i.e. Flinders/Gammon Ranges, Olary Ranges, Gawler Ranges, New South Wales) should be treated as separate ecological and operational significant units. This would provide greater legislative protection and more appropriate targeted action in populations when required. Broad-scale management actions may extend to translocations to more suitable habitat (Batson *et al*. 2015; Langridge *et al*. 2020), supplementation of food and water resources during droughts (West *et al*. 2017), and human-mediated population admixture to boost genetic diversity and adaptive potential (Mills 2012). If substantial population decreases occur, it may also be necessary to establish captive insurance populations to maintain unique genetic profiles for future translocations/reintroductions to suitable habitat elsewhere.

Development of additional species distribution modelling inputs would help identify specific colonies which might be at greatest risk of population declines. As YFRW habitat characteristics significantly differed between geographic regions (see chapter five), model inputs relating to habitat-specific functions (i.e. refugia use, survival resources) in each region, and how these vary in response to environmental conditions (e.g. droughts versus high rainfall events), would enable spatially explicit future SDM analyses. This information would also inform the timing of on-ground actions as some

refugia factors relate to resource accumulation (i.e. retention of moisture or energy), and therefore effective plans can be implemented with conservation outcomes maximised.

The Final Word:

The findings in this thesis have highlighted how well YFRWs are adapted to their harsh, semi-arid environment, but also revealed that some aspects of their ecology make them vulnerable to climate change. As such, there are conservation management actions which are recommended to be implemented now which can minimise future efforts and aid in securing the YFRWs viability. This is particularly important for small, isolated colonies, such as those in the Gawler/Olary Ranges and New South Wales. On-going monitoring across the YFRW distribution is needed to re-enforce the findings of this thesis, and be used to inform and maintain the validity of future conservation management plans.

This project has highlighted the suite of ecological characteristics unique to YFRW colonies in highly connected or isolated habitat. It is therefore strongly recommended that the management of YFRWs be tailored to reflect the colony's ecological characteristics and future needs.

Supplementary Chapter

"A guide to trapping, handling, and processing small to medium sized macropodids: with examples from the iconic, semi-arid adapted yellow-footed rock-wallaby (*Petrogale xanthopus*)"



"*They are shy; but this is probably an acquired trait, as in distant parts of the colony I have watched them basking in the sun, feeding and playing together for an hour at a time*"

– Fountain 1907

Statement of Authorship

Title of Paper	A guide to trapping, handling, and processing small to medium sized macropodids: with examples from the iconic, semi-arid adapted yellow-footed rock-wallaby (*Petrogale xanthopus*)
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Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	14/10/2024

Co-Author Contributions

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

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

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A guide to trapping, handling, and processing small to medium sized macropodids: with examples from the iconic, semi-arid adapted yellow-footed rock-wallaby (*Petrogale xanthopus*)

Werner L.E, Kleemann S.L, Dix S.K, Taggart D.A.

Abstract:

Wildlife monitoring programs can lead to important insights into population dynamics, fluctuations in abundance, and population health. Underpinning all of these monitoring programs is trapping, where physical examinations and biological samples are obtained from the target species. This guide offers a step-by-step protocol for trapping, handling, and obtaining biological samples from small to medium sized macropodids, with specific examples provided from a yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) trapping program. An important factor in establishing a trapping program is planning and preparation. Some macropodid species are often difficult to locate, and therefore the placement and free-feeding of traps prior to active trapping is needed to ensure the success of the program. The animal handling techniques described here have been developed over the years to ensure the physical safety of the researchers and animals involved, as well as to limit the animal's stress whilst processing and obtaining biological samples. The technical detail provided in this guide on all aspects of trapping, handling, and processing can be utilised across a range of macropodid species, and other marsupial species with similar physical characteristics. The detailed data that can be gathered in trapping programs can greatly benefit the species by increasing ecological and biological knowledge, and underpin conservation management efforts.

Introduction:

Non-invasive monitoring of wildlife species is growing ever more popular (Zemanova 2020; Kleemann *et al.* 2021; Pannoni *et al*. 2022; Schilling *et al*. 2022; Treloar *et al*. 2023), but monitoring of this nature is limited in the information it can provide about a population. However, to understand how animals are being affected by their environment, physical hands-on health and reproductive assessments are needed, and this can only be done by actively trapping, handling and processing an animal. As trapping and handling can be highly stressful for an animal, it needs to be undertaken carefully by experts which have been appropriately trained in order to minimise stress and reduce the risk of injury to the animal, or any research/veterinary personnel involved.

The following is a guide to trapping, handling, and processing small to medium sized macropodids, using the yellow-footed rock-wallaby (YFRW; *Petrogale xanthopus*) as a model species. The YFRW lives in the semi-arid, rocky ranges and outcrops of South Australia, western New South Wales, and central-western Queensland (Copley 1983; Gordon *et al.* 1993; Dovey *et al*. 1997), and is classified as Vulnerable (EPBC Act 1999, Woinarski *et al*. 2014). The examples included in this guide were obtained from a YFRW trapping program being conducted in South Australia.

Trapping to gain individual and species-specific information is key to tracking and understanding population ecology, demographics, reproduction and general health in most vertebrate species. This information can also be used to assess changes due to changing environmental conditions, drought, or climate change into the future. The YFRW trapping program which is referred to throughout this guide was established as the monitoring of this species in South Australia is currently largely restricted to periodic aerial counts (Lethbridge *et al*. 2019), which does not provide a good indication of population dynamics, or how the physical health of a population is changing over time. The most comprehensive way to understand population characteristics, for YFRWs and other macropodids, is to physically assess a subset of the animals within the population. However, this must be done in a way which ensures the safety of the animals and the researchers involved.

The aim of this technical paper is to outline the best methodology for trapping, handling, and processing small to medium sized macropodids. Although centred around examples from the YFRW, these techniques can also be applied to other macropodids and a range of marsupials with similar morphological characteristics.

Methods:

Trap Design and Setup:

The selection of trapping locations will naturally vary depending on the target species, and incorporating knowledge of the species' ecology and habitat preferences is needed when planning. However, once trapping locations have been determined, the following guidelines will apply generally, regardless of species.

Before active trapping commences, traps should be placed into the environment for animals to become accustomed to. This will alleviate any neophobic tendencies animals may have towards the traps. Ideally, for YFRWs this should be conducted approximately 4-6 weeks prior to trapping. The traps used for trapping YFRWs, and what can be used for most small to medium sized macropodids, are soft-sided steel-framed cage traps (80cm length x 40cm width x 40cm depth), made specifically for capturing macropodids (Figure 1; Crestware Industries, South Australia).

The steel frame of the trap is wrapped in shade-cloth to help provide the animal with shelter from environmental extremes, reduce the risk of trap related injury, and provide a sense of cover and protection which acts to calm the animal, thus reducing stress. Shade-cloth which has been manufactured with an edging to avoid fraying is best. Medium-gauge wire mesh is attached to the outside of the trap frame with metal zip ties, ensuring the trapped animal is secure, and that there are no large gaps or spaces where the animal can get entangled whilst in the trap. Traps are triggered by pressure being applied to a treadle which releases a latch, allowing the door to close. A drop bar falls with the door and secures it so it cannot be opened from the inside. These traps also have a solid metal slide at the back of the trap which provides access into, and out of, the end of the trap for simple extraction of the trapped animal.

The exact number of traps to be used for any given trapping program should be based on the size of the target area to be trapped, the topography, and the number of experienced researchers/assistants available for trap monitoring, animal handling and processing. The aim here being to ensure animal and researcher safety and to minimize the time taken to remove and process each animal captured.

In the case of the YFRW, this equates to a group of four researchers on a trapping line of approximately 300 meters long in moderately steep terrain, where ten traps can be positioned and accessed rapidly and efficiently each trapping morning. Each trap should be positioned in an area where there is sign of active occupation (i.e. areas with high/fresh scat counts) and spaced approximately 20-50m apart depending on the terrain. For instance, areas of active YFRW occupation tend to have ledges, which animals use for sunbaking or resting, and crevices and overhangs used for shelter (Copley 1981; Lim *et al*. 1987). Adjacent to these areas are often places where vegetation is available for feeding.

When positioning a trap care should be taken to ensure that the trap will be shaded from environmental extremes, and particularly from the morning sun to minimise the risk of the animal overheating. When placing traps in steep, rocky terrain, each trap should be secured in place with rocks and strong cord or anchored to the ground with metal stakes to protect against the trap tipping or rolling once the animal has been captured (Figure 1). Once in position, the trap door should be locked in an open position with heavy duty cable ties, wire and/or padlocks and the drop bar secured to stop it from closing in the event that the door fixings fail. Each trap should also be marked with a GPS and flagging tape.

Once in place, traps should be free baited every 3-5 days, but ideally at least once a week. The bait which is commonly used for all small to medium sized macropodids is a mixture of macropod pellets (Wombaroo or Laucke Mills), carrots, apples and sweet potatoes. Aniseed oil and peanut butter can also be utilised as a scent lure to bring animals close to the trap. Although, avoid using lures which are too sweet (i.e. honey) as it may attract ants towards the trap. During free feeding, some of the bait can

be placed outside of the traps to lure animals closer, but during active trapping all the bait should be placed inside the trap (Figure 1).



Figure 1: The set up of soft-sided cage traps which can be used to capture small to medium sized macropodids. *Top and bottom left:* A close-up image of a soft-sided rock-wallaby trap set in place, showing the cord tying the trap securely in place. *Top right:* A trap setup ready to capture an animal with fresh bait inside. *Bottom right:* The inside of a soft-sided cage trap with bait in place, noting the majority of bait is behind the treadle. *Note*: all traps are located on a flat surface and secured in place with rocks and cord.

Active Trapping:

Four nights trapping per location is recommended. This timing is based on the decline in the proportion of new animals caught with each night's trapping. It also limits re-trapping events, and reduces the general disturbance caused to the animals at a fixed location. In the late afternoon (approximately 4pm) prior to the first night of trapping, the fixings used to hold the trap doors open should be removed, enabling the trap to be engaged. At least 80% of the bait should be placed past the triggering treadle. Always ensure that the drop bar is freed from all fixings and the door can fall and close without obstructions (i.e. no small rocks, dirt mounds, sticks).

All traps should be cleared within 18 hours of setting, and preferably by 9am the following morning. At least two people should attend each trap whilst checking for captures. Any animals captured should be transferred into close-weave heavy duty cotton bags (approximately 80cm long x 60cm wide for YFRWs), ideally made of heavy-duty cotton drill fabric, and marked with the trap number (Figure 2). These specific bags are used for their durability, breathability, and the lack of visibility, blocking almost all light from passing through the fabric and stimulating the animal. Handlers should have enough trapping bags with them to accommodate capturing females with pouch young.

Approaching a triggered trap should be done slowly, quietly and from behind (where possible). A quick examination of the animal inside should be done to assess if it is male or female, or on subsequent trapping mornings, a recapture from the previous days' trapping. Recaptures should be released immediately.

It is recommended to have a small trapping bag ready on approach to a trap in case there is a pouch young loose in the trap. Any pouch young or juveniles which are found loose in the trap should be removed from the trap immediately and placed into a trapping bag before attempting to extract the mother. The young should be removed via the slide door at the back of the trap whilst a second person watches the mother from the front of the trap. If the animal has been identified as a female, the pouch should be checked from a distance (binoculars, if possible) for a mound/lump which may indicate a young still present in the pouch. If the captured animal is a male, it can be extracted from the trap without further examination.

The person designated to remove the animal from the trap should be positioned at the rear end (solid slide end) of the trap and the person designate to bag the animal at the door end of the trap. The slide in the back of the trap can be lifted about 3cm by the extractor to see where the animal is positioned in the trap. The two researchers should quietly communicate to each other during this process about the position of the animal (e.g. facing the front or rear of the trap). When access to the base of the tail is presented near the rear end of the trap, the extractor can lift the slide ~10-15cm, put their hand through, and grasp the base of the tail. The trap slide can then be lifted out of the back of the trap, and the animal pulled out for bagging. If it is a large animal and requires two hands to lift or is potentially a female with pouch young still in the pouch, then a hand should be placed between the rear legs (over the pouch/scrotal area) to aide in getting the animal out the trap and prevent any young from being thrown from the pouch. This can also be done if the animal tries to wedge themselves in the trap with their legs.

Once out of the trap, the animal should be held firmly by the base of the tail, upside down, and with their hindlegs facing away from both people doing the bagging (i.e. the animal's side should be facing the person bagging, with its head down). The bagger should then ensure the bag is held open, and that the opening is wide enough for the animal to be directed inside. In one quick sweeping motion from

low to high, the bagger should then scoop the animal into the bag. This process should be done as quickly as possible as handling can be highly stressful for the animal. The bag should be tied up securely, and the trap location/number should then be recorded on the bag (i.e. on a tag or flagging tape tied onto the bag) to ensure the animal is later returned to the correct location after processing. Whilst bagging an animal, if it begins to thrash around or spiral, lower the animal towards the ground until their front legs rest on the ground, this will stabilise the animal and stop it moving around, and then it can be quickly lifted up for bagging again once stabilised.



Figure 2: A typical heavy-duty cotton drill fabric bag used for bagging and holding rock-wallabies whilst processing.

Processing:

It is recommended that, if possible, all captured animals should be brought to a central location for processing and the collection of biological samples. This minimises the time animals are in traps, provides a controlled environment with all equipment required on hand, and allows the animal time to calm down after the bagging process. This has been shown to help reduce the amount of bleeding after tissue sampling (e.g. ear biopsy) in YFRWs with the majority of animals not bleeding at all after sampling (*pers. obs.* D. Taggart, L. Werner). All captured animals should be hung in their bags in a cool, shaded location away from the processing area to eliminate any stress associated with proximity to humans (i.e. scent and sounds). Females and their young should be hung together in close

proximity to one another but in separate bags. Always ensure that the pouch young cannot be squashed by the mother.

At least two, preferably three, people should be present for processing: a handler, a processer, and a record keeper/assistant. Before opening the bag, the animal should be weighed using hand-held scales. The weight of the empty bag can be subtracted from the total weight later. Any small to medium sized macropodid can be handled whilst the handler is sitting in a chair or kneeling on the ground depending on the size of the animal. Larger, stronger, or more anxious animals should be processed on the ground, whilst smaller, more relaxed animals can be processed whilst sitting (Figure 3). Females with pouch young should always be processed first and released once processing is complete. Large, furred pouch young can be processed as an independent individual, but if the young are small and still attached to the teat, they should not be removed and the young's measurements included with the female's processing data. Once weighed, the animal can be scanned for a microchip through the bag to determine if it is a recapture from a previous trip or trapping days. When the animal has been restrained, the bag can then be untied, and the animal checked for the presence of an ear tag. During processing, the animal should always be kept in the bag with the eyes covered as this reduces stress.

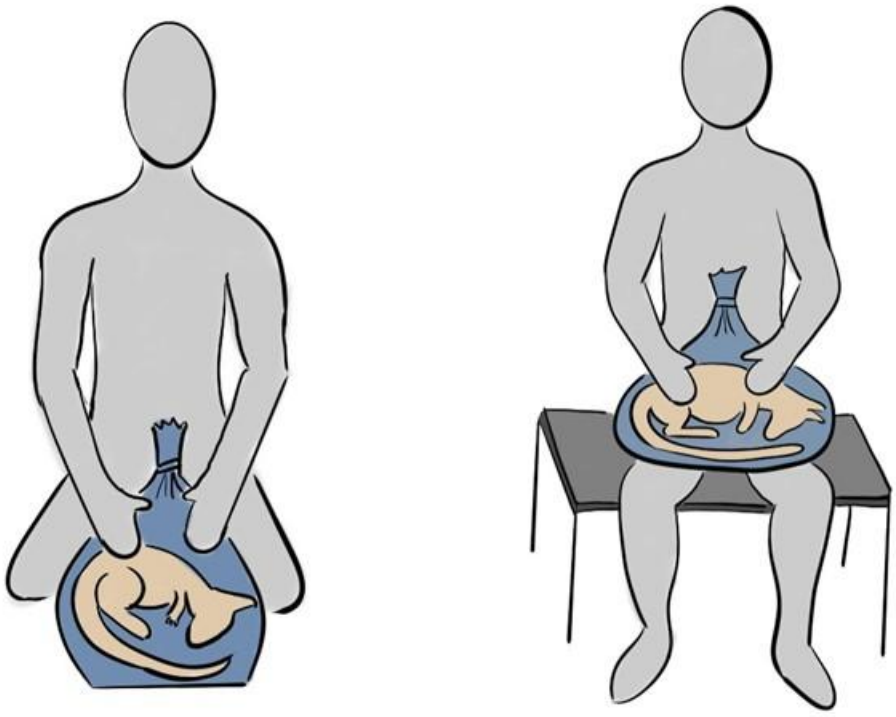


Figure 3: Two of the positions which handlers can use to safely control small to medium sized macropodids whilst processing. For larger, stronger, or more active individuals, handling should occur on the ground (*left*) to ensure greater control over the animal. Smaller individuals can be handled whilst sitting (*right*).

Order of Observations and Morphometric Sampling:

The following order should be followed when processing any small or medium sized macropodid, depending on the samples or information required for the project. Data should be collected from the rear of the animal first, before progressing towards the head.

The information which can be obtained, if required, and an explanation as to how to proceed to gather this information is detailed below.

1. Check sex of animal
2. Females: take microbiome swab before touching the pouch, check for pouch young. If a pouch young is present, measure it (head width/length, pes length, tail length, assess sex, weight off the teat). Make a note of pouch conditions
3. Males: measure scrotal diameter
4. Measure pes length
5. Take note of parasites in groin area
6. Measure tail length
7. Measure ear length
8. Take microbiome oral swab
9. Measure head width and length
10. Take note of parasites behind ears
11. Administer microchip
12. Collect ear tissue biopsy
13. Administer ear tag
14. Take blood sample
15. Administer vitamin E injection

Note: females with large pouch young may need to have their pouch young returned back to the pouch and/or reattached to the teat (Taggart et al. 2005; 2010) before females are given a vitamin E injection and released at their site of capture.

Measurements:

All measurements should be collected using vernier callipers and recorded in millimetres, except for tail length which is collected using a flexible measuring tape (i.e. dressmakers measuring tape) and usually recorded in centimetres.

Hind-end Measurements:

Pes:

For measuring pes length, one hind foot can be exposed in the bag by the handler and measurements taken from the base of the heel to the top of the longest toe, not including the toenail (Figure 4). The pes can then be returned into the bag. Be sure to keep the head in the dark within the bag.



Figure 4: The hindfoot of a yellow-footed rock-wallaby (*Petrogale xanthopus*) being held for obtaining pes length. This method of measuring pes length can also be used for other small or medium sized macropodids.

Tail:

The tail can be extracted from the bag for measuring tail length. Tail length measurements are taken from the base of the tail where it joins the body to the tip of the tail bone, not including the fur at the end of the tail (Figure 5). The tail can then be returned into the bag.



Figure 5: The process of measuring tail length of small to medium sized macropodids. *Note*: the tape measure should reach from the very base of the tail where it joins the body to the tip of the tail vertebrae (not the fur).

Pouch Assessment and Measurement of Pouch Young:

Whilst the animal is lying on its side and still secure in the bag (Figure 3), pull the animal's top hindleg (should be the animal's right hindleg) backwards, to expose the pouch region. The person processing can then examine the inside of the pouch by gently opening the skin at the pouch entrance. Notes should be made on the condition of the pouch (i.e. dry, moist, dirty, clean, deep, shallow), if there is a pouch young present, and if there is an enlarged mammary gland (Figure 6). If there is a pouch young present, measurements of head length, head width, and pes length (if possible) should be obtained carefully using callipers. Ensure that any small, unfurred pouch young which are not able to reattach themselves to the teat (i.e. incomplete lip formation) are not dislodged. If small pouch young are dislodged from the teat, they can be careful reattached using blunt, fine-tipped forceps as described in Taggart *et al*. (2005). The sex of the pouch young and teat to which it is attached to should also be noted. Teat position should be recorded relative to the animal (i.e. front left teat, back right teat), not from the person processing's perspective.



Figure 6: Images showing the inside of a yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) pouch. *Top*: a pouch with a small pouch young attached to a teat. *Bottom:* a pouch with a small pouch young and an enlarged mammary gland with an elongated teat, associated with the young currently being weaned.

Scrotal Measurements:

To obtain scrotal measurements, the animal should be on its side with the top hindleg pulled backwards in a similar manner as if exposing a female's pouch. The person processing can then use vernier callipers to measure the scrotal diameter (width) and length, if required. For scrotal diameter, this is done by lifting the scrotum slightly away from the body and measuring with callipers, so that they just brush the widest points of the scrotum (Figure 7). Avoid pushing the testes together.



Figure 7: The technique used to measure the scrotal diameter of small to medium sized macropodids. *Note*: the scrotum is being held slightly away from the body to allow measurements to be taken, and that the callipers are gently brushing the edges of the scrotum.

Anterior-end Measurements:

The person handling the animal should only prepare the animal to obtain head or ear measurements, biological samples, and give identification markings if confident in doing so and experienced with this process. These are the most stressful processing steps for the animal and the time when animals are most likely to try to escape. Handling should occur on the ground with the animal upright in the bag (Figure 8). A blindfold (i.e. folded cotton bag) should be used across the animal's eyes when exposing the animal's head to eliminate light exposure. Measurements of head length, head width and ear length should then be taken and recorded in millimetres.

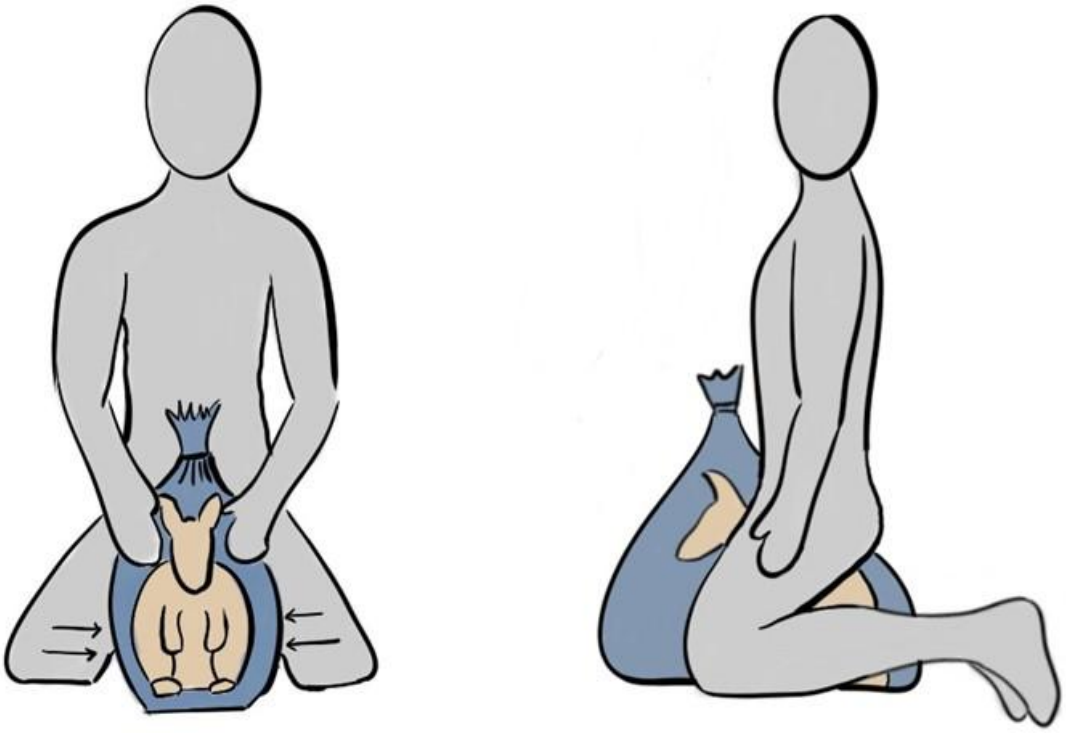


Figure 8: The positions which can be used to obtain head measurements or oral swabs from small to medium sized macropodids. *Note*: the handler's thighs and calves are used to corral the animal to restrict movement. The feet should be crossed behind the animal to avoid escape from pushing backwards.

Ear length:

Ear length is measured with the prongs of the callipers from the inside of the base of the ear to the furthest tip of the ear. The ear alone can be extracted from the bag for this measurement. After each measurement, the animal's body part should be returned to the bag to minimise light reaching the animal's eyes and to ensure there is no prolonged opening of the bag allowing for escape.



Figure 9: The process of measuring ear length of small to medium sized macropodids. *Note*: the curved prongs of the callipers are being used.

Head Width/Length:

Whilst restraining the animal on the ground (Figure 8) place a blindfold over the animal's eyes (but not its nose) whilst still in the bag (i.e. a small cotton bag folded up, approximately 8-10cm wide) and hold the blindfold in place. This is done by reaching into the bag whilst holding the blindfold length-ways, following up the animal's back towards their head and feeling for the ears. Once you've found the ears, the blindfold can be slid in front of the ears, which will naturally cover the animal's eyes.

The blindfold is then held firmly in place by holding beneath the animal's chin. The handler must ensure that during this process no light gets into the bag to stimulate the animal. The bag must only be pulled open to expose the animal's head once the blindfold is secured in place. Do not remove any other part of the animal's body from the bag. Head width should be measured from the widest point of the head, located just behind the eyes. Head length is measured from the tip of the nose to the backmost point of the skull (Figure 10).



Figure 10: Images showing the process of obtaining head measurements of small to medium sized macropodids. *Left:* a blindfold firmly held in place over the eyes of a yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*). *Top right:* measuring head width. *Bottom right:* measuring head length. *Note*: only the head is extracted from the bag for this process, the rest of the body remains in the bag.

Sample Acquisition:

Microbiome:

Samples for assessing an animal's microbiome can be obtained from faeces, and from swabs taken from the pouch, the skin around the pouch and the oral cavity. Samples of particular interest might be faecal samples to compare the animal's faecal microbiome with the diet or microbial differences across seasons. Pouch swabs taken from females with pouch young of varying ages, and oral swabs from females and their pouch young can provide insights to female-offspring microbial relationships.

Faecal:

Faecal microbiome samples must be fresh and can be obtained from the trap which the animal was caught in. Faeces should be collected from the trap once the animal has been removed by placing an inverted snap-lock bag over your hand to pick up the scat. Later the scat can be transferred into a 1.5mL Eppendorf tube containing 70-100% ethanol whilst wearing gloves to avoid skin contamination. Ensure that you label both the snap-lock bag and Eppendorf tube well. Once the faeces is in the ethanol tube, shake well to disperse the ethanol throughout the sample (Eisenhofer *et al*. 2021).

Pouch:

Swabs from the pouch, and skin around pouch should be obtained before any standard animal processing has occurred (i.e. before opening the pouch to check pouch conditions). All swab samples should be obtained whilst wearing gloves and using a sterile swab. Pouch skin swabs should be gathered first by wiping the swab around the opening of the pouch. Inner pouch swabs should be collected by holding open the pouch and wiping the swab across the skin lining the inner pouch. Separate swabs should be used for areas of interest (i.e. one for skin surrounding pouch, another for inside the pouch).

Oral:

Oral swabs should also be collected prior to standard animal processing (i.e. taking head measurements). Swabs are taken be pushing the swab inside the corner of the macropodid's mouth and rotating the swab in the cheek/gum cavity (Figure 6). Ensure that the swab does not touch any facial skin and only touches the lips and inside of the animal's mouth.

Storage:

The end of all swabs used to collect microbiome samples should be cut off directly into a 1.5mL Eppendorf tube containing DNA/RNA shield (Zymo Research, USA) immediately following collection. Field blank swabs should also be collected from each site and each day by waving a clean

swab in the air and then storing as previously described. This can later be used to identify contaminants in the air which may alter microbial results (Eisenhofer *et al*. 2019; 2021).



Figure 11: Two techniques for obtaining oral microbiome swabs from small to medium sized macropodids. *Left:* an oral swab being obtained from a juvenile yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) whilst on the handler's lap. *Right:* an oral swab being obtained from a large individual which is being handled on the ground for greater control of the animal (refer to Figure 8 and Figure 10).

Tissue:

Tissue for genetic analysis from small to medium sized macropodids is generally collected from the ear. To do this, the animal should be positioned in the bag on its feet, or for very quiet or smaller animals, on its side (Figure 3; Figure 8). Only the ear should be extracted from the bag, ensuring the eyes are still covered. As a general convention, tissue samples are taken from the right ear for males and left ear for females.

The inside and outside of the ear should be cleaned thoroughly using a gauze swab and 70-100% ethanol. The sample should be taken from the middle section of the ear. When looking closely at the inside of the ear, you can see that it is covered in small dimples. When deciding where to take a biopsy from, you must look for an area where there are no dimples (Figure 12) which is indicative of an area where there are no major blood vessels. Once the area has been identified, keep the tip of your

thumb in position to mark the target area. The sample is then taken using a leather punch tool and a small piece of folded cardboard, or a similar single-use equivalent.

If using a leather punch, it should be set to obtain a sample of approximately 3-4mm diameter (2mm for large pouch young) and be cleaned thoroughly with 70-100% ethanol before and after use. The cardboard should be placed on the back of the ear between the ear and the punch (Figure 12). Clamp down strongly on the leather punch to obtain the sample, there should be an audible click as the punch goes through the ear tissue and cardboard. To be sure, take a little pressure off the leather punch, rotate 90 degrees, and clamp down again. This ensures that the tissue has been cut through cleanly. Release the leather punch and extract the tissue sample from inside the leather punch.

Samples should be stored in a 1.5mL Eppendorf tube in 70-100% ethanol and kept frozen as soon as possible. If the ear bleeds, apply pressure with gauze pads directly to the ear until bleeding stops. Veterinary anti-septic spray can be applied if required.



Figure 12: The positioning of a macropodid ear for collection of an ear biopsy. *Left:* the process of examining for a section of tissue which has no dimples indicating a lack of major veins. *Right:* taking an ear biopsy using a leather punch tool. *Note*: the cardboard is placed on the opposite side of the ear from the punch.

Blood:

Blood samples from small to medium sized macropodids can be readily collected from the lateral caudal tail vein, at the base of the tail. This vein runs laterally along either side of the tail. The animal should be positioned on its side with the tail out of the bag. The animal should be wrapped tightly in the bag to stop it from kicking or moving during the procedure. This process should be conducted with an animal handler sitting in a chair, and the person obtaining the blood sample sitting opposite, holding the unused length of the tail under their arm or between their legs to ensure it cannot move. A scalpel blade or another shaving instrument can then be used to shave an area of fur on the lateral side of the base of the tail (Figure 13). In YFRWs, this often corresponds to where the fur changes colour. The area to be shaved will depend on the species, but for YFRWs this area is approximately 4cm wide by 8cm long, and about 15cm down from the base of the tail. This enables ready identification of the vein. The area should be cleaned using gauze and 70-100% ethanol prior to sampling. Apply a torniquet to the base of the tail at the highest point possible, where the tail joins the body. You should see the vein protrude up as a result of its constriction (Figure 13).

Once the vein is visible, use a suitably sized needle and syringe for the species (For YFRW, 22G needle and 2mL syringe) to obtain the blood sample. To do this, whilst holding the vein between the tips of your thumb and finger, insert the needle at a 45-degree angle to the skin with the needle bevel pointing upwards initially (Figure 13). Once blood fills the small chamber at the top of the needle, turn the needle/syringe 180 degrees (now the bevel will be pointing down toward the tail), lower the syringe so it is roughly perpendicular to the tail, and begin to slowly apply pressure to the syringe plunger to extract the sample (Figure 13). If the blood flow ceases, manoeuvre the needle/syringe until blood flows again. A sample of 2mL can be collected from any YFRW above 3kg in weight. Once the required amount of blood has been obtained, remove the needle and the torniquet, and immediately apply pressure to the puncture site with a gauze swab. Maintain pressure on the site until it stops bleeding, which can take several minutes.

Blood samples should be stored in a blood tube which is suitable for the analysis which is required. Where necessary, blood samples can be stored for short periods at 4ºC. If required, samples should be spun in a centrifuge as soon as possible after collection, ideally within a few minutes, to separate plasma from red blood cells. The quicker centrifuging occurs, the lesser the risk of sample haemolysis occurring. Whole blood and plasma samples should be kept frozen as soon as practically possible.



Figure 13: The process of obtaining a blood sample from the lateral tail vein of a small to medium sized macropodid. *Top left:* lateral caudal tail vein with torniquet. *Top right:* vein being held between fingers to stop it from being pushed aside when inserting the needle. *Bottom left:* the angle (approximately 45 degrees from the tail) of the needle when inserted into the vein. *Bottom right:* blood being extracted from the tail vein with the needle now approximately parallel to the tail.

Identification and Marking:

The most common ways of marking small to medium sized macropodids for future identification is with ear tags, ear notching, and/or microchips. Ear notching for wild animals can be misleading and can cause mis-identifications due to naturally occurring damage to ears (i.e. from fighting). For this reason, ear tags and microchips are the preferred methods.

Microchips:

Microchips are given in the back of the neck, between the shoulder blades, just beneath the skin. To do this, restrain the animal on its feet, or for quieter animals, on their side (Figure 3; Figure 8) in the bag. The neck and shoulder blade area should be accessible from the bag opening. Clean the back of the neck area thoroughly with 70-100% ethanol or another disinfecting agent. Tent the skin between the animal's shoulder blades with your fingers to create a pocket for the chip to be inserted into. Insert the needle into this skin pocket in the direction of head to rump (Figure 14). You should feel the

needle push through the skin and once under the skin, plunge the microchip applicator to insert the microchip. Do not pull the needle out. With the hand which was tenting the skin, now hold either side of the needle with the index finger and thumb whilst under the skin, then pull the needle out (Figure 14). This stops the microchip from being pulled out of the skin with the needle of the applicator. Check that the microchip has remained in place by scanning with a microchip reader.



Figure 14: A microchip being inserted into a yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*). *Note*: once the needle is inserted and microchip given, that the thumb and forefinger hold the needle whilst being removed from the skin, to ensure the microchip stays in place.

Ear tags:

Ear tags used for many small to medium sized macropodids are generally made of plastic. For YFRWs, a two-piece, round sheep tag is used (Figure 15) which can be sourced online. These tags are then cut smaller, and the cut edges smoothed to ensure that they did not rub or irritate the animal's ear (Figure 15). Ear tags should be applied to the ear at the site where an ear biopsy has been taken. While the animal is still in position and the ear extracted from the bag after having a tissue sample taken,a pply the ear tag using the appropriate applicator. If there is some bleeding after application, apply pressure with gauze pads until bleeding stops.



Figure 15: Ear tags which can be used for tagging small to medium sized macropodids. *Top:* Ear tag components before (*left*) and after (*right*) being cut down to a smaller size. *Bottom:* Ear-tag components once in place in the ear of a yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*).

Return of Furred Pouch Young:

Any furred pouch young found out of the pouch upon capture, needs to be returned to the pouch before release. This ensures that the female and young will be together on release, and that the young won't be abandoned.

To place any pouch young back into the pouch, the female must be held in the bag with the pouch exposed (Figure 16). The handling technique to do this is critical, as the pouch can be difficult to open wide enough to return the pouch young as the female can hold it tight, often making the opening too small for a large pouch young to enter easily. To do this, the bag should be positioned so that the animal is sitting upright facing away from the handler, with the female's back towards the handler's chest. The bag should be manoeuvred so that the bag opening is adjacent to the pouch opening. The handler should sit upright with the animal's tail held between their legs. The handler should then hold the animal so that one arm is used to restrain the hind legs pulled anteriorly (whilst still in the bag), and the other arm is restraining the forelimbs (whilst still in the bag). The bag can then be opened to expose the pouch area (Figure 16). The head of the female should be kept in the bag in darkness throughout this process.

Once restrained properly and pouch is exposed, gently open the pouch, and push the pouch young inside. The best way to do this is by pushing the pouch young into the pouch headfirst in a rotating motion to stimulate rolling into the pouch. At this point it is handy to have an assistant helping to manoeuvre the pouch around the pouch young. Once in the pouch, use tape (i.e. sports rigid strapping tape or veterinary tape) to close the pouch. This is done to ensure that the young is not ejected from the pouch of the panicked mother upon release. Ensure that there are no gaps big enough for the young to get out. Females with pouch young taped in place should be released instantly to ensure that there is no time for the tape to be removed by the mother or young. The process of replacing the pouch young should be done in situ to the release site so the female can be released immediately once the young is returned to the pouch. This can also be done for females which have smaller young in the pouch to ensure that they are not dislodged from the teat.

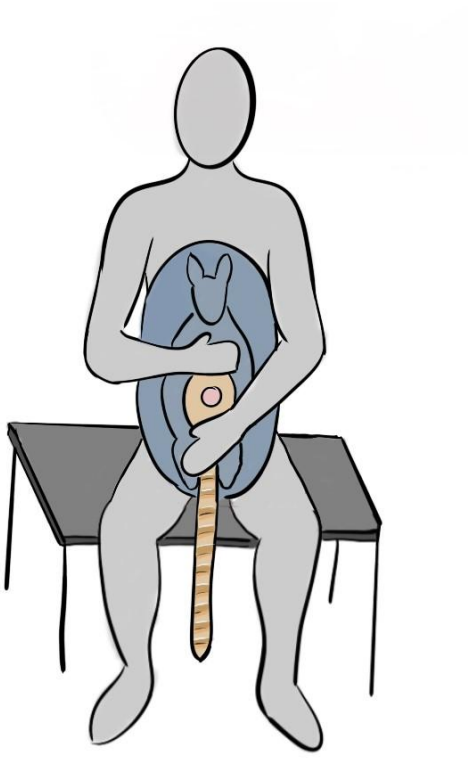


Figure 16: The positioning of the handler whilst returning a pouch young to the pouch of a rock-wallaby.

Vitamin E Injection:

Vitamin E can be administered to macropodids before release as it helps reduce the risk of animals developing capture myopathy, a buildup of toxins which can inhibit muscle and organ function sometimes resulting in death (Green-Barber *et al*. 2018; Breed *et al*. 2019 Câmara *et al*. 2020). Vitamin E is an antioxidant, and its administration often helps to dissipate these toxins.

All sub-adult and adult YFRWs should be given vitamin E prior to release. Juveniles/pouch young do not require a vitamin E dosage. A vitamin E injectable should be drawn into a suitably sized needle and syringe (For YFRWs, 25G needle and 1mL syringe) and administered intra-muscular, usually in the rump (Figure 17). Dosage rates will depend on the species and specific serum which is used, and should be assessed as per the manufacturer's instructions or following consultation with a veterinarian.

Vitamin E injections should be given once processing is complete, or for females with the pouch young, once the young has been replaced. Release should occur as quickly as possible after the injection has been administered.



Figure 17: Administration of a vitamin E injection after processing and prior to releasing a small to medium sized macropodid.

Releasing:

All animals should be released at the site of capture, or as close to the capture site as possible. Females with pouch young should always be processed and released first. As a general rule, when at the site of capture, find a flat position with a natural 'pathway' for the animal to escape along. Whilst kneeling, position the closed bag in front of you on the ground with the opening facing away. Manoeuvre the animal so that they are standing on their feet, and you can feel that the head is facing the opening. Untie the bag but hold it closed until ready to release. Place one foot, or knee, onto the corner of the bag to stop the animal getting caught up in the bag and taking the bag with it. Open the bag and pull the opening back to expose the animal's head. Wait until it leaves the bag on its own accord.

Main Points and Conclusions:

Trapping, handling, and processing small and medium sized macropodids, including YFRWs, requires specific skills and techniques which if done competently, can provide a variety of useful data, whilst minimising stress to the animal.

The key points from this guide are:

- Traps should be placed in areas where animals are likely to be spending time (based on presence of scats), and positioned on flat ground where the trap can be adequately secured.
- Free-feeding traps for at least one month prior to active trapping will boost the likelihood of capture.
- Bagging of small to medium sized macropodids should be done quickly and calmly to ensure the safety of the animal and handlers.
- Processing animals at a central location gives the animal time to calm down after being extracted from the trap which can result in less (or no) bleeding after tissue sampling and keeps the animal much calmer throughout.
- Always keep the head/and or eyes covered during processing and sampling, and keep a firm hold of the animal whilst processing as they are prone to sudden erratic movements.
- When obtaining a tissue sample, take the ear biopsy from the middle region of the ear where there are no dimples in the skin.
- Teamwork between researchers when processing is vital for data and samples to be obtained quickly and efficiently.
- Obtain as many samples as possible from any captured animal to maximise the data which can be gained, you never know when something may be useful in the future.
- Safe handling techniques, such as those described here, are vital for reducing stress on the animal and risks to staff.

The trapping, handling, and processing of small to medium sized macropodids should never be undertaken by anyone who is not trained in doing so, or if there is not an experienced macropodid researcher involved. Any trapping and handling of animals should always be approved by the relevant animal ethics committee and with the appropriate permits granted. Animals can be extremely strong, and so mishandling could result in harm to the animal or staff.

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Appendices

Appendix 1:

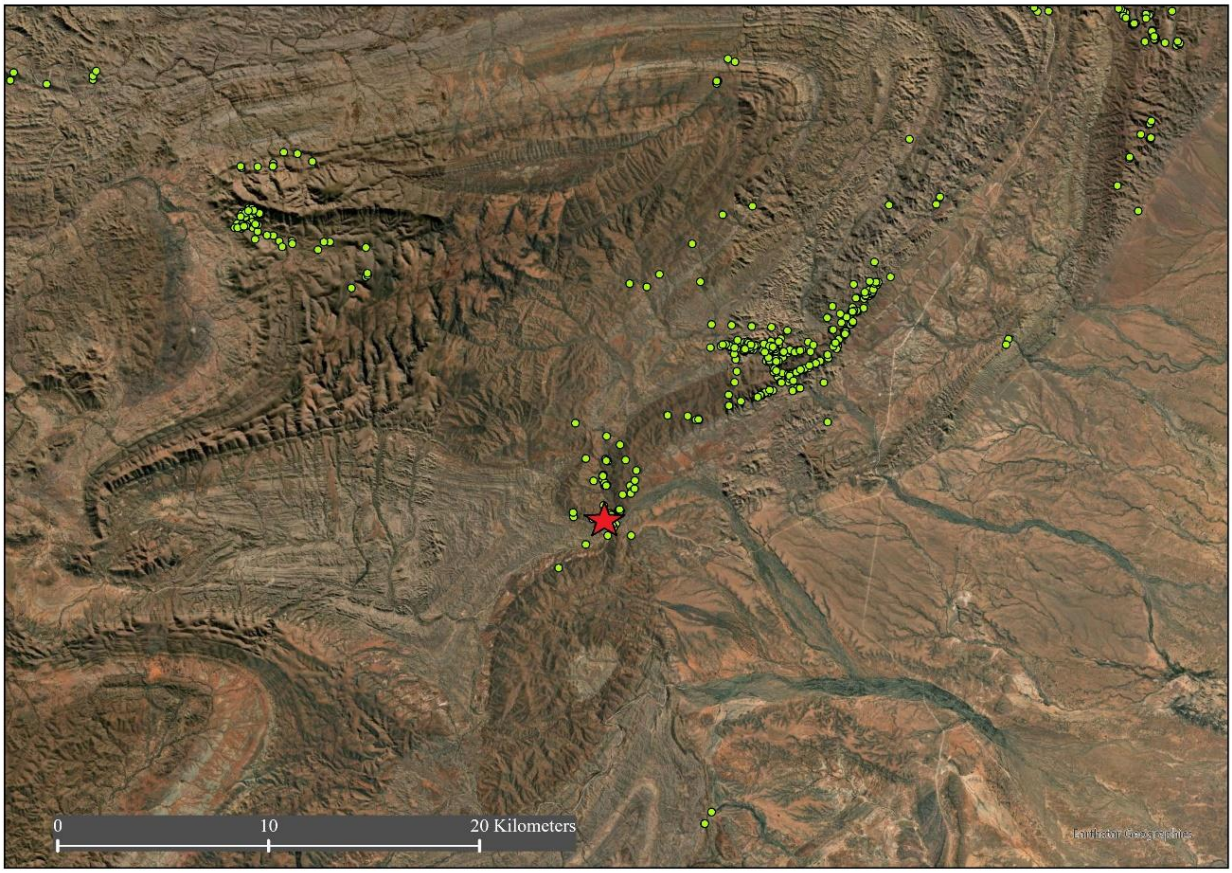


Figure S1: Yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) occurrence points (green points) obtained from the Atlas of Living Australia (www.ala.org.au) in the Gammon Ranges region, South Australia. The sampling site in this study, Italowie Gap, is indicated (red star).

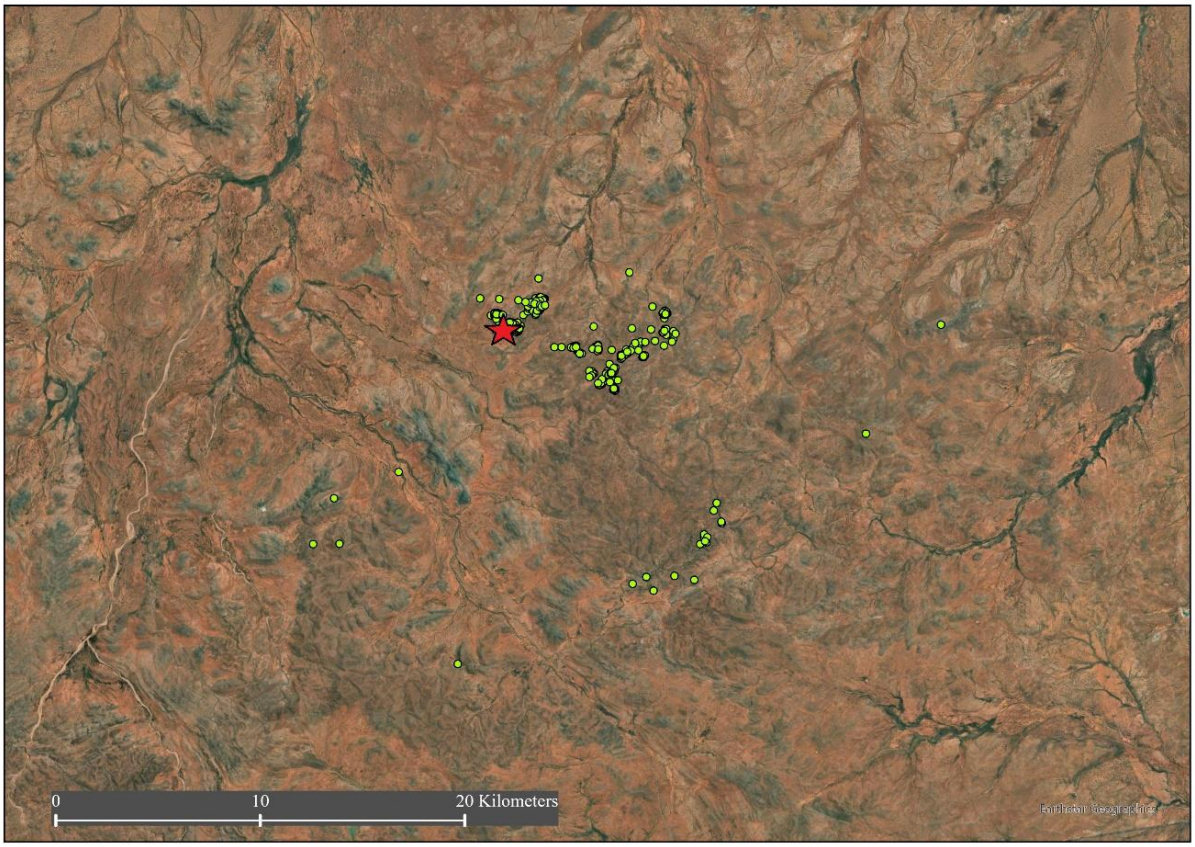


Figure S2: Yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) occurrence points (green points) obtained from the Atlas of Living Australia (www.ala.org.au) in the Olary Ranges region, South Australia. The sampling site in this study, Bimba Hill, is indicated (red star).

Table S1: Adult yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) blood biochemistry values from Italowie Gap and Bimba Bill during spring 2021 and autumn 2022. Reference interval is determined using a 95% confidence interval of results.

Adult	Mean (± S.E)				Reference Interval (95% CI)
	Italowie Gap		Bimba Hill		
	Spring 2021	*Autumn 2022*	*Spring 2021*	*Autumn 2022*	
FRAP (mmol/L)	0.60 (± 0.02)	0.54 (± 0.02)	0.70 (± 0.05)	0.94 (± 0.11)	0.63 – 0.84
TEAC (mmol/L)	2.28 (± 0.04)	2.28 (± 0.01)	2.27 (± 0.04)	2.26 (± 0.02)	2.24 – 2.29
TBARS (µmol/L)	2.54 (± 0.29)	1.65 (± 0.08)	2.82 (± 0.24)	2.05 (± 0.15)	1.96 – 2.44
Ascorbic Acid (µmol/L)	20.60 (± 1.55)	20.62 (± 1.23)	20.46 (± 1.29)	23.14 (± 0.86)	20.38 – 22.73
Retinol (µg/mL)	0.03 (± 0.01)	0.06 (± 0.02)	0.08 (± 0.03)	0.12 (± 0.04)	0.04 – 0.12
a-tocopherol (µg/mL)	5.46 (± 1.61)	9.20 (± 0.59)	5.91 (± 0.75)	8.71 (± 0.52)	6.68 – 8.64
Sodium (mmol/L)	139.6 (± 1.57)	141.5 (± 1.23)	141.2 (± 0.8)	135.8 (± 0.74)	137.5 – 140.27
Potassium (mmol/L)	4 (± 0.08)	3.82 (± 0.17)	4 (± 0.06)	4.28 (± 0.17)	3.90 – 4.23
Chloride (mmol/L)	91.2 (± 1.83)	94.5 (± 1.75)	93.6 (± 1.03)	93.6 (± 0.82)	92.08 – 94.61
Phosphorus (mmol/L)	1.52 (± 0.08)	1.31 (± 0.14)	1.61 (± 0.2)	1.33 (± 0.15)	1.27 – 1.57
Magnesium (mmol/L)	0.76 (± 0.05)	1.06 (± 0.09)	0.91 (± 0.04)	1.00 (± 0.05)	0.88 – 1.02
Calcium (mmol/L)	2.26 (± 0.05)	2.40 (± 0.05)	2.29 (± 0.06)	2.28 (± 0.02)	2.26 – 2.35
Iron (µmol/L)	27.18 (± 6.57)	37.52 (± 6.57)	33.7 (± 6.35)	20.72 (± 2.31)	23.07 – 33.6
Bicarbonate (mmol/L)	27.2 (± 0.97)	30.33 (± 1.05)	23.2 (± 0.74)	24 (± 0.79)	24.55 – 27.29
Total Protein (g/L)	60.9 (± 1.79)	59.25 (± 0.87)	59.56 (± 2.26)	57.29 (± 1.25)	57.38 – 60.37
Albumin (g/L)	41.46 (± 1.76)	39.33 (± 1.08)	40.62 (± 2.0)	39.17 (± 0.73)	38.72 – 41.14
Globulin (g/L)	19.44 (± 1.03)	19.92 (± 1.39)	18.94 (± 0.36)	18.12 (± 1.26)	17.76 – 20.14
Cholesterol (mmol/L)	3.54 (± 0.2)	3.21 (± 0.27)	4.01 (± 0.19)	3.90 (± 0.33)	3.38 – 4.0
Triglycerides (mmol/L)	0.432 (± 0.04)	0.66 (± 0.16)	0.88 (± 0.13)	0.85 (± 0.14)	0.58 – 0.88
Glucose (mmol/L)	6.54 (± 0.6)	10.05 (± 1.05)	10.38 (± 1.42)	10.28 (± 0.92)	8.41 – 10.65
Amylase (IU/L)	209 (± 15.37)	241.33 (± 35.47)	134 (± 26.04)	117.5 (± 16.91)	136.71 – 196.98
Urea (mmol/L)	11.98 (± 0.69)	9.45 (± 0.66)	12.06 (± 0.51)	13.8 (± 0.96)	11.08 – 13.15
Creatinine (µmol/L)	97.2 (± 8.41)	76.50 (± 4.8)	90.6 (± 6.27)	110.3 (± 3.62)	89.08 – 103.3
Uric Acid (µmol/L)	23.6 (± 4.63)	26.33 (± 3.04)	49.8 (± 13.68)	40.6 (± 4.8)	28.51 – 43.12
Alkaline Phosphatase (ALP) (IU/L)	234.2 (± 79.9)	845.83 (± 228.75)	220.6 (± 22.47)	328.5 (± 86.78)	256.69 – 561.31
Alanine aminotransferase (ALT) (IU/L)	34.4 (± 5.24)	23.33 (± 1.33)	45.8 (± 7.42)	41.3 (± 5.85)	30.49 – 42.89
Aspartate transaminase (AST) (IU/L)	271.8 (± 61.75)	149 (± 14.44)	385 (± 70)	438.9 (± 77.64)	249.97 – 409.03
Gamma-glutamyltransferase (GGT) (IU/L)	22.6 (± 1.17)	26 (± 4.57)	27.2 (± 2.48)	31.2 (± 3.18)	24.18 – 30.98
Cholinesterase (ChE) (IU/L)	984.8 (± 165.66)	1105 (± 103)	1185.2 (± 273.26)	1096.4 (± 155.74)	928.31 – 1259.7
Lactate dehydrogenase (LDH) (IU/L)	1220.4 (± 162.42)	900.83 (± 159.04)	1714.2 (± 306.23)	1696.3 (± 272.8)	1148.85 – 1700.46
Creatine kinase (CK) (IU/L)	21614 (± 2980.44)	4445.67 (± 1589.52)	15890 (± 3727.28)	17049 (± 3557.09)	10289.98 – 18164.68

Table S2: Sub-adult and juvenile yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) blood biochemistry values from Italowie Gap and Bimba Bill during autumn 2021, spring 2021 and autumn 2022.

Sub-adult / Juvenile	Mean (± S.E)			
	Italowie Gap		Bimba Hill	
	Spring 2021	***Autumn 2021***	***Spring 2021***	***Autumn 2022***
FRAP (mmol/L)	0.59 (± 0.04)	1.07 (± 0.1)	0.94 (± 0.19)	0.7 (± 0.1)
TEAC (mmol/L)	2.1 (± 0.07)	2.18 (± 0.03)	2.23 (± 0.05)	2.21 (± 0.02)
TBARS (µmol/L)	3.38 (± 0.28)	3.32 (± 0.23)	3.17 (± 0.37)	1.63 (± 0.14)
Ascorbic Acid (µmol/L)	24.67 (± 1.24)	21.18 (± 0.62)	22.85 (± 1.51)	19.86 (± 0.99)
Retinol (µg/mL)	0.057 (± 0.04)	0.054 (± 0.01)	0.049 (± 0.01)	0.083 (± 0.01)
a-tocopherol (µg/mL)	9.02 (± 0.24)	6.49 (± 0.6)	3.4 (± 1.01)	9.2 (± 0.99)
Sodium (mmol/L)	139 (± 0)	136.17 (± 1.35)	139 (± 1.68)	133.8 (± 1.96)
Potassium (mmol/L)	4.17 (± 0.42)	6.12 (± 0.33)	4.85 (± 0.48)	4.38 (± 0.13)
Chloride (mmol/L)	99.33 (± 2.33)	94.33 (± 1.38)	94.75 (± 0.63)	91.8 (± 1.39)
Phosphorus (mmol/L)	1.9 (± 0.22)	2 (± 0.12)	2.38 (± 0.2)	1.49 (± 0.17)
Magnesium (mmol/L)	0.89 (± 0.16)	0.83 (± 0.07)	0.82 (± 0.06)	0.98 (± 0.04)
Calcium (mmol/L)	1.92 (± 0.43)	2.17 (± 0.16)	2.2 (± 0.1)	2.24 (± 0.06)
Iron (µmol/L)	29.63 (± 5.26)	18.78 (± 4.66)	19.95 (± 2.66)	11.82 (± 2.8)
Bicarbonate (mmol/L)	20.67 (± 1.33)	19 (± 0.85)	20 (± 1.1)	27.2 (± 1.59)
Total Protein (g/L)	48.97 (± 3.3)	48.5 (± 0.57)	53.98 (± 1.5)	51.3 (± 0.86)
Albumin (g/L)	36.97 (± 2.81)	37.42 (± 0.58)	40.55 (± 0.97)	37.8 (± 0.98)
Globulin (g/L)	12 (± 0.49)	11.08 (± 0.18)	13.43 (± 0.67)	13.5 (± 0.38)
Cholesterol (mmol/L)	3.26 (± 0.17)	3.74 (± 0.07)	2.94 (± 0.35)	3.78 (± 0.43)
Triglycerides (mmol/L)	0.65 (± 0.11)	0.39 (± 0.04)	0.41 (± 0.02)	0.41 (± 0.03)
Glucose (mmol/L)	8.5 (± 0.75)	7.28 (± 1.27)	10.43 (± 0.34)	8.42 (± 1.26)
Amylase (IU/L)	228.67 (± 51.67)	187.33 (± 33.27)	163.5 (± 45.2)	142.8 (± 37.62)
Urea (mmol/L)	10.3 (± 1.88)	9.8 (± 0.56)	15.28 (± 1.08)	14.34 (± 1.29)
Creatinine (µmol/L)	68.67 (± 1.86)	61 (± 2.77)	63.75 (± 4.8)	83 (± 6.16)
Uric Acid (µmol/L)	24.67 (± 2.33)	55.5 (± 4.29)	49.25 (± 4.87)	23 (± 6.2)
Alkaline Phosphatase (ALP) (IU/L)	1121.33 (± 52.78)	821.5 (± 41.38)	886.5 (± 56.14)	843 (± 70.87)
Alanine aminotransferase (ALT) (IU/L)	25.67 (± 4.18)	46.83 (± 4.47)	40 (± 3.34)	126.4 (± 73.16)
Aspartate transaminase (AST) (IU/L)	128.33 (± 17.02)	338.5 (± 48.82)	238.5 (± 31.92)	1075 (± 582.23)
Gamma-glutamyltransferase (GGT) (IU/L)	31 (± 8.02)	20 (± 4.31)	22.25 (± 4.03)	29 (± 1.84)
Cholinesterase (ChE) (IU/L)	1273 (± 25.11)	822.83 (± 145.56)	1224 (± 270.35)	997.4 (± 185.75)
Lactate dehydrogenase (LDH) (IU/L)	852.67 (± 61.41)	1256 (± 197.79)	1530.25 (± 195.69)	2226.4 (± 725.4)
Creatine kinase (CK) (IU/L)	4743.67 (± 1618.95)	9382.33 (± 2784.9)	9139.75 (± 2255.7)	12619 (± 5726.95)

Appendix 2:

Table S1: The number of individuals from each age class and the sex ratio of yellow-footed rock-wallabies (*Petrogale xanthopus xanthopus*) which were captured at Italowie Gap and Bimba Hill during autumn 2021, spring 2022 and autumn 2022. Recaptures are individuals which had been caught in previous trapping trips.

	First Trapping (Autumn 2021)		**Second Trapping (Spring 2021)**		**Third Trapping (Autumn 2022)**	
	Italowie	Bimba	Italowie	Bimba	Italowie	Bimba
Adults	5	0	6	5	7	10
Sub-adults	1	6	3	4	0	5
Juveniles	1	2	3	2	3	2
Total	7	8	12	11	10	17
Sexes	3F : 4M	3F : 5M	6F : 6M	4F : 7M	7F : 3M	8F : 9M
Recaptures			3	0	2	8

Appendix 3:

Italowie Gap

YFRW

October 2020

Grass
Cenchrus sp
Enneapogon caerulescens
Enneapogon lindleyanus
Enneapogon polyphyllus
Enneapogon sp
Eragrostis setifolia
Eragrostis sp
Eragrostis tenellula
Setaria dielsii
Sporobolus actinocladus
Triodia scariosa
Tripogonella loliiformis
Triraphis mollis

Herb (Perennial)
Boerhavia sp
Commicarpus australis
Sclerolaena sp
Sclerolaena uniflora
Sida fibulifera
Sida sp
Sida trichopoda
Tribulus sp

Tree
Acacia aneura
Capparis mitchellii
Casuarina pauper
Melaleuca lanceolata

Herb (Annual)
Calandrinia eremaea
Euphorbia australis
Euphorbia drummondii
Indigofera colutea
Portulaca oleracea
Sisymbrium erysimoides
Stenopetalum lineare

Shrub
Abutilon sp
Acacia burkittii
Acacia ligulata
Acacia oswaldii
Acacia rivalis
Acacia tetragonophylla
Acacia victoriae
Hibiscus sturtii
Indigofera australis
Marsdenia australis
Nitraria billardierei
Parkinsonia aculeata
Petalostylis labicheoides
Senna artemisioides

Italowie Gap

YFRW

March 2021:

Grass
Astrebla lappacea
Cenchrus sp
Digitaria brownii
Enneapogon lindleyanus
Eragrostis xerophilla
Setaria dielsii

Herb (Perennial)
Boerhavia sp
Convolvulus remotus
Malvastrum americanum

Herb (Annual)
Malva parviflora
Portulaca oleracea
Trianthema triquetrum

Shrub
Abutilon cryptopetalum
Abutilon sp
Acacia ligulata
Acacia victoriae
Hibiscus sturtii

Sida fibulifera
Sida sp
Solanum esuriale
Solanum sp

Italowie Gap

YFRW

August 2021

Grass
Cenchrus sp
Cymbopogon ambiguus
Digitaria brownii
Enneapogon lindleyanus
Panicum decompositum
Schismus barbatus
Setaria dielsii
Osteocarpum dipterocarpum

Herb (Perennial)
Convolvulus remotus
Goodenia pinnatifida
Malvastrum americanum
Rumex sp
Sclerolaena patenticuspis
Sclerolaena sp
Sclerolaena uniflora
Sida fibulifera
Sida sp
Sida trichopoda
Solanum esuriale

Tree
Acacia aneura
Allocausaurina sp

Herb (Annual)
Calandrinia eremaea
Glinus lotoides
Gnephosis arachnoidea
Heliotropium tanythrix
Indigofera colutea
Malva parviflora
Nicotiana velutina
Portulaca oleracea
Ptilotus exaltatus
Trianthema triquetrum

Shrub
Abutilon sp
Acacia burkittii
Acacia ligulata
Acacia oswaldii
Acacia rivalis
Acacia tetragonophylla
Acacia victoriae
Calytrix tetragona
Enchylaena tomentosa
Lysiana exocarpi
Maireana pyramidata
Parkinsonia aculeata
Petalostylis labicheoides
Ptilotus obovatus
Scaevola sp
Sclerolaena eriacantha
Senna artemisioides

Italowie Gap

YFRW

February 2022

Grass
Setaria dielsii
Malva parviflora
Portulaca oleracea

Herb (Annual)
Amaranthus sp

Herb (Perennial)
Boerhavia sp
Commicarpus australis
Malvastrum americanum
Sclerolaena uniflora
Sida fibulifera
Sida sp
Sida trichopoda

Shrub
Abutilon cryptopetalum
Abutilon sp
Ptilotus obovatus

Italowie Gap

YFRW

November 2022

Grass
Cenchrus sp
Digitaria brownii
Setaria dielsii
Nicotiana velutina

Herb (Perennial)
Boerhavia sp
Commicarpus australis
Glossocardia bidens
Malvastrum americanum
Olearia sp
Rumex sp
Sida sp
Sida trichopoda
Solanum esuriale
Solanum nigrum
Solanum sp

Tree
Melaleuca lanceolata

Herb (Annual)
Datura leichhardtii
Dysphania sp
Malva parviflora
Sigesbeckia australiensis
Sonchus oleraceus
Xanthium spinosum

Shrub
Abutilon cryptopetalum
Abutilon sp
Acacia burkittii
Acacia ligulata
Acacia oswaldii
Acacia sp
Acacia tetragonophylla
Acacia victoriae
Hibiscus sturtii
Lycium australe
Nitraria billardierei
Parkinsonia aculeata
Petalostylis labicheoides
Senna artemisioides

Bimba Hill

YFRW

December 2020

Herb (Annual)
Datura leichhardtii
Euphorbia drummondii
Nicotiana velutina
Portulaca oleracea
Roepera sp
Tetragonia tetragonoides
Trianthema triquetrum

Herb (Perennial)
Boerhavia sp
Solanum esuriale
Solanum nigrum
Solanum sp
Tribulus sp

Shrub
Lycium australe
Solanum ferocissimum

Bimba Hill

YFRW

March 2021

Grass
Cenchrus sp
Enneapogon polyphyllus
Enneapogon sp
Eragrostis xerophila
Setaria sp
Tetragonia tetragonoides
Trianthema triquetrum

Herb (Annual)
Calandrinia eremaea
Dysphania sp
Glinus lotoides
Malva parviflora
Portulaca oleracea

Herb (Perennial)
Boerhavia sp
Commicarpus australis
Lysimachia arvensis
Sclerolaena patenticuspis
Sclerolaena uniflora
Solanum esuriale
Solanum sp

Shrub
Enchylaena tomentosa
Gunniopsis quadrifida
Lycium australe
Maireana pyrimidata
Neobassia sp
Sclerolaena eriacantha
Solanum ferocissimum

Bimba Hill

YFRW

August 2021

Grass
Hordeum sp
Rytidosperma sp
Schismus barbatus
Glinus lotoides
Gnephosis arachnoidea
Lemooria burkittii
Lepidium sp
Myriocephalus pluriflorus
Osteocarpum dipterocarpum
Portulaca oleracea
Sisymbrium erysimoides
Sonchus oleraceus
Tetragonia tetragonoides

Herb (Annual)
Calandrinia eremaea
Calotis sp
Dysphania sp

Shrub
Acacia burkittii
Acacia oswaldii
Acacia sp
Acacia tetragonophylla
Acacia victoriae
Enchylaena tomentosa
Maireana pyrimidata

Herb (Perennial)
Brachyscome sp
Eriochiton sclerolaenoides
Malvastrum americanum
Olearia sp
Sclerolaena cuneata
Sclerolaena patenticuspis
Sclerolaena sp
Sclerolaena uniflora
Tecticornia indica
Vittadinia sp
Parkinsonia aculeata
Sclerolaena eriacantha
Sclerolaena glabra
Senecio sp
Senna artemisioides
Maireana sp
Maireana villosa
Neobassia sp

Tree
Acacia aneura

Bimba Hill

YFRW

February 2022

Grass
Enneapogon sp
Cenchrus sp
Setaria sp

Herb (Perennial)
Alternanthera denticulata
Boerhavia sp
Commicarpus australis
Malvastrum americanum
Sclerolaena uniflora
Sida fibulifera
Sida sp
Sida trichopoda
Solanum esuriale
Solanum sp

Tree
Santalum lanceolatum

Herb (Annual)
Actinobole uliginosum
Angianthus brachypappus
Dysphania sp
Glinus lotoides
Gnephosis arachnoidea
Lemooria burkittii
Portulaca oleracea
Rhodanthe floribunda
Rhodanthe sp
Stuartina muelleri
Trianthema triquetrum

Shrub
Abutilon cryptopetalum
Abutilon sp
Enchylaena tomentosa
Hibiscus sturtii

Bimba Hill

YFRW

November 2022

Herb (Annual)
Datura leichhardtii
Euphorbia drummondii
Malva parviflora
Nicotiana velutina
Portulaca oleracea
Tetragonia tetragonoides
Solanum sp

Herb (Perennial)
Lysimachia arvensis
Sida fibulifera
Sida sp
Sida trichopoda
Solanum esuriale
Solanum nigrum

Shrub
Abutilon cryptopetalum
Abutilon fraseri
Abutilon sp
Lycium australe
Solanum ferocissimum

Italowie Gap

Euro

November 2022

Grass
Enneapogon lindleyanus
Malva parviflora

Herb (Annual)
Daucaus glochidiatus

Herb (Perennial)
Malvastrum americanum
Sida sp
Wahlenbergia stricta
Acacia tetragonophylla
Alyogyne hakeifolia
Hibiscus sturtii

Shrub
Abutilon cryptopetalum
Abutilon sp
Acacia ligulata

Bimba Hill

Euro

December 2020

Grass
Cenchrus sp
Digitaria brownie
Enneapogon polyphyllus
Enneapogon sp
Eragrostis pergracillis
Eragrostis setifolia

Herb (Annual)
Brassica tournfortii
Euphorbia drummondii
Leucochrysum molle
Portulaca oleracea
Pycnosorus pleiocephalus
Rhodanthe floribunda

Eragrostis sp
Eragrostis tenellula
Rytidosperma sp
Schismus barbatus
Setaria sp
Themeda triandra
Triodia scariosa
Tripogonella loliiformis

Herb (Perennial)
Boerhavia sp
Commicarpus australis
Convolvulus remotus
Sclerolaena cuneata
Sclerolaena patenticuspis
Sclerolaena sp
Sclerolaena uniflora
Solanum sp

Rhodanthe sp
Sisymbrium erysimoides
Sonchus oleraceus
Stenopetalum lineare

Shrub
Acacia victoriae
Enchylaena tomentosa
Maireana pyrimidata
Sclerolaena eriacantha
Solanum ferocissimum

Bimba Hill

Euro

November 2022

Grass
Dactyloctenium radulans
Eragrostis setifolia
Triodia scariosa

Herb (Perennial)
Reichardia tingitana
Senecio sp

Herb (Annual)
Sonchus oleraceus

Shrub
Dodonea viscosa

Italowie Gap

Feral Goat

November 2022

Grass
Cenchrus sp
Setaria dielsii

Herb (Perennial)
Boerhavia sp
Chrysocephalum sp
Pterocaulon sphacelatum
Rumex sp
Solanum esuriale

Herb (Annual)
Actinobole uliginosum
Citrullus lanatus
Datura leichhardtii
Euphorbia australis
Euphorbia drummondii
Helianthus annuus
Nicotiana velutina
Portulaca oleracea
Schoenia ramosissima
Sigesbeckia australiensis

Tree
Acacia aneura
Casuarina pauper
Eucalyptus largiflorens
Eucalyptus sp
Melaleuca lanceolata

Shrub
Acacia burkittii
Acacia ligulata
Acacia oswaldii
Acacia sp
Acacia tetragonophylla
Acacia victoriae
Parkinsonia aculeata
Petalostylis labicheoides
Ptilotus obovatus

Bimba Hill

Feral Goat

December 2020

Herb (Annual)
Brassica tournefortii
Carrichtera annua
Erodium carolinianum
Euphorbia australis
Euphorbia drummondii
Euphorbia tannensis
Lepidium sp
Nicotiana velutina
Portulaca oleracea
Sisymbrium erysimoides
Stenopetalum lineare

Tree
Acacia aneura

Herb (Perennial)
Euphorbia stevenii
Malvastrum americanum
Solanum esuriale

Shrub
Acacia burkittii
Acacia oswaldii
Acacia sp
Acacia tetragonophylla
Acacia victoriae
Adriana quadripartita
Lycium australe
Parkinsonia aculeata
Petalostylis labicheoides
Senna artemisioides
Solanum ferocissimum

Bimba Hill

Feral Goat

March 2021

Herb (Annual)
Datura leichhardtii
Euphorbia australis
Euphorbia drummondii
Nicotiana velutina
Portulaca oleracea

Shrub

Herb (Perennial)
Euphorbia stevenii
Lysimachia arvensis
Solanum esuriale
Solanum sp

Tree

Acacia burkittii
Acacia oswaldii
Acacia sp
Acacia tetragonophylla
Acacia victoriae
Lycium australe
Parkinsonia aculeata
Petalostylis labicheoides
Solanum ferocissimum
Acacia aneura
Callitris glaucaphylla
Santalum lanceolatum

Bimba Hill

Feral Goat

August 2021

Grass
Cenchrus sp

Herb (Perennial)
Sclerolaena uniflora

Tree
Acacia aneura
Santalum acuminatum
Santalum lanceolatum

Herb (Annual)
Parietaria cardiostegia

Shrub
Acacia burkittii
Acacia oswaldii
Acacia sp
Acacia tetragonophylla
Acacia victoriae
Dodonea viscosa

Bimba Hill

Feral Goat

February 2022

Grass
Cenchrus sp
Setaria sp

Tree
Acacia aneura
Santalum acuminatum
Santalum lanceolatum

Shrub
Acacia burkittii
Acacia oswaldii
Acacia sp
Acacia tetragonophylla
Acacia victoriae
Parkinsonia aculeata
Pimelea stricta
Senna artemisioides

Bimba Hill

Feral Goat

November 2022

Herb (Perennial)
Sida fibulifera
Sida trichopoda
Solanum esuriale
Solanum nigrum
Verbena sp

Shrub
Acacia burkittii
Acacia sp
Acacia tetragonophylla
Acacia victoriae
Lycium australe

Tree
Acacia aneura
Santalum lanceolatum

Appendix 4:

Italowie Gap:

Autumn 2021:

"Judd"
Boerhavia diffusa
Enneapogon lindleyanus
Eragrostis tenellula
Indigofera colutea
Malvastrum americanum
Neobassia procerifloria
Scaevola sp
Schlerolaena patenticuspis
Schlerolaena uniflora
Sida fibulifera
Sida sp
Sida trichopoda
Tetragonia tetragonoides

"Zara"
Portulaca oleracea

"Ringo"
Acacia oswaldii
Acacia tetragonophylla
Acacia victoriae
Boerhavia diffusa
Commicarpus australis
Enneapogon lindleyanus
Portulaca oleracea
Ptilotus obovatus

"Eileen"
Abutilon sp
Alternanthera denticulate
Boerhavia diffusa
Indigofera colutea
Malva parviflora
Malvastrum americanum

"Elton"
Acacia aneura
Acacia burkittii
Acacia oswaldii
Acacia ligulata
Acacia tetragonophylla
Cenchrus sp
Digitaria brownii
Lysiana exocarpi
Malvastrum americanum
Portulaca oleracea
Setaria dielsii
Sida fibulfera

Spring 2021:

"Max"
Acacia ligulata
Acacia tetragonophylla
Acacia victoriae
Hibiscus sturtii
Portulaca oleracea
Trianthema triquetrum
Sida trichopoda
Solanum nigrum
Solanum sp

"Lola"
Acacia burkittii
Acacia ligulata
Acacia tetragonophylla
Acacia victoriae
Roepera sp
Sida fibulifera

"Meatloaf"

"Zara"

Acacia burkittii
Acacia ligulata
Acacia tetragonophylla
Malvastrum americanum
Myoporum montanum
Scaevola sp
Sida sp
Sida trichopoda

Acacia burkittii
Acacia tetragonophylla
Acacia victoriae
Ptilotus obovatus
Sida fibulifera
Sida trichopoda

"Eileen"
Acacia ligulata
Acacia sp
Acacia victoriae
Sclerolaena sp
Sclerolaena uniflora
Portulaca oleracea
Acacia victoriae
Capparis mitchellii
Dysphania sp
Parkinsonia aculeata
Sclerolaena sp
Sclerolaena uniflora
Sida fibulifera

"Rosey"
Abutilon sp
Acacia aneura
Acacia burkittii
Acacia ligulata
Acacia oswaldii
Acacia tetragonophylla

Autumn 2022:

"Tags"
Acacia sp
Boerhavia diffusa
Commicarpus australis
Malvastrum Americanum
Opuntia sp
Portulaca oleracea
Senna artemisioides
Sida fibulifera
Tribulus sp
Triraphis mollis

"Valerie"
Acacia aneura
Acacia burkittii
Acacia sp
Acacia tetragonophylla
Enneapogon sp
Parkinsonia aculeata
Portulaca oleracea

"Adele"
Acacia aneura
Acacia sp
Acacia tetragonophylla
Digitaria brownii
Dysphania sp
Malva parviflora
Malvastrum americanum
Pterocaulon sphacelatum
Scaevola sp
Setaria dielsii
Sida fibulifera
Sida sp
Sida trichopoda
Sonchus oleraceus

"Brandy"
Acacia tetragonophylla
Portulaca oleracea
Scaevola sp
Sida fibulifera
Sida trichopoda

"Cecilia"
Acacia sp
Acacia tetragonophylla
Hordeum sp
Lycium australe
Portulaca oleracea
Solanum esuriale
Solanum sp

"Eileen"
Enchylaena tomentosa
Eriochiton sclerolaenoides
Helianthus annuus
Maireana pyrimidata
Malvastrum americanum
Portulaca oleracea
Pterocaulon sphacelatum

Trianthema triquetrum
Sclerolaena patenticuspis
Sclerolaena sp
Sclerolaena uniflora
Sigesbeckia australiensis

Rumex sp

Bimba Hill:
Spring 2021:

"Patrick"
Enchylaena tomentosa
Eriochiton sclerolaenoides
Hydrocotyle trachycarpa
Maireana integra
Maireana pyramidata
Maireana sp
Neobassia proceriflora
Sclerolaena eriacantha
Scleroleana sp
Scleroleana uniflora
Tetragonia tetragonoides
Tragus australianus

"Pumpkin"
Calandrinia eremaea
Crassula sp
Dysphania sp
Eragrostis dielsii
Eragrotis setifolia
Eragrotis tenellula
Eragrotis xerophila
Hydrocotyle trachycarpa
Silene sp
Sonchus oleraceus

"Polly"
Calandrinia eremaea
Enchylaena tomentosa
Hordeum sp
Maireana georgei
Maireana pyrimidata
Maireana villosa
Sclerolaena patenticuspis
Sclerolaena sp
Sclerolaena uniflora

"Peter"
Acacia victoriae
Calandrinia eremaea
Santalum lanceolatum

Autumn 2022:

"Pickle"
Acacia burkittii
Acacia ligulata
Acacia oswaldii
Acacia tetragonophylla
Acacia victoriae
Boerhavia diffusa
Commicarpus australis
Dysphania sp
Setaria sp
Tetragonia tetragonoides
Themeda triandra

"Pedro"
Acacia burkittii
Acacia ligulata
Acacia tetragonophylla
Dysphania sp
Eragrostis tenellula
Setaria sp

"Pumpkin"
Abutilon sp
Acacia ligulata
Acacia tetragonophylla
Parkinsonia aculeata
Setaria sp

"Petra"
Acacia oswaldii
Acacia tetragonophylla
Dysphania sp
Parkinsonia aculeata

Appendix 5:

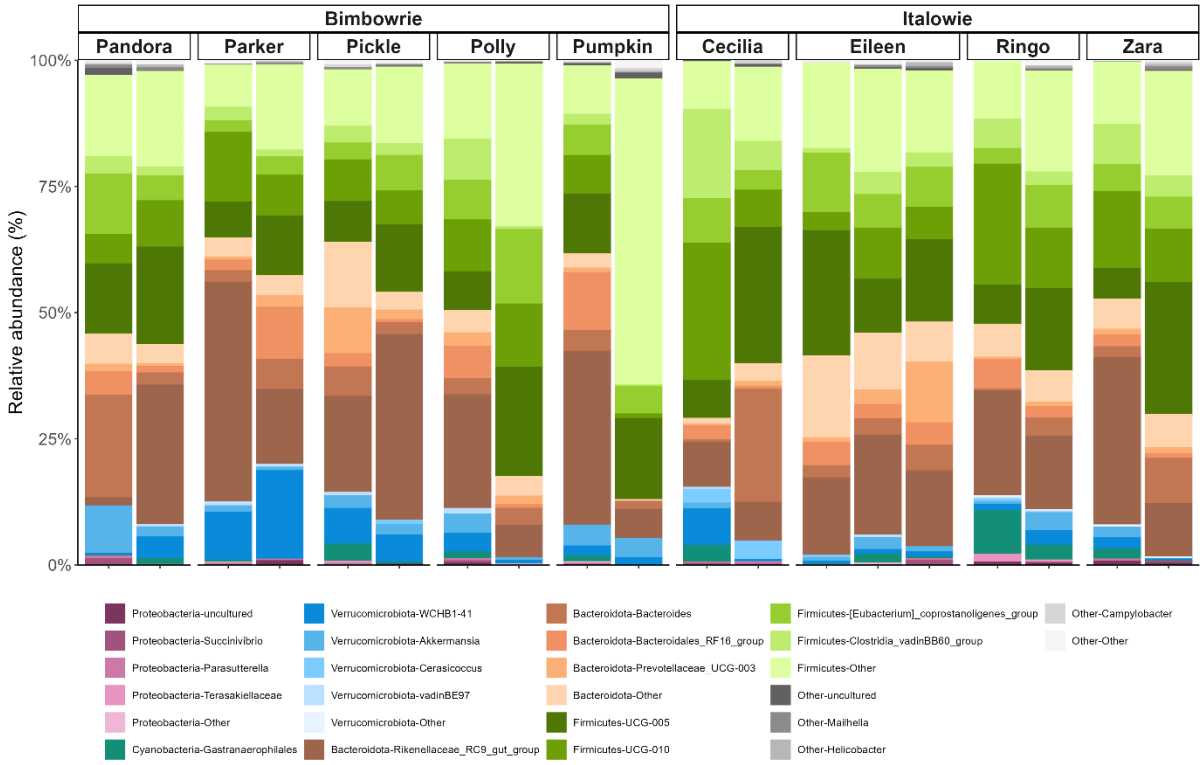


Figure S1: The relative abundance (%) of yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) faecal microbes which have been sampled from the same individual over multiple time periods from Italowie Gap and Bimba Hill. Bimbowrie = Bimba Hill, Italowie = Italowie Gap.

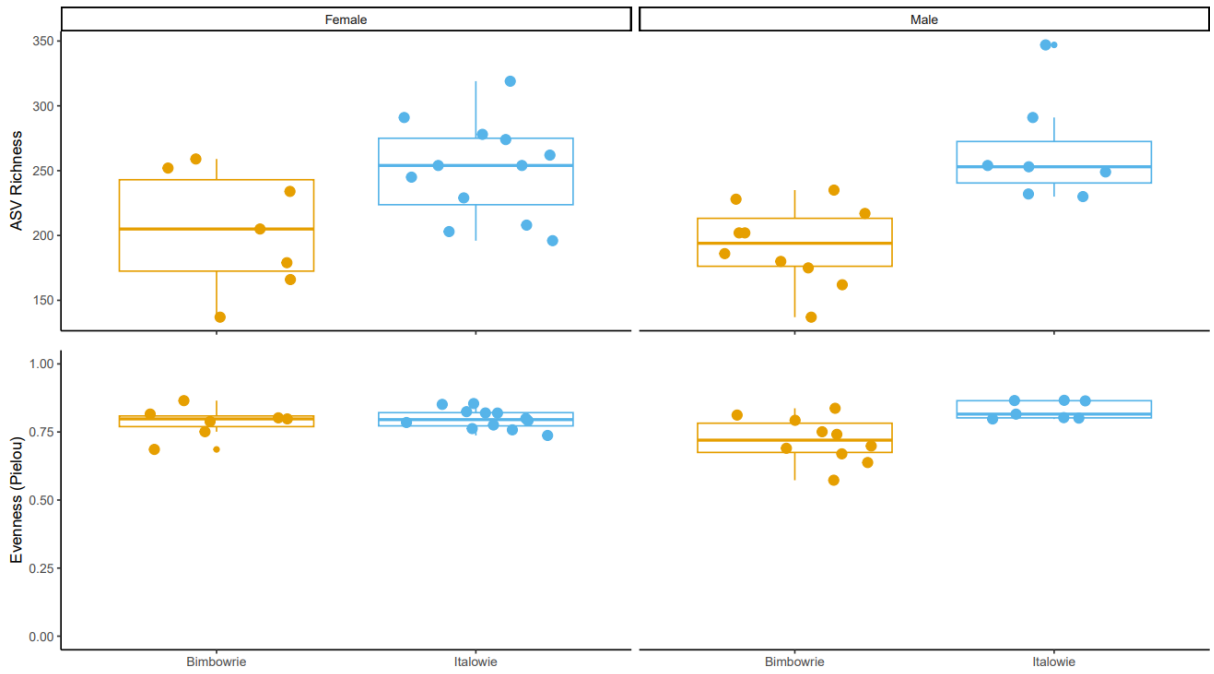


Figure S2: The faecal microbial alpha diversity ASV richness (top) and evenness (bottom) for female and male yellow-footed rock-wallabies (*Petrogale xanthopus xanthopus*) at two different study sites (Italowie Gap and Bimba Hill).

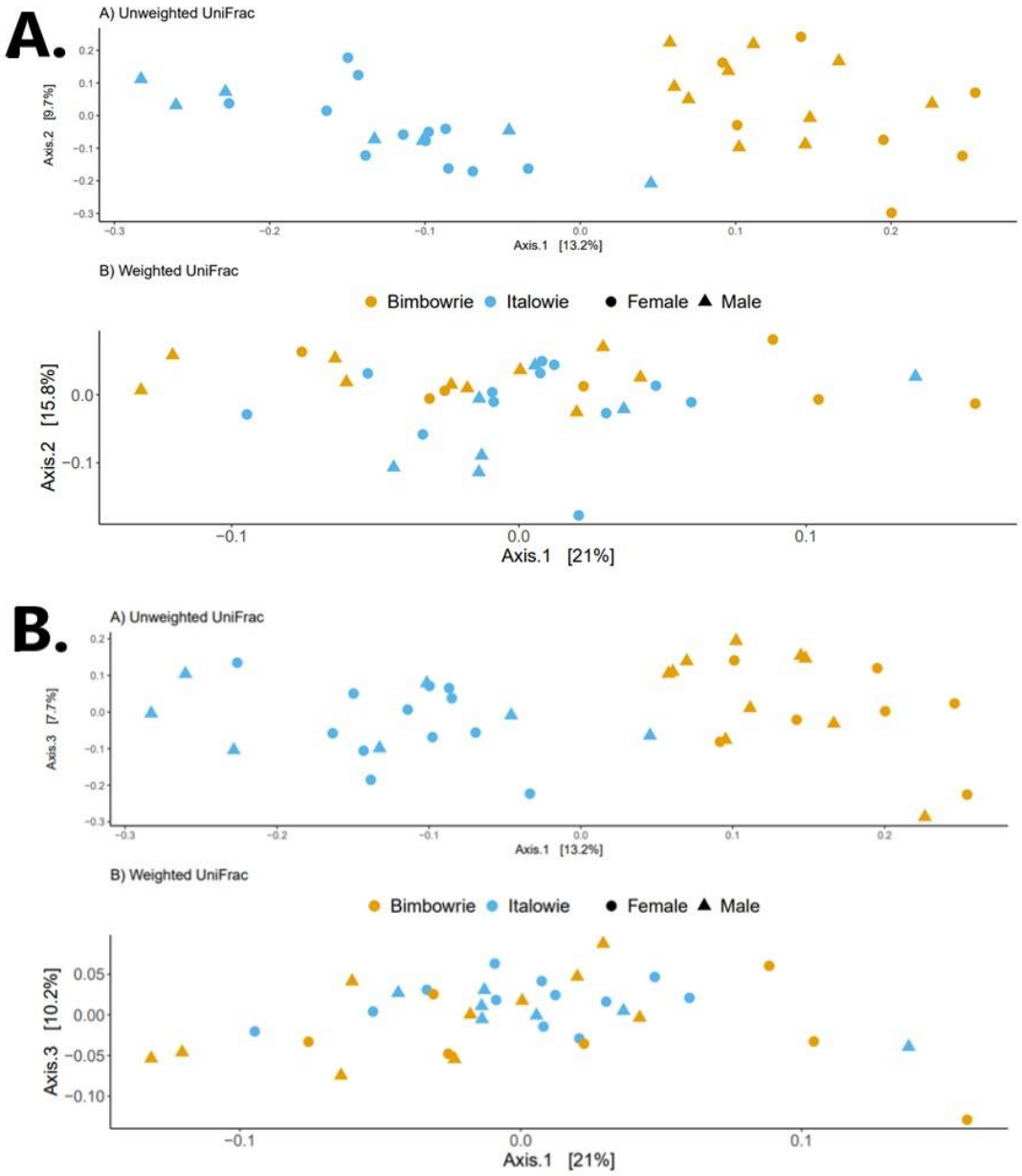


Figure S3: The beta diversity of faecal microbes from female and male yellow-footed rock-wallabies (*Petrogale xanthopus xanthopus*) from two different sites, Italowie Gap and Bimba Hill using Unweighted UniFrac (top) and Weighted UniFrac (bottom) distances. (a) Axis 1 vs 2. (b) Axis 1 vs 3.

Appendix 6:

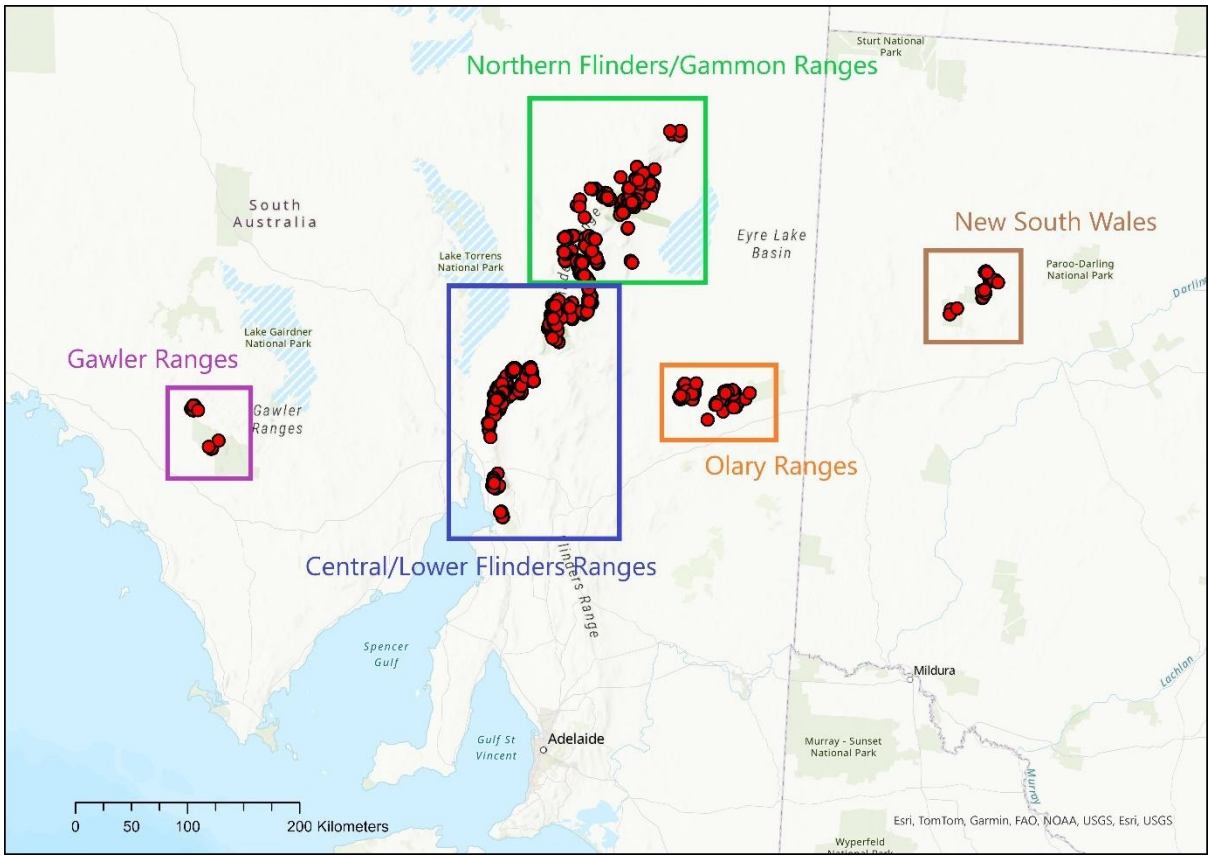


Figure S1: The geographic regions of South Australia and New South Wales where yellow-footed rock-wallabies (*Petrogale xanthopus xanthopus*) are found. Red points indicate Atlas of Living Australia (www.ala.org.au) species observations. The regions of the Gawler Ranges (purple), northern Flinders/Gammon Ranges (green), central/lower Flinders Ranges (blue), Olary Ranges (orange), and New South Wales (brown) are indicated.

Table S1: A table showing the number of replicated yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) scat counts in each of the survey regions, Flinders/Gammon and Olary Ranges, South Australia.

Scat Count	Flinders / Gammon Ranges	Olary Ranges
1	390	306
2	268	302
3	222	189
4	137	169
5	152	168
6	76	100
7	47	47
8	53	64
9	14	33
10	70	73
11	14	17
12	28	38
13	7	14
14	9	14
15	43	41
16	7	9
17	5	14
18	5	11
19	3	5
20	36	43
21	2	6
22	1	6
23	1	9
24	0	4
25	14	14
26	1	2
27	0	3
28	1	4
29	0	2
30	19	21
31	2	2
32	0	2
33	0	2

34	0	1
35	4	4
37	1	4
39	0	1
40	9	17
41	0	3
42	0	2
43	0	4
44	0	1
45	1	4
47	0	1
50	10	26
52	0	2
53	1	3
55	1	0
57	0	1
59	0	1
60	2	9
61	0	1
66	0	1
70	3	7
75	0	1
80	4	7
90	1	1
100	2	28
110	0	1
120	0	4
150	0	1
180	0	3
200	0	1

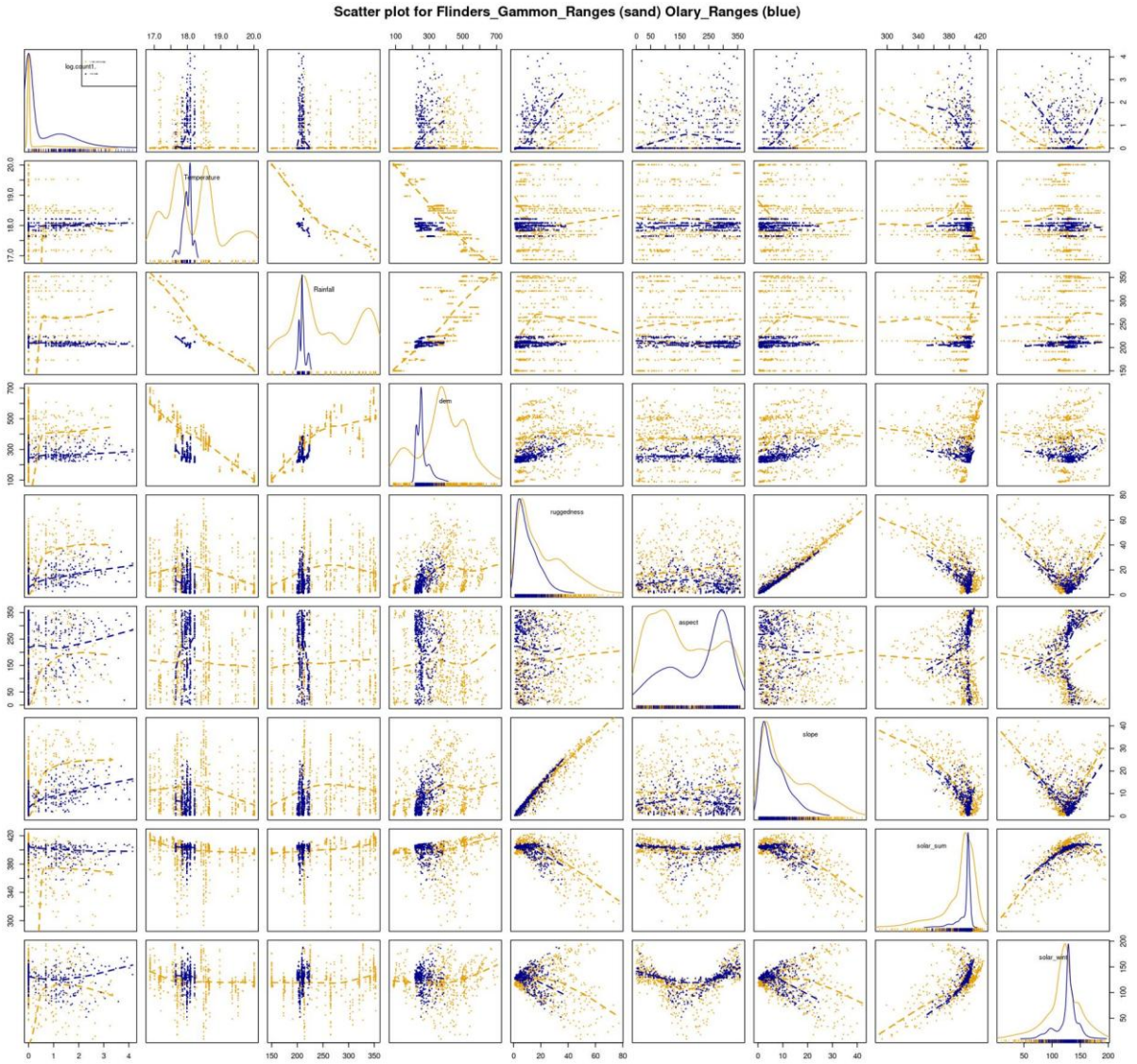


Figure S2: Correlation scatterplots examining the interactions between yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) presence and habitat parameters (annual temperature, rainfall, elevation [DEM], ruggedness, slope, aspect, winter solar radiation, and summer solar radiation). Data has been separated into the Flinders/Gammon Ranges (sand) and Olary Ranges (blue).

Table S2: The summary statistics of a generalized additive model for assessing yellow-footed rock-wallaby (*Petrogale xanthopus xanthopus*) presence from scat survey data (log[scat_count] ~ te[temperature, ruggedness, by = Region]) across two geographical regions (Flinders/Gammon and Olary Ranges) in South Australia. Model diagnostics reported are the *k*-value (*k`*), the effective degrees of freedom (*edf*), the reference degrees of freedom (*Ref. df*), the *k*-index, *F* value (*F*), and *p*-value. Model summary statistics reported are the adjusted R-squared value (*R-sq.* [*Adj*]), and deviance explained (%).

log(scat_count) ~ te(temperature, ruggedness, by = Region)						
Model Diagnostics and Summary						
Region	**k`**	**edf**	**Ref. df**	**k-index**	**F**	***p*-value**
Flinders/Gammon Ranges	24	14.8	17.5	0.63	32.2	***
Olary Ranges	24	10.2	11.5	0.62	97.4	***
R-sq. (Adj)			**Deviance Explained**			
0.434			49.3%			

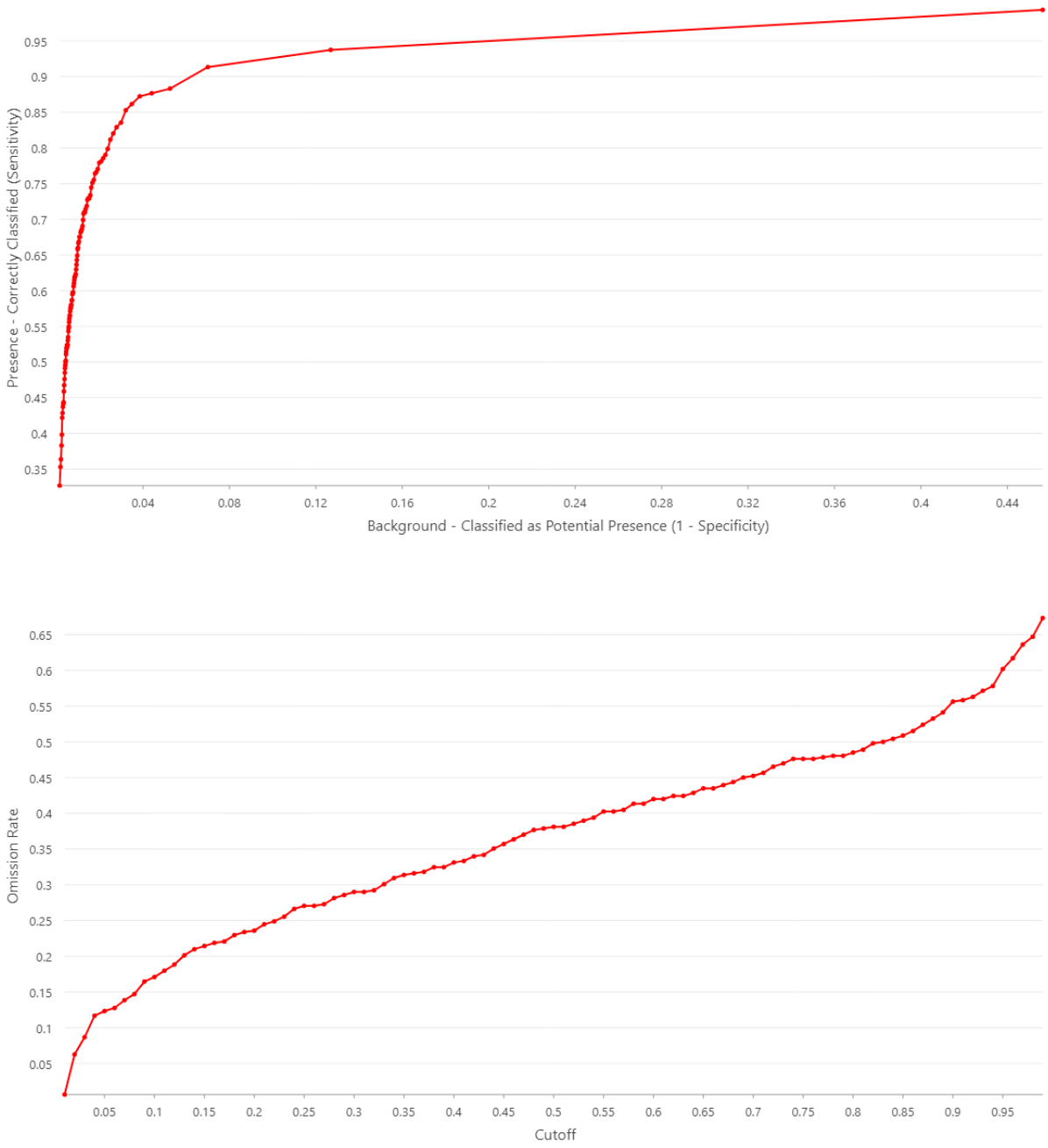


Figure S3: *Top*: A receiver operating characteristic curve plot output from a species distribution model for yellow-footed rock-wallabies (*Petrogale xanthopus xanthopus*). *Bottom*: An omission rates plot from a species distribution model for yellow-footed rock-wallabies, which used a 0.5 cutoff value.