The ecology of parasite transmission during fauna translocations: Observations from the woylie (*Bettongia penicillata*)

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"The question is, are we happy to suppose that our grandchildren may never be able to see an elephant except in a picture book?"

Sir David Attenborough

Author's Declaration

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

Amy Susan Northover

Abstract

Fauna translocations play a pivotal role in the management of threatened wildlife, though we are limited by our understanding of how the host-parasite community changes during translocation and in response to antiparasitic drug treatment. This project aimed to quantify changes in parasite community structure and host health in woylies following translocation, and in response to ivermectin treatment. This is the first study to evaluate changes to the broader parasite community in translocated and resident animals following translocation. During two fauna translocations to three different locations within south-western Australia, woylies were sampled for blood-borne, ecto- and gastrointestinal parasites, and morphometrics were measured. Prior to translocation, half of the translocated woylies were treated with ivermectin. Post-translocation monitoring was undertaken for up to 12 months following translocation.

Destination site and time since translocation had the strongest effect on parasite dynamics and host health following translocation. Significant changes to the parasite community occurred within the first few months after translocation, and the parasite communities of translocated and resident woylies generally converged to become more similar over time, with failure of some parasite taxa to persist and new host-parasite associations emerging. *Trypanosoma* spp. richness and the prevalence of haemoparasite coinfection increased after translocation. Ivermectin treatment did not significantly reduce the prevalence/abundance of target parasites, or improve body condition in treated hosts. In translocated woylies, the presence of coccidia during the first three months following translocation, and increasing *Strongyloides*-like egg counts were associated with lower body condition. Results from this study highlight the importance of long-term parasite monitoring to better understand the biological implications (for individuals, populations and ecosystems) of wildlife translocations on host-parasite ecology. Insights gained from this study are broadly applicable to the management of threatened fauna and their parasite taxa, and enhance our fundamental understanding of the potential ecosystem impacts of wildlife translocations.

Acknowledgements

I am extremely grateful to my supervisors Andrew Thompson (Principal Supervisor), Stephanie Godfrey, Alan Lymbery and Adrian Wayne for imparting their knowledge and skills, providing guidance and support, and assisting (willingly) with remote field work. Stephanie Godfrey, thank you for teaching me the art of R studio; your statistical advice, direction and patience were fundamental to the development of four of the chapters presented in this thesis (chapters 3, 5, 6 and 8).

To our research assistant and my friend Sarah Keatley, I cannot thank you enough for your support with all aspects of this project, in particular your molecular expertise. I appreciate all the time that you have spent centrifuging, extracting, screening, sequencing, re-extracting, trouble-shooting, and compiling results. Thank you for ensuring the smooth-running of our field trips, I couldn't have done it without you - literally, I would still be lost in the bush!

I feel privileged to have undertaken this field-based research project in collaboration with staff from the Department of Biodiversity, Conservation and Attractions (DBCA). Spending time in the field with such enthusiastic, knowledgeable and inspiring people was a highlight for me. To Adrian Wayne, Marika Maxwell, Colin Ward, Chris Vellios, Peter Wnuk, Malcolm Ovans, Brian Macmahon, Julia Wayne and Keith Morris, your advice, expertise and logistical support were instrumental in the design and implementation of this project. Thank you for bearing with us while we learned to roll peanut butter bait balls and became accustomed to setting Sheffield traps. The skills I have developed in native fauna identification, small mammal trapping/tagging, physical restraint and health evaluation are a credit to you, and an asset to me as a veterinarian.

Undertaking such a large scale collaborative project would not have been possible without the assistance of Murdoch University staff and a large number of dedicated volunteers. Data collection involved spending 31 weeks in the field and I would like to thank each and every one of the volunteers that gave up their spare time to assist with sample collection and processing; in particular James Barr, Jade Kelly, Siobhon Egan and Amy Robey. I would like to thank John Lawson for his support with fieldwork in Dryandra and provision of photographs; and Alison Hillman for assisting with field anaesthesia. To Amanda Ash and Aileen Elliot; thank you for assisting with the morphological identification of hundreds (and hundreds) of ectoparasites, and providing advice regarding the development of a faecal flotation protocol. Thank you for teaching me the basics of

endo- and ectoparasite identification. To Louise Pallant, the logistical magic you worked to keep this project in order will not be forgotten; neither will your advice and encouragement.

I would like to express gratitude to the funding bodies that enabled this project to become a reality. This project was primarily funded by the Australian Research Council (LP130101073) but has also been supported by grants from the Holsworth Wildlife Research Endowment (HOLSW2015-1-F149) and the Ecological Society of Australia, The Royal Zoological Society of New South Wales (Paddy Pallin Grant) and the Australian Wildlife Society. Costs associated with the translocation of woylies were covered by DBCA, our collaborative partner, who provided the opportunity for this project to take place with the aim of delivering better conservation outcomes for threatened wildlife.

Lastly, I would like to thank my family, friends and parasitology colleagues for their support during this journey, in particular my husband Matthew Summers for encouraging me to take on this challenge. To my three year old son Owen, who accompanied me on various field trips, and our newest addition, Liam; I dedicate this research to you - may your future also be filled with a love for the great outdoors and an appreciation for our native wildlife.

List of publications, and work in progress, arising from this project

Chapter 1

Northover, A.S., Godfrey, S.S., Lymbery, A.J., Wayne, A.F., Thompson, R.C.A., 2018. The hidden consequences of altering host-parasite relationships during fauna translocations. Biological Conservation **220**: 140-148. https://doi.org/10.1016/j.biocon.2017.12.037.

Chapter 3

Northover, A.S., Thompson, R.C.A., Lymbery, A.J., Wayne, A.F., Keatley, S., Ash, A., Elliot, A., Morris, K., Godfrey, S.S., 2019. Altered parasite community structure in an endangered marsupial following translocation (*submitted for publication*)

Chapter 4

Northover, A.S., Keatley, S., Godfrey, S.S., Lymbery, A.J., Wayne, A.F., Elliot, A., Thompson, R.C.A., 2019. Identification of a novel *Eimeria* species from the woylie (*Bettongia penicillata*) and the genetic characterisation of *Eimeria gaimardi*, *Eimeria potoroi* and *Eimeria mundayi* from other potoroid marsupials (*submitted for publication*)

Chapter 5

Northover, A.S., Keatley, S., Godfrey, S.S., Lymbery, A.J., Wayne, A.F., Cooper, C., Pallant, L., Morris, K., Thompson, R.C.A., 2019. Increased *Trypanosoma* spp. richness and prevalence of haemoparasite co-infection following translocation. Parasites & Vectors **12**: 126. https://doi.org/10.1186/s13071-019-3370-6.

Chapter 6

Northover, A.S., Thompson, R.C.A., Lymbery, A.J., Wayne, A.F., Keatley, S., Barr, J., Morris, K., Godfrey, S.S., 2019. Parasite infection and ivermectin treatment have minimal impact on woylie health during fauna translocation (*intended for journal submission*)

Chapter 7

Northover, A.S., Elliot, A., Keatley, S., Lim, Z., Botero, A., Amanda, A., Godfrey, S.S., Lymbery, A.J., Wayne, A.F., Thompson, R.C.A., 2018. Debilitating disease in a polyparasitised woylie (*Bettongia penicillata*): a diagnostic investigation. International Journal for Parasitology: Parasites and Wildlife **7**: 274-279. https://doi.org/10.1016/j.ijppaw.2018.07.004.

Chapter 8

Northover, A.S., Godfrey, S.S., Lymbery, A.J., Morris, K., Wayne, A.F., Thompson, R.C.A., 2017. Evaluating the effects of ivermectin treatment on communities of gastrointestinal parasites in translocated woylies (*Bettongia penicillata*). EcoHealth **14**(S1): 117-127. https://doi.org/10.1007/s10393-015-1088-2. Epub 2015 Dec 30.

List of other publications arising from this project

Original research articles:

Cooper, C., Keatley, S., Northover, A., Gofton, A.W., Brigg, F., Lymbery, A.J., Pallant, L., Clode, P.L., Thompson, R.C.A., 2018. Next generation sequencing reveals widespread trypanosome diversity and polyparasitism in marsupials from Western Australia. International Journal for Parasitology: Parasites and Wildlife **7**: 58-67. https://doi.org/10.1016/j.ijppaw.2018.01.005

Ash, A., Elliot, A., Godfrey, S., Burmej, H., Abdad, M.Y., Northover, A., Wayne, A., Morris, K., Clode, P., Lymbery, A., Thompson, R.C.A., 2017. Morphological and molecular description of *Ixodes woyliei* n. sp. (Ixodidae) with consideration for co-extinction with its critically endangered marsupial host. Parasites & Vectors **10**:70. https://dx.doi.org/10.1186/s13071-017-1997-8

Hing, S., Northover, A.S., Narayan, E.J., Wayne, A.F., Jones, K.L., Keatley, S., Thompson, R.C.A., Godfrey, S.S., 2017. Evaluating stress physiology and parasite infection parameters in the translocation of critically endangered woylies (*Bettongia penicillata*). EcoHealth **14**: 128-138. https://doi.org/10.1007/s10393-017-1214-4

Conference abstracts:

Northover, A.S., Godfrey, S.S., Lymbery, A.J., Wayne, A.F., Maxwell, M., Thompson, R.C.A., 2014. Investigating the incidence of polyparasitism in translocated woylies (*Bettongia penicillata*): Impact on host fitness and translocation success. *Australian Society for Parasitology 50th Anniversary Conference, 30 June-3 July 2014, Canberra, Australia* (Poster presentation)

Northover, A.S., Godfrey, S.S., Lymbery, A.J., Wayne, A.F., Maxwell, M., Thompson, R.C.A., 2014. The ecology of parasite transmission in fauna translocations: Investigating the impact of polyparasitism on host fitness and translocation success. *International Congress on Parasites of Wildlife, 14-18 September 2014 Kruger National Park, South Africa* (Poster presentation).

Northover, A.S., Godfrey, S.S., Lymbery, A.J., Wayne, A.F., Morris, K., Thompson, R.C.A., 2015. To treat or not to treat? Investigating the impact of polyparasitism in translocated woylies (*Bettongia*

penicillata), and the effect of anti-parasite treatment on host fitness and survivability. *Zoological Society of London Symposium: Health and Disease in Translocated Wild Animals, 14-15 May 2015, London, England* (Poster presentation).

Keatley, S., Northover, A.S., Godfrey, S.S., Lymbery, A.J., Wayne, A.F., Thompson, R.C.A., 2015. Investigating the ecology of parasite transmission in fauna translocations and the impact of polyparasitism in translocated woylies (*Bettongia penicillata*). *New Zealand Society for Parasitology and Australian Society for Parasitology Annual Conference, 29 June-2 July, Auckland, New Zealand* (Oral presentation, presented by S. Keatley)

Northover, A.S., Godfrey, S.S., Lymbery, A.J., Wayne, A.F., Morris, K., Thompson, R.C.A., 2015. To treat or not to treat? Investigating the impact of polyparasitism in translocated woylies (*Bettongia penicillata*), and the effect of anti-parasite treatment on host fitness and survivability. *64th International Conference of the Wildlife Disease Association, 26-30 July 2015, Queensland, Australia* (Poster presentation).

McCallum, H., Thompson, R.C.A., Godfrey, S.S., Keatley, S., Lymbery, A.J., Northover, A.S., Wayne, A.F., Muths, E., 2017. Parasites and pathogens in translocations and reintroductions. *28th International Congress for Conservation Biology, 23-27 July 2017, Cartagena, Colombia* (Oral presentation, presented by H. McCallum).

Godfrey, S.S., Lymbery, A.J., Wayne, A.F., Northover, A.S., Thompson, R.C.A., 2018. Conserving hosts at the peril of their parasites? Conservation and parasite extinction in the woylie. *Ecological Society of Australia Annual Conference: Ecology in the Anthropocene, 25-29 November 2018, Queensland, Australia* (Oral presentation, presented by S. Godfrey)

Statement of animal ethics approval

This project was undertaken in collaboration with the Department of Biodiversity, Conservation and Attractions under DBCA Scientific License's (Regulation 4: written notice of lawful authority; and 17: licence to take fauna for scientific purposes) and in accordance with Murdoch University Animal Ethics Approval (RW2659/14).

Statement of contributions

This thesis consists of nine chapters, which include four published papers, two papers that have been submitted for publication, and a draft paper intended for journal submission. In each case, A. Northover was the first author and significantly contributed to the content of each paper, including all fieldwork, sample collection and analysis. All text, tables and images were developed and written by A. Northover under the editorial guidance of R.C.A Thompson (principal supervisor), A.J. Lymbery, A.F. Wayne and S.S. Godfrey (co-supervisors). For published papers, A. Northover coordinated the editorial changes suggested by journal reviewers. A. Northover under the direction of S.S. Godfrey and A.J. Lymbery carried out data analysis (Chapters 3, 5, 6 and 8). All supervisors assisted with fieldwork/sample collection. The input of additional co-authors is outlined below:

Chapter 3

A. Elliot and A. Ash morphologically identified endoparasites and ectoparasites, respectively. K. Morris played an instrumental role in the design of this study. S. Keatley performed all molecular laboratory work (i.e. detection of *Trypanosoma* spp. infection) and undertook fieldwork and sample collection. All co-authors provided editorial input.

Chapter 4

S. Keatley and R. Yang genetically characterised *E. woyliei, E. gaimardi, E. mundayi* and *E. potoroi.* A. Elliot and R. Hobbs assisted with the morphological description and photography of *Eimeria* spp. oocysts. A. Northover and A. Elliot performed $NaNO_3$ and $ZnSO_4$ faecal flotation, respectively. A. Northover performed all oocyst/sporocyst measurements. S. Keatley undertook fieldwork and sample collection. All co-authors provided editorial input.

Chapter 5

S. Keatley, with the assistance of C. Cooper, performed all molecular laboratory work (i.e. identification of haemoparasite infection). L. Pallant provided technical laboratory assistance. S. Keatley undertook fieldwork and sample collection. All co-authors provided editorial input.

Chapter 6

S. Keatley performed all molecular laboratory work (as above). J. Barr performed in-field centrifugation and refractometry to obtain PCV/TPP measurements. S. Keatley and J. Barr

undertook fieldwork and sample collection. K. Morris played an active role in the design of this study. All co-authors provided editorial input.

Chapter 7

A. Elliot and A. Ash morphologically identified endoparasites and ectoparasites, respectively. Z. Lim performed the post-mortem examination. S. Keatley and A. Botero provided technical laboratory assistance. All co-authors provided editorial input.

Chapter 8

K. Morris played an instrumental role in the design of this study.

For chapters 1, 5, 7 and 8 (published papers) a link to each of these papers is provided in the prelude to this thesis (pages viii-ix) and at the start of each relevant chapter. A PDF copy of these papers could not be included within this thesis due to copyright restrictions.

Signatures of Co-Authors:

1. R.C.A. Thompson

2. S.S. Godfrey

3. A.J. Lymbery

4. A.F. Wayne

5. S. Keatley

6. K. Morris

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12. R. Hobbs

13. R. Yang

14. A. Botero

15. Z. Lim

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List of abbreviations

A/G	Albumin to globulin ratio
ANOSIM	Analysis of similarities
ARC	Australian Research Council
BCI	Body condition index
BCS	Body condition score
BOHB	Beta-hydroxybutyrate
CD3	Cluster of differentiation 3
CDV	Canine Distemper Virus
CI	Confidence interval
COI	Cytochrome oxidase
DBCA	Department of Biodiversity, Conservation and Attractions
dGTP	Deoxyguanosine triphosphate
dNTPs	Deoxyribonucleotide triphosphate
EDTA	Ethylenediaminetetraacetic acid
FEC	Faecal egg count
G1	Genotype 1
G2	Genotype 2
GC	Guanine and cytosine
GLDH	Glutamate dehydrogenase
HCT	Haematocrit
IFN-γ	Interferon gamma
IUCN	International Union for Conservation of Nature
$MgCl_2$	Magnesium chloride
Na/K	Sodium to potassium ratio
$NaNO_3$	Sodium nitrate
NCBI	National Center for Biotechnology Information

PAS	Periodic acid-Schiff
PCR	Polymerase chain reaction
PCV	Packed cell volume
PERMANOVA	Permutational analysis of variance
PIT	Passive integrated transponder
PRIMER	Plymouth Routines in Multivariate Ecological Research
RBC	Red blood cell
rDNA	Ribosomal DNA (deoxyribonucleic acid)
SC	Subcutaneous
Taq	Taq polymerase
TPP	Total plasma protein
TST	Time since translocation
$ZnSO_4$	Zinc sulphate

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Thesis structure

Thesis layout

All papers included in this thesis are re-formatted copies of published manuscripts [or manuscripts submitted (or intended) for publication] and are presented in separate chapters. All formalities (i.e. acknowledgements, declarations of interest and references) are presented after the main content of each chapter. Tables and figures are included at the appropriate point within each chapter (with the exception of chapter 2, methods) for ease of reference. Given the nature of this thesis, there is some unavoidable repetition of content, in particular the materials and methods.

Project background

The results presented in this thesis were obtained during the course of a project primarily funded by the Australian Research Council (ARC Linkage project number LP130101073) and undertaken in collaboration with the Department of Biodiversity, Conservation and Attractions. This project builds upon previous collaborative research with DBCA (ARC Linkage project LP0775356), which examined the nature, diversity and potential impacts of infectious agents in threatened mammals from Western Australia. This project expands on the above preliminary research by (a) examining changes in parasite community structure in translocated and resident woylies following translocation; (b) evaluating the efficacy of ivermectin treatment; and (c) examining how parasite infection and ivermectin treatment influence host health.

Chapter One

General Introduction

Chapter 1: General introduction

The conservation management of threatened wildlife increasingly relies upon fauna translocations to augment populations (Fischer and Lindenmayer, 2000). For critically endangered species such as the woylie (syn. brush-tailed bettong, *Bettongia penicillata*) in which only remnant wild indigenous populations remain, translocations are crucial for sustaining genetic diversity, population health and ensuring the long-term survival of this species (Wayne et al., 2015). During the course of translocating a host (and their infracommunity of parasites) from one location to another however, pre-existing host-parasite relationships are unavoidably disrupted (Corn and Nettles, 2001; Telfer et al., 2010; Moir et al., 2012). Changes to the composition of the parasite community may significantly impact host health, population dynamics and thus translocation outcomes (Thompson et al., 2010). Parasites are well known for their ability to adversely influence host health, and parasitic disease is a significant concern during the translocation of wildlife (Viggers et al., 1993; Cunningham, 1996; Kock et al., 2010; Sainsbury and Vaughan-Higgins, 2012). Parasites also form a vital component of biodiversity (Hudson et al., 2006) and there is growing evidence to suggest that host immunity and translocation outcomes may be enhanced if parasites are conserved, rather than eradicated, during translocation (Pizzi, 2009; McGill et al., 2010; Boyce et al., 2011; Rideout et al., 2016).

Regrettably, fauna translocation protocols rarely incorporate host-parasite studies, thus the way in which fauna translocations impact host-parasite dynamics and polyparasitism within a host is poorly understood, as are the consequences of such perturbations on host health and translocation outcomes. In this thesis, I evaluate changes in parasite community structure and host health in woylies following translocation, and in response to ivermectin treatment, in order to advance our fundamental understanding of perturbations in host-parasite systems during translocation.

Chapter 1 is comprised of three sections. Section 1.1 *The hidden consequences of altering host-parasite relationships during fauna translocations* is a published paper, which gives a general overview of fauna translocations and the mechanisms by which fauna translocation and antiparasitic drug treatment may influence the parasite community within a host; including potential consequences for host health and translocation success. Importantly, this manuscript highlights the paucity of knowledge regarding the impacts of translocation and antiparasitic drug treatment on parasite assemblages in translocated wildlife. The following section (1.2) provides background information on the woylie,

while section 1.3 outlines the thesis structure in the context of the aims and hypotheses of this study.

1.1 The hidden consequences of altering host-parasite relationships during translocation

The following is a published paper:

Northover, A.S., Godfrey, S.S., Lymbery, A.J., Wayne, A.F., Thompson, R.C.A., 2018. The hidden consequences of altering host-parasite relationships during fauna translocations. Biological Conservation **220**: 140-148. https://doi.org/10.1016/j.biocon.2017.12.037.

Introduction

Fauna translocations have become a widely employed conservation tool for the management of threatened species worldwide (IUCN, 2013). Translocations for conservation are occurring at an ever-increasing frequency (Seddon et al., 2007) with their value extending beyond conservation management, by also benefiting conservation and biological research, ecosystem restoration and the wider human community (Parker, 2008). Despite their pertinent role, the success rate of fauna translocations remains poor (Fischer and Lindenmayer, 2000). In a recent assessment of species relocations within Australia (Sheean et al., 2012), only 46% were successful. While there are a range of factors influencing translocation success, it is increasingly (albeit inconsistently) recognised that parasites (using the term broadly to include viruses, bacteria, fungi, protozoa, helminths and arthropods; Viney and Graham, 2013) impose a risk to host fitness and translocation success (Griffith et al., 1993; Viggers et al., 1993; Cunningham, 1996; Sainsbury and Vaughan-Higgins, 2012).

Hosts are usually infected by multiple parasite species (polyparasitism). In essence, therefore, fauna translocations involve the movement of complete "biological packages" from one ecosystem to another, during which the disruption of normal host-parasite relationships can have various outcomes for both the host and the parasites it carries (Corn and Nettles, 2001; Telfer et al., 2010, Moir et al., 2012). In contrast to the widely recognised disease risks associated with translocating wildlife (see Table 1 for examples), the way in which fauna translocations disrupt the dynamics of within-host parasite communities (infracommunities) is far less well understood, as is the impact of such perturbations on host fitness and translocation success.

Table 1: Examples of disease transmission risks during fauna translocations.

Risk	Example	Effect / Outcome	References
Translocated host introduces novel parasite into naïve wild population	Parapoxvirus introduced into the United Kingdom by grey squirrels (*Sciuris carolinensis*)	Debilitating skin disease, catastrophic mortality and local extinction of native red squirrels (*Sciurus vulgaris*)	Tompkins et al., 2002 Sainsbury et al., 2008
	Crayfish plague (*Aphanomyces astaci*) introduced into Europe by infected North American crayfish.	Local extinction of native European crayfish (*Austropotamobius pallipes*).	Holdich and Reeve., 1991 Prenter et al., 2004
Translocated host exposed to local endemic parasite	Caribou (*Rangifer tarandus*) and moose (*Alces americana*) translocated into areas inhabited by white-tailed deer (*Odocoileus virginianus*) exposed to the meningeal worm *Parelaphostrongylus tenuis*.	Major morbidity and mortality amongst translocated hosts due to the development of cerebrospinal nematodosis (the meningeal worm does not affect local white-tailed deer, which have coevolved with this parasite).	Anderson., 1972 Viggers et al., 1993
	North American elk (*Cervus canadensis*) translocated into the Gila Forest, New Mexico, exposed to the arterial worm (*Elaeophora schneideri*), which is endemic in local mule deer (*Odocoileus hemionus*)	Morbidity (blindness, neurological symptoms, facial gangrene and abnormal antler growth) and mortality of elk calves (15-20% survival rate). Arterial worm infection is asymptomatic in mule deer.	Viggers et al., 1993 Hibler et al., 1969
Parasite spillover from translocated wild host to domestic/human host and vice versa	Translocated bighorn sheep (*Ovis canadensis*) contracted pasteurellosis from direct contact with healthy domestic sheep.	Bighorn sheep developed fatal *Mannhaeimia haemolytica* pneumonia.	Foreyt, 1989 Kock et al., 2010
	Brush-tailed possums (*Trichosurus vulpecula*) translocated from Australia to New Zealand acquired bovine tuberculosis (*Mycobacterium bovis*) from infected dairy cattle.	Possums became a new reservoir host for the disease (i.e. amplified parasite transmission) with significant economic consequences for the New Zealand dairy industry.	Coleman, 1988 Daszak et al., 2001 Kock et al., 2010

We know for example, that a translocated host can acquire novel parasites (using the term "novel" to refer to any parasite that an individual has not previously encountered) within the destination site with devastating consequences for host health and survival. What we don't know is the mechanism behind this outcome. The presence of both canine distemper virus (CDV) and *Sarcoptes scabiei* (mange) reduced pack growth rates of Yellowstone's reintroduced grey wolves (*Canis lupus*), and in severe cases mange was associated with pack extinctions (Almberg et al., 2012). While host density and connectivity appeared to influence the spatio-temporal spread of sarcoptic mange, there were some packs that remained mange-free despite close proximity or territorial overlap with other infected packs. Likewise the spread and severity of mange varied among individuals within the same pack.

Inconsistencies such as these raise a number of important questions with regard to host-parasite dynamics and resistance to infection within an individual. For example, does the presence of *S. scabiei* directly influence host health? What role does stress and immune function play in enabling *S. scabiei* acquisition and persistence, particularly in the presence of CDV? Does exposure to *S. scabiei* (or any other novel parasite) affect the abundance or pathogenicity of other pre-existing parasites within a host? One can see how this aspect of host-parasite ecology is of great importance, yet there is a lack of research evaluating how fauna translocations influence host-parasite assemblages at the individual level; and the serious, often permanent consequences of using anti-parasitic drugs to remove parasites during translocation.

Our aim in this paper is to illustrate how fauna translocations have the potential to alter within host-parasite relationships and how translocation-induced perturbations to parasite infracommunities may affect host health and translocation success. We also highlight the potential positive and negative consequences of anti-parasitic drug treatment and investigate the possible benefits of conserving parasites during translocation.

Polyparasitism and infracommunity interactions

In the past, studies have focused on the effects of single parasite species within a host (Bordes and Morand, 2011; Holmstad et al., 2005) despite polyparasitism (co-infection, multiparasitism or concomitant infection) being the norm in wild animal populations (Keusch and Migasena, 1982; Graham, 2008). Theoretical studies have suggested that polyparasitism will reduce host fitness more than single infections (Bordes and Morand, 2011). This may occur because polyparasitism can lead to competitive interactions between parasite species or strains, resulting in increased virulence, which we define as the degree of parasite-induced reduction in host fitness (Lymbery and Thompson, 2012). For example, experimental coinfection of laboratory rats (*Rattus norvegicus*) with *Trypanosoma lewisi* and *Toxoplasma gondii* resulted in higher numbers of *T. gondii* tachyzoites compared to rats infected with *T. gondii* alone (Guerrero et al., 1997; Catarinella et al., 1998), suggesting that in co-infected hosts *T. gondii* had increased virulence.

In addition, polyparasitism has the potential to reduce host fitness and increase susceptibility to predation or disease through synergistic effects on the course and severity of infection (Irvine, 2006). Observational studies in wildlife have detected a negative correlation between polyparasitism and host body condition (Holmstad et al., 2005; Lello et al., 2005; Jolles et al., 2008); although a causal connection has rarely been demonstrated experimentally. Gibson et al.

(2011) found that California sea lions (*Zalophus californianus*) parasitised with *Sarcocystis neurona* and *T. gondii* succumbed to severe protozoal encephalitis and death, while sea lions with single *S. neurona* infections showed no disease symptoms. Likewise, domestic piglets (*Sus scrofa domesticus*) experimentally infected with *Ascaris suum* and *Escherichia coli* displayed severe signs of respiratory disease and weight loss, due to migrating *A. suum* larvae transporting *E. coli* to the lungs (Adedeji et al., 1989).

On the other hand, interactions between parasites may suppress pathogenicity within a host, reducing the impact of disease. In a murine coinfection model, prior infection with the filarial nematode *Litomosoides sigmodontis* protected the host against malarial (*Plasmodium berghei*) pathology via immunomodulation (Ruiz-Fernández, 2008). Mixed trypanosome infections in woylies (*Bettongia penicillata*) also suggest that interspecific competition may sometimes be important in reducing pathogenic effects on the host (Thompson et al., 2014). Woylies initially infected with *Trypanosoma vegrandis* never developed subsequent *Trypanosoma copemani* infections, while several woylies that initially tested positive to *T. copemani*, later also tested positive to *T. vegrandis*. This is of significance because *T. copemani* appears to be more pathogenic than other trypanosome species found in woylies, because of its propensity for intracellular invasion (Botero et al., 2013).

Fauna translocations and within-host-parasite dynamics

Because wildlife are host to a variety of parasites, there is inherent difficulty in not only determining what parasite species and/or strains are present, but also predicting how these parasites interact with each other and their host during translocation (Sainsbury and Vaughn-Higgins, 2012; Aiello et al., 2014). Translocating fauna can alter existing cycles of parasite transmission among translocated hosts, and establish new transmission cycles between translocated hosts and the recipient host community, thereby altering parasite infracommunity structure and establishing new parasite interactions within hosts. To demonstrate the basic processes by which translocation may influence within-host parasite interactions, parasite persistence and host health, we provide a conceptual framework (Figure 1), which we refer to in the sections below.

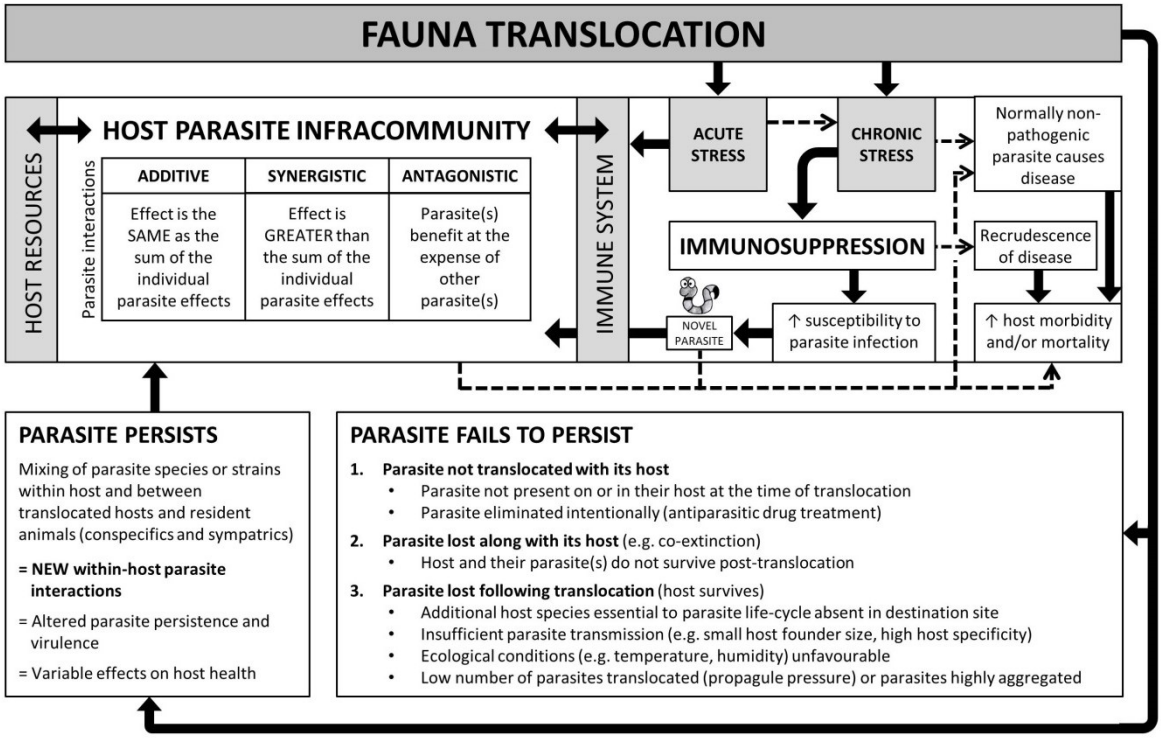

Figure 1: Conceptual flowchart depicting how fauna translocations may influence within-host parasite interactions, parasite persistence and host health. Solid arrows represent known, predictable outcomes (e.g. translocation almost always causes acute stress), whereas dashed arrows indicate potential, less predictable outcomes (e.g. acute stress may lead to chronic stress, but, this is not always the case).

To better understand how fauna translocations may influence polyparasitism within a host, a basic understanding of within-host competitive interactions is required. Romansic et al. (2011) highlight that the presence of multiple parasites within a single host can have additive, synergistic, or antagonistic effects; and competition between parasites may be direct (e.g. competition for physical space), or indirect via "bottom-up" (e.g. antagonism wafor mutual host resources) or "top-down" processes (e.g. immune-mediated competition or collaboration) (Pedersen and Fenton, 2006; Knowles et al., 2013) (Figure 1). Interactions between parasites, particularly helminth-microparasite coinfection, have been described using a "hypothetical within-host-parasite community interaction network" (Pedersen and Fenton, 2006) comprising three levels of trophic structure; host resources, parasite community and host immune system (Pedersen and Fenton, 2006; Graham, 2008). This network can be used to predict the outcome of polyparasitism using ecological first principles. In helminth-microparasite coinfected mice for example, Graham (2008) demonstrates how "bottom-up" resource-based (Figure 2A) and "top-down" immunological control (Figure 2B) can regulate microparasite population size.

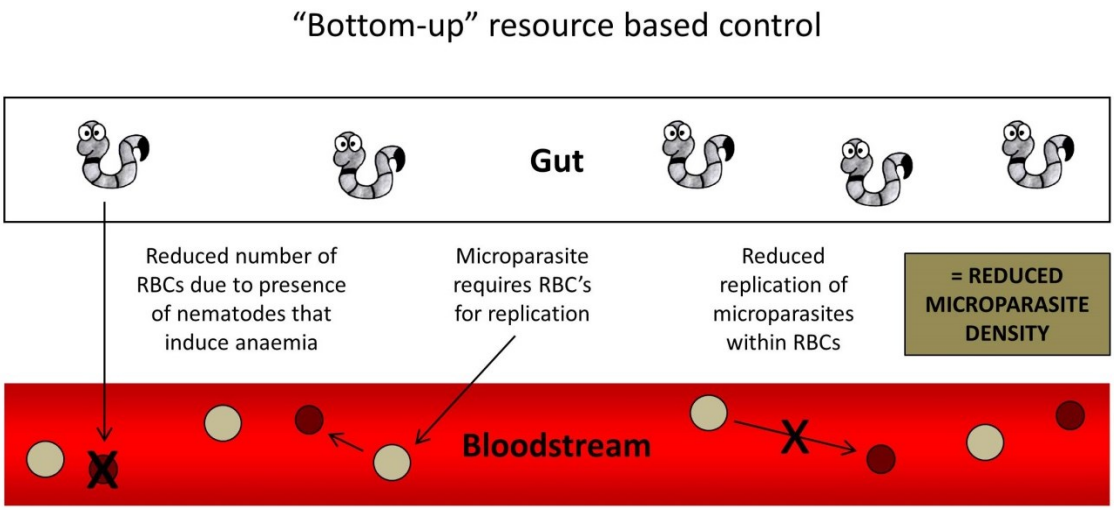

Figure 2A: A schematic representation of "bottom-up" resource-based control. In this scenario, helminth-induced changes in RBC availability impose "bottom-up" control of microparasites that require RBCs for replication (i.e. resource limitation), decreasing microparasite population size.

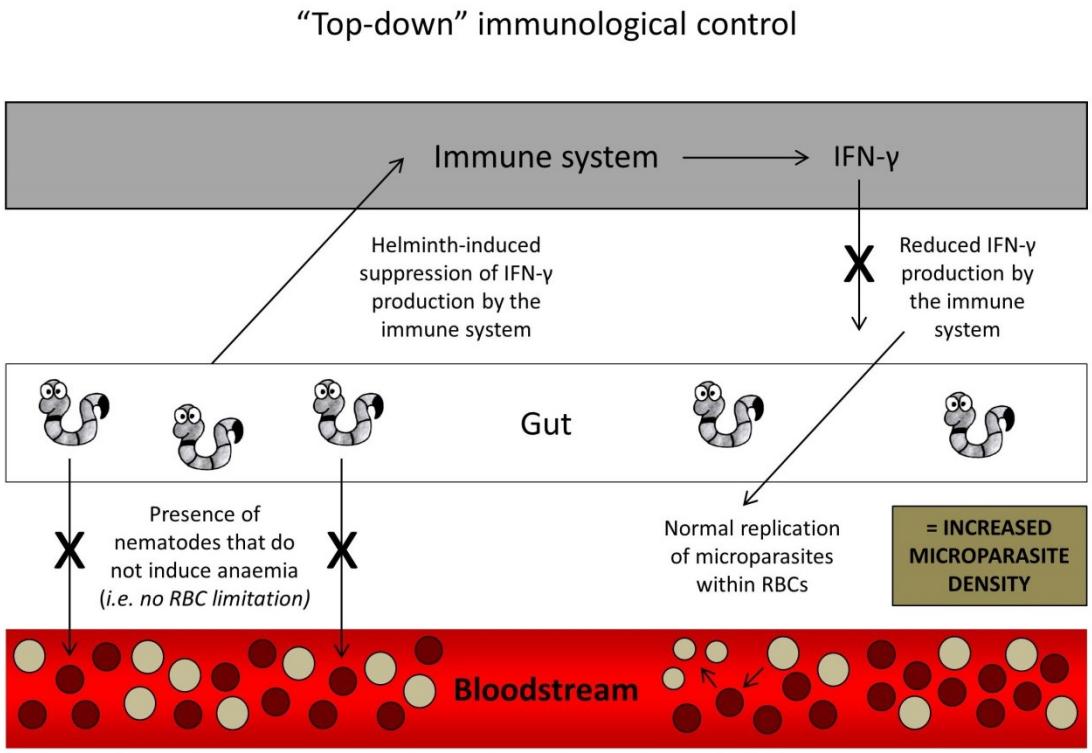

Figure 2B: A schematic representation of "top-down" immunological control. In this coinfection model, helminth-induced suppression of IFN-γ predictably increases microparasite population size. Importantly, this picture also illustrates how microparasites may be positively influenced by helminth co-infection in the absence of resource limitation.

While ecological first principles provide a theoretical framework that enables us to comprehend how "bottom-up" and "top-down" processes may influence parasite interactions within a host, the immunological response to different parasites varies considerably both between and within-hosts depending on the parasite(s) in question [see Cox (2001) and Supali et al. (2010) for examples]; thus the outcome of infection is not easily predictable. For translocated hosts with known parasites, knowledge of how each parasite may influence immune function could theoretically be used to inform parasite management protocols using ecological first principles. While this is by no means straight-forward, it provides a starting point for decision making, particularly with regard to the use of anti-parasitic drugs (see section 6).

Stress and immunocompetence of the host

As the epidemiology of individual parasite species within a host is governed by interactions between co-infecting parasites, we must also consider how resistance to infection may be influenced by immunocompetence of the host; an important concept for translocated hosts. During translocation, prolonged or recurrent exposure to multiple acute stressors (e.g. capture, clinical examination, transportation, captivity and release into a novel environment) can cause chronic stress, which may depress immunity and reduce the ability of a host to resist infection (Dickens et al., 2009; Poulin et al., 2011; Aiello et al., 2014) (Figure 1). Captivity in particular has been identified as a "critical step" in inducing chronic stress in birds (Dickens et al., 2009), which may compromise post-translocation survival by enhancing vulnerability to disease (Dickens et al., 2010). Stress associated with handling, captivity and release into a new environment has been linked to high mortality rates in translocated beavers (*Castor fiber*), which succumbed to leptospirosis and yersiniosis infection post-translocation (Nolet et al., 1997). Stress-mediated immunosuppression has also been associated with recrudescence of latent disease in zoo translocations (e.g. toxoplasmosis in macropods; Adkesson et al., 2007; Bermudez et al., 2009). While there is a link between chronic stress and disease during translocation, the precise mechanisms by which stress influences within-host-parasite relationships is yet to be determined.

Translocations involving captive-bred animals also suggest that immunological naivety is linked with decreased survival post-translocation (Jule et al., 2008; Faria et al., 2010; Boyce et al., 2011; Ewen et al., 2012a). During captivity, regular contact with parasites is lost, which enhances vulnerability to infection due to loss of acquired immunogenic variation (Viggers et al., 1993; Mathews et al., 2006, Faria et al., 2010). Parasites may also be removed intentionally, which may have adverse consequences for the host when they are released into the wild. In captive-bred

guppies (*Poecilia reticulata*) that underwent experimental reintroduction, those that were pre-exposed to native parasites had significantly lower parasite loads at the end of the experiment and their ability to eliminate parasite infection was higher, compared to naïve guppies (Faria et al., 2010). These effects may be exacerbated in endangered species that often have reduced immunocompetence associated with inbreeding and low genetic diversity (e.g. Cassinello et al., 2001).

Factors influencing parasite persistence

Selection pressures imposed on parasites during and post-translocation may lead to parasite extinction, and parasite loss, whether incidental or intentional, is commonplace (Torchin et al., 2003; MacLeod et al., 2010). In reintroduced Eastern bettongs (*Bettongia gaimardi*), five ectoparasite species recorded at the point of translocation failed to persist following translocation (Portas et al., 2016). Parasites may fail to persist because they are either not translocated with their host in the first place, or they do not survive following translocation (Figure 1; Torchin et al., 2003; MacLeod et al., 2010). For chewing lice on New Zealand's introduced bird species, 40% of native parasite genera were absent post-introduction and parasites were more likely to be lost following translocation than not translocated with their host (MacLeod et al., 2010).

Parasite loss during and post-translocation may have important consequences for coinfecting parasites and host health, where the presence of one parasite may indirectly regulate another (Figure 3). For example, if parasite A (nematode) were lost during translocation, parasite B (microparasite) may thrive and negatively influence host health. This has been demonstrated experimentally, with elimination of *H. polygyrus* in wild wood mice (*Apodemus sylvaticus*) resulting in a 15-fold increase in *Eimeria* spp. intensity, suggesting dynamic localised competition between *H. polygyrus* and *Eimeria* spp. (Knowles et al., 2013). As *Eimeria* spp. are classified as "high risk" parasites during the stress of translocation for some species (e.g. *E. reichenowi* in cranes, Ellis et al., 1996), the loss of a coinfecting regulatory parasite may intensify the negative effects of infection with *Eimeria* spp. Equally, resistance to one parasite may confer resistance against other parasites that the host may encounter following translocation (Spencer and Zuk, 2016). In flies (*Drosophila melanogaster*), the experimental removal of the bacterium *Wolbachia pipientis* reduced the survival of its virus-challenged hosts, suggesting protection against virus-induced mortality (Hedges et al., 2008). In mice, bacteria (e.g. *Corynebacterium parvum*) and bacterial products likewise protect their host from intraerythrocytic protozoal infections (e.g. *Plasmodium falciparum*) via immunomodulatory mediators (Cox, 1975).

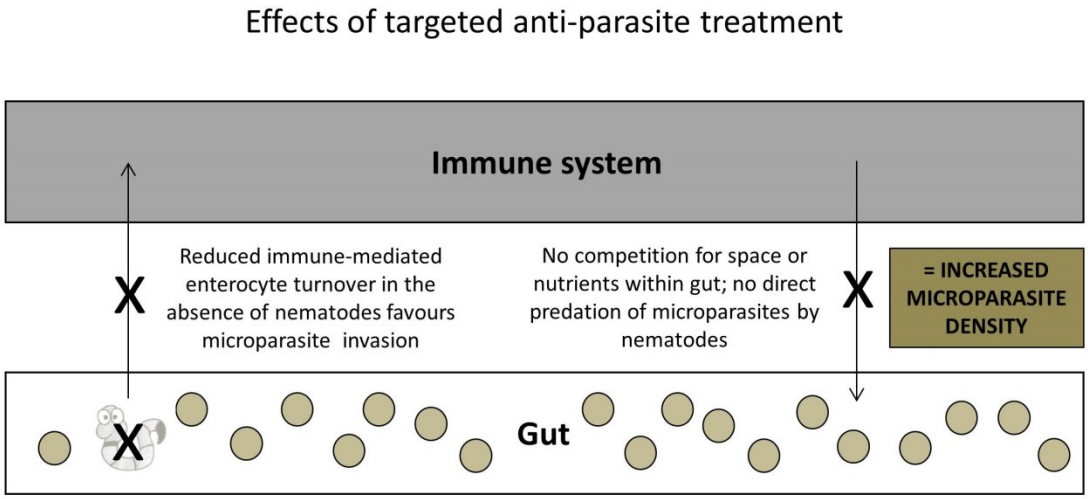

Figure 3: Mechanisms by which anti-parasitic drug treatment can indirectly affect non-target microparasites within a host. In this scenario, the elimination of nematodes alters the within-host-parasite network to benefit microparasites within the gastrointestinal tract.

Anti-parasitic drug treatment

In an effort to enhance host health and translocation outcomes, wildlife are often treated with anti-parasitic drugs prior to translocation. For critically endangered species with parasites that impose a risk to host survival, or when every individual is imperative for species survival (e.g. small population size), parasite treatment may be warranted (Stringer and Linklater, 2014). The decision regarding whether or not to implement parasite control during fauna translocations however, is constrained by our lack of knowledge about most parasite infracommunities of wildlife. One of the main limitations in using anti-parasitic drugs in wildlife is that few clinical trials have been carried out to evaluate the safety or efficacy of these drugs. Dose rates, routes of administration, and dosage regimes are often extrapolated from closely related species, and may not be feasible in the context of translocating fauna (e.g. administering repeat doses). Thus we cannot be certain whether (a) anti-parasitic drug treatment will actually work, and (b) what parasite species will be affected (directly or indirectly) by treatment.

Regrettably, anti-parasitic drugs are also often administered in an inconsistent manner without any attempt to assess the effectiveness of treatment following translocation (Pedersen and Fenton, 2015). Routine translocation protocols often use parasite control in an analogous manner to the enemy release hypothesis, by inferring that treated individuals may benefit from reduced parasite burden post-release (Almberg et al., 2012). Studies in invasive animal species show that invaders

have fewer parasites (Torchin et al., 2003), and the absence of such parasites may explain their success. Introduced populations of the European green crab (*Carcinus maenas*) for instance, have fewer parasites than native European populations, and are larger and heavier than native crabs (Torchin et al., 2002). However, while there is evidence to support the fact that translocated hosts have fewer parasites, there are no equivalent studies in translocated hosts that empirically demonstrate that parasite loss offers any competitive advantage post-translocation, thus this outdated practice can no longer be justified.

In studies that do evaluate the efficacy of anti-parasitic drug treatment in wildlife, there isn't always a benefit of treatment to host health. Experimental anthelmintic treatment in juvenile eastern grey kangaroos (*Macropus giganteus*) did not significantly improve body condition, weight or growth rates (leg or pes length), despite oral albendazole significantly reducing strongylid egg counts in treated individuals (Cripps et al., 2014). Equally, ivermectin administered to woylies prior to translocation did not improve body condition post-translocation (Northover et al., 2017), nor did it alleviate physiological stress (measured using faecal cortisol metabolites) associated with parasite infection (Hing et al., 2017). Thomas and Morgan (2013) reported reduced host health (i.e. poorer live weight gain) in anthelmintic treated alpacas compared to control animals.

Despite efforts to 'control' parasites during translocation, the effect of anti-parasitic drug treatment is unpredictable and only transitory, and translocated hosts will in due course be exposed to an array of parasites with variable outcomes. Almberg et al. (2012) found that vaccination and anti-parasitic drug treatment only conferred short-term benefits to reintroduced grey wolves (*Canis lupus*), which eventually succumbed to infection with endemic parasites, thus highlighting that this strategy does not eliminate the risk of translocated hosts acquiring parasites from the resident host community (Larkin et al., 2003). While the effects of anti-parasitic treatment are infrequently examined during and/or post-translocation, field studies in non-translocated populations also highlight the transient nature of these drugs. Ivermectin treated free-ranging Australian sea lions (*Neophoca cinerea*) for example, were re-infected with lice (*Antarctophthirus microchir*) at the same prevalence and intensity as control animals two months post-treatment (Marcus et al., 2015). Cripps et al. (2014) also found a transient reduction in strongyle egg counts in juvenile eastern grey kangaroos following albendazole treatment, however mean faecal egg counts were significantly greater in the treated group compared to the control group at some points post-treatment.

Anti-parasitic drug treatment and within-host-parasite dynamics

The administration of any anti-parasitic drug prior to translocation will to some degree disrupt parasite infracommunity structure. Even in the case of 'targeted' anti-parasitic drug treatment, the drugs selected often target more than one parasite group (e.g. ivermectin; nematodes and arthropods) and treatment is in fact not targeted at all. This raises a number of important questions. Does targeting one part of the parasite community create more 'space' for coinfecting parasites to thrive or facilitate parasite invasion? What are the effects of treatment in non-target parasites? Notably, there will also be instances where we have identified parasites of concern (e.g. *T. copemani* in woylies; Thompson et al., 2014), however drugs are not available to treat these parasites in wildlife. Targeting other parasite genera and disrupting host-parasite relationships may inadvertently potentiate the adverse effects of such parasites.

Experimental studies in mice demonstrate how anti-parasitic drug treatment can indirectly influence the abundance of coinfecting parasites. In white-footed mice (*Peromyscus leucopus*), anthelmintic treatment resulted in a reciprocal increase in coccidia prevalence (Pedersen and Antonovics, 2013). As the presence of gastrointestinal nematodes may elicit some form of protective immunity for the host against coinfecting microparasites, or down-regulate their negative impact on host health, anthelmintic treatment has the potential to negatively influence host health (Fenton, 2013). In free-living yellow-necked mice (*Apodemus flavicollis*), anthelmintic treatment unexpectedly increased non-target tick (*Ixodes ricinus*) numbers, an effect that may negatively influence host health (*I. ricinus* is the vector for tick-borne encephalitis virus; Labuda et al., 1997) and potentially impact tick-borne disease dynamics within the population (Ferrari et al., 2009).

Parasite treatment may even enhance host susceptibility to disease upon re-exposure (Viggers et al., 1993). Hosts reintroduced to supplement wild populations that are likely to encounter parasites that have evolved to exploit them, may be particularly vulnerable to parasite invasion (Almberg et al., 2012). Thus in circumstances where the host is likely to encounter a host-specific parasite with a prolonged period of coevolution, eradication of the parasite prior to translocation is not recommended (De Leo and Dobson, 2002; McGill et al., 2010).

Justification for anti-parasitic drug use

As "treating for good health" and eliminating parasites can disrupt parasite community composition (Pedersen and Antonovics, 2013), enhance susceptibility to disease (Viggers at al.

1993; Hedges et al., 2008), and increase morbidity and mortality post-translocation (Almberg et al., 2012), there needs to be strong justification for the use of anti-parasitic drugs, such as targeting a parasite with demonstrated negative effects on host health. Host monitoring should also be carried out to determine whether parasite treatment has (a) controlled the parasite of concern, and (b) whether treatment has benefited host health. Monitoring non-target parasite genera is also important for identifying anyindirect effects of parasite treatment. While adopting an appropriate experimental framework (e.g. having treatment and control cohorts, replicates and repeated trials over space and time) would be ideal for reliably improving our knowledge in this area and informing parasite management protocols in future translocations, this expectation is likely to be unrealistic when dealing with small numbers of threatened species. Undertaking studies such as these in closely related or more common species however, may help fill the knowledge gap in this area. In cases where the effects of anti-parasitic drug treatment are poorly understood, or there is no clear justification for their use, a precautionary approach is advised and parasite conservation, rather than parasite elimination, should be considered.

Ideally parasite control must offer benefits to host health that outweigh the cost of disrupting host-parasite relationships (Stringer and Linklater, 2014) and should aim to minimise disease rather than eliminate parasites in their entirety. For parasites that are capable of inducing epidemic disease in their host during the stress of translocation (e.g. coccidia), translocation outcomes have improved following the implementation of such protocols. Immature Eurasian Cranes (*Grus grus*) receive prophylactic treatment to reduce but not eliminate coccidian parasites during captive-rearing, as exposure to this parasite during development stimulates immunity and reduces the likelihood of disease outbreaks during translocation (Sainsbury and Vaughan-Higgins, 2012). Strict hygiene and prophylactic treatment to control disease but not eradicate the coccidian parasite *Isospora normanlevinei* has similarly contributed to the success of cirl bunting (*Emberiza cirlus*) translocations, even though this parasite has been associated with mortality of translocated hosts in the past (McGill et al., 2010). In captive black-footed ferrets (*Mustela nigripes*), two species of *Eimeria* were likewise conserved rather than eradicated from the population (Williams et al., 1992; Gompper and Williams, 1998). Exposure to low levels of *Eimeria* spp. during captivity is believed to stimulate immunity, which serves to protect the host during re-exposure in the wild.

Parasite conservation

More recently, studies have started to acknowledge the benefit of conserving parasites (Hudson et al., 2006; Gomez et al., 2012; Hatcher et al., 2012; Spencer and Zuk, 2016). Given the innate ability

of parasites to influence host health and population dynamics, parasite conservation may be crucial for preserving overall ecosystem integrity (Thompson et al., 2010; Gomez et al., 2012). In addition, the loss of parasites is likely to affect the evolutionary trajectory of a host population. Parasites and their host are classically described as being in an 'arms race' in which selective pressures placed on one species by another drive the process of evolution. Continual adaptation between a parasite and its host or between parasites within a host, elicit selection for adaptations that enhance competitive fitness and therefore survival (Strickberger, 2000). Translocation and anti-parasitic drug treatment can interfere with this host-parasite arms race, which ultimately disrupts host adaptation and evolution (Nunn et al., 2004), and may have unintended impacts on non-target parasite species (Spratt, 1997).

Of particular concern for threatened species, is that translocation may induce parasite extinction cascades for host-specific parasites that are likely to be endangered themselves (Colwell et al., 2012) and anti-parasitic drug treatment may further compound this effect. For example, the host-specific louse *Rallicola* (*Aptericola*) *pilgrimi* did not survive translocation to predator-free islands along with its host the spotted kiwi (*Apteryx owenii*) and is now extinct (Buckley et al., 2012). Targeted ectoparasite removal in black-footed ferrets (*Mustela nigripes*) is suspected of causing the extinction of the host-specific louse (*Neotrichodectes* sp) and this species is now host to a low diversity of generalist ectoparasites (Harris et al., 2014). Delousing treatment is also believed to be responsible for the extinction of the host-specific louse *Colpocephalum californici* in the California condor (*Gymnogyps californianus*) (Rozsa and Vas, 2015). A recently discovered species of tick (*Ixodes woyliei*) found almost exclusively on critically endangered woylies is considered to be at risk of coextinction due to its apparent host specificity and the extensive use of fauna translocations in the management of its host; a risk that is heightened by the use of anti-parasitic drugs (Ash et al., 2017). Thus, parasites may be particularly vulnerable to extinction through processes designed to conserve the host.

Embracing the concept of parasite co-introduction

With our increasing awareness of the ecological and evolutionary importance of parasites (Almberg et al., 2012), the concept of parasite co-introduction has recently come into focus. Essentially we cannot translocate a species in order to "save" it without considering what else needs to be translocated with it, thus we can no longer view a translocated host as an entity on its own. We must consider the entire "biological package." Parasites are now considered meaningful conservation targets (Gomez and Nichols, 2013) and the IUCN Species Survival Commission

appeals for deliberation of parasite co-introduction during fauna translocations (IUCN/SSC 2013). Rebuilding ecosystems is one of the aims of translocating fauna, and parasite co-introduction should be encouraged for native parasites that have coevolved with their host (Jørgensen, 2014; Rideout et al., 2016). For endangered species with host-specific parasites, in which the likelihood of parasite extinction outweighs that of the host, co-introduction offers a means of conserving biological diversity (Moir et al., 2012) and preserving the host-parasite arms race, which promotes the maintenance of genetic diversity (Stringer and Linklater, 2014).

The host-specific louse, *Felicola (Lorisicola) isidoroi*, found on the endangered Iberian lynx (*Lynx pardinus*) is now considered a conservation target, and any live lice that are found on lynx during translocation are manually removed and transferred to captive lynx for preservation (Perez et al., 2013). In the case of translocating fauna, maintaining host-parasite relationships can enhance host immunity (Pizzi 2009; McGill et al., 2010; Boyce et al., 2011), which ultimately reduces disease susceptibility and thus morbidity and mortality, thereby improving translocation outcomes (Rideout et al., 2016). During captive breeding and translocation of the brush-tailed rock wallaby (*Petrogale penicillata*), *Eimeria* spp. community structure within wild, captive bred and supplemented populations has been maintained by not administering anti-coccidial drugs to hosts before release or during translocation between sites (Vermeulen et al., 2016). Similarly, the Save the Tasmanian Devil Program aims to conserve parasites and symbionts during captive management and translocations to enhance immunity, a practice that is particularly important for a species with low genetic diversity, which may be more susceptible to disease (Wait et al., 2017). Tasmanian devil insurance populations were routinely treated with prophylactic anti-parasitic drugs in the past (Jones et al., 2007); this is no longer the case (Wait et al., 2017).

While the concept of parasite conservation has been given significant attention, the practicalities of implementing parasite conservation are largely neglected, with consideration needed for how dependent parasite species will survive on their host within a new environment (summarised by MacLeod et al., 2010). In many cases, the number of translocated hosts may be too small to support sustainable populations of dependent parasite species (Moir et al., 2012), or ecological conditions (e.g. temperature, humidity) may be unfavourable. Parasites with high host specificity such as *Eimeria* spp. (Duszynski and Wilber. 1997) are strongly influenced by host density and are more likely to undergo declines with their host. This is also true for parasites with complex life-cycles (e.g. helminths). If a host population is too small for adequate transmission, parasites may fail to persist following translocation (Figure 1). The absence of coccidia in Gunner's Quoin night

geckos (*Nactus coindemirensis*) has been attributed to a population bottleneck, where the host population size fell below the threshold density required to sustain infection (Leinwand et al., 2005). Likewise, when parasite control programs aim to reduce but not eliminate certain parasites, they may in fact cause the extinction of species due to suboptimal numbers being translocated. As the very act of translocating fauna decreases the diversity of dependent parasite species (Moir et al., 2012), parasite control may exacerbate this process.

In an effort to conserve host-parasite assemblages and thus ecological function post-translocation, the IUCN Guidelines for Reintroductions and Other Conservation Translocations (IUCN/SSC, 2013) provide a framework for maintaining and/or restoring host-parasite relationships, while minimising disease risk. The ten key questions proposed by Armstrong and Seddon (2007) likewise provide an integrated approach to fauna reintroduction biology, with four of these questions being applicable to parasite management (Ewen et al., 2012b; see Box 1). It is important to note there are cases where (a) parasites have been identified as a threat, (b) parasites are verified not to be a threat (e.g. non-pathogenic commensal parasites), or (c) the potential pathogenicity of a parasite is uncertain. The decision regarding whether or not to implement anti-parasitic drug treatment will vary in each case. To gain a greater understanding of host-parasite assemblages and their impact on host health post-translocation, the value of parasite monitoring cannot be over-emphasised. Evaluating the efficacy of anti-parasitic drugs in wildlife is also of paramount importance. Once we have a better understanding of the parasite infracommunities we are dealing with, guidelines such as those proposed above can be used as an evolving tool (i.e. an adaptive management approach) and form the basis for decision making with the overall aim of improving the outcome of wildlife translocations.

Box 1

Parasite management during fauna translocations - Key questions:

1. How is post-release survival affected by parasite management?
2. How do parasites within the release site affect suitability for translocation?
3. Are parasites native to the ecosystem?
4. How will the ecosystem be affected by the parasites?

Concluding remarks

Determining the best way to manage parasites during fauna translocations requires a multifaceted approach. On the one hand, parasites provide vital ecosystem services, drive host adaptation and

evolution, and constitute an important component of biodiversity; on the other hand parasites are capable of compromising host health and population survival post-translocation. We highlight the importance of the fundamental host-parasite relationship and demonstrate how translocation- and treatment-induced perturbations to host-parasite assemblages can negatively influence host health and translocation outcomes. We also stress the need for parasite conservation and preservation of host-parasite relationships during fauna translocations, where it is deemed safe to do so. While we do not discredit the value of anti-parasitic drug treatment, we do question the ad-hoc use of anti-parasitic drugs without clear purpose or adequate monitoring to validate treatment efficacy. Given the potential ramifications of anti-parasitic drugs in both target and non-target species, parasite control must be justified and preservation of the host-parasite relationship should be a key consideration in the design and implementation of fauna translocation programs. We identify the need for ongoing surveillance and screening of native wildlife in conjunction with field-based studies to further elucidate the impacts of translocation and anti-parasitic drug treatment on parasite assemblages in translocated hosts and co-habiting species.

1.2 The woylie (*Bettongia penicillata*)

The woylie or brush-tailed bettong *Bettongia penicillata* (Gray) (Figure 4) is an endemic Australian macropod marsupial (Family Potoroidae), which is currently listed as critically endangered by the international Union for Conservation of Nature (Woinarski and Burbidge, 2016). Adult woylies weigh between 1.0-1.6kg, measure approximately 600 mm in length (nose to tail tip) and are continuous breeders (Richardson, 2012). Woylies have been documented to reach sexual maturity as early as 122 days (Thompson et al., 2015). Woylies are nocturnal, foraging for hypogeal fungi (truffle-like mushrooms), tubers, bulbs and seeds at night; and sheltering in nests built from plant material during the day (Richardson, 2012). The foraging activity of the woylie facilitates healthy ecosystem functioning, in particular fungal sporulation and seed dispersal (Fleming et al., 2014).

Figure 4: An adult female woylie from Dryandra Woodland (image courtesy of John Lawson).

Once abundant across most of southern and central Australia, indigenous woylie populations declined following European settlement (de Tores and Start 2008). By the 1960's remaining woylie populations were confined to three isolated areas in south-western Australia (Sampson, 1971). In response to conservation efforts (i.e. feral predator control and translocations), woylie numbers recovered, and in 1996 the woylie became the first Australian taxon to have its conservation status downgraded (Start et al., 1998). Unexpectedly, woylie populations then underwent a 90% decline

in population size between 1999 and 2006, and are now restricted to three wild populations (Kingston, Perup and Dryandra) located within the south-west of Western Australia (Wayne et al., 2015). Both the Kingston and Perup woylie populations are situated within the Upper Warren region, where woylie population declines were most pronounced (95% decline between 2002 and 2010; Wayne et al., 2013). In 2010, DBCA constructed Perup Sanctuary, a 423 ha fenced reserve located within the Tone-Perup Nature Reserve near Manjimup, to support an insurance population of woylies during the decline (Wayne et al., 2013). The initial founding population of woylies (n = 41; introduced November-December 2010) were obtained from various sites within the Upper Warren. In July 2013, an additional 36 woylies were translocated into Perup Sanctuary from Dryandra. Since then, Perup Sanctuary has been sporadically supplemented with woylies from various sites including offspring from the now extinct Tutanning woylie population (Wayne et al., 2013).

While numerous hypotheses have been proposed to explain the population declines including mesopredator release of feral cats (*Felis catus*) in response to red fox (*Vulpes vulpes*) baiting (Marlow et al., 2015; Wayne et al., 2015), the spatio-temporal pattern of decline suggests the potential role of an infectious disease agent (Wayne et al., 2015). Monitoring carried out immediately prior to, and during the decline, detected a high prevalence of woylies with skin disease, but despite thorough investigation a causative disease agent could not be identified (Wayne et al., 2013). Since then, the focus of investigation has shifted toward the potential role of other disease agents. *Trypanosoma* spp. have been of particular interest, given the demonstrated pathogenicity of *T. copemani* genotype 2 (G2) and the association between *T. copemani*, *Trypanosoma* spp. coinfection and declining woylie populations (Smith et al., 2008; Botero et al., 2013; Thompson et al., 2014; Godfrey et al., 2018). It has been proposed that disease associated with *T. copemani* infection may enhance vulnerability to predation by introduced predators (Wayne et al., 2015; Thompson et al., 2014).

1.3 Project aims, objectives and hypotheses

Fauna translocations have the potential to disrupt the parasite community within a host, which may change the host-parasite balance to the detriment of either the host (Telfer et al., 2010) or the parasite (Moir et al., 2012). Unfortunately, knowledge regarding the parasite species infecting Western Australia's native fauna is limited (Wayne et al., 2015), as is our understanding of how fauna translocation and antiparasitic drug treatment influence the parasite community within a host. The primary aim of this project was to investigate how the parasite community of translocated and resident woylies changes following translocation. In particular, this thesis aims to (a) identify gastrointestinal, ectoparasite and haemoparasite species infecting woylies; (b) investigate how ivermectin treatment impacts the prevalence of these parasites, and parasite infracommunity structure within a host; and (c), determine how parasite infection and ivermectin treatment impact woylie health. In Chapter 2, I outline the methods that were used to identify parasites, and quantify parasite infection in woylies during two fauna translocations, including an overview of our study sites and the 'ivermectin treatment trial.'

Given the current conservation status of the woylie and the reliance of this species on periodic translocations, enhancing our fundamental understanding of how parasite community structure changes following translocation and in response to ivermectin treatment will help to improve translocation outcomes and assist DBCA in the planning and implementation of future fauna translocations. Importantly, the insights gained from this project will be broadly applicable to other wildlife conservation agencies for the management of fauna translocations, which will assist in the conservation management of Australia's threatened fauna and ensure the sustainability of wildlife and ecosystem health.

In Chapter 3, I examine how the parasite community of translocated and resident woylies changes following translocation and in response to ivermectin treatment (translocated hosts only). This chapter is presented as a paper (submitted for publication), which specifically aims to evaluate the parasite community on a broader scale (i.e. gastrointestinal, blood-borne and ectoparasite taxa). As parasite loss commonly occurs during fauna reintroductions (Torchin et al., 2003; MacLeod et al., 2010), and ivermectin treatment should theoretically reduce the burden of nematodes and arthropods, I expected parasite infracommunity richness to decrease following translocation, and that this effect would be most pronounced in treated woylies. In Chapter 4, I present a paper (submitted for publication), which morphologically describes and genetically characterises a novel

coccidian parasite, *Eimeria woyliei*, which was identified from woylies during this study. This paper also genetically characterises three other *Eimeria* spp. (*E. gaimardi*, *E. potoroi*, and *E. mundayi*) infecting other potoroid marsupials; no potoroid *Eimeria* spp. had been genetically characterised prior to this study.

To evaluate how fauna translocation impacts the parasite community at a finer level, I also monitored changes to haemoparasite species [*Trypanosoma* spp. and piroplasms (*Theileria* and *Babesia* spp.)] following translocation and in response to ivermectin treatment using the same translocation experiments presented in Chapter 3. This study is presented as a published paper in Chapter 5, and includes the identification of three novel haemoparasites including *Trypanosoma* sp. ANU2 (a putative new species genetically characterised by Cooper et al., 2018), *Theileria apogeana* genotype ANO2 and *Bodo* sp. ANO4. *Trypanosoma gilletti* was also identified in woylies for the first time. As previous molecular studies (Smith et al., 2008; Botero et al., 2013; Thompson et al. 2014; Godfrey et al., 2018) have detected an association between trypanosomes and declining woylie populations within the Upper Warren region, it is important to investigate how trypanosomes may be impacted during translocation, including the indirect effects of ivermectin treatment. Although ivermectin does not directly "target" haemoparasites, a reciprocal increase in "non-target" parasites (e.g. *Eimeria* spp.; Knowles et al., 2013; Pedersen and Antonovics, 2013) has been demonstrated experimentally in other species. Ivermectin treatment may also impact ectoparasite survival, and thus potential *Trypanosoma* spp. transmission. Hence the effects of ivermectin treatment were also investigated here.

As we lack an understanding of how translocation and antiparasitic drug treatment impact host health, Chapter 6 examines the influence of parasite infection and ivermectin treatment on woylie health. This chapter is presented as a paper (intended for journal submission), which investigates whether parasites infecting the woylie adversely influence host health (as measured using BCI, PCV and TPP) during translocation. Importantly this chapter also evaluates whether ivermectin treatment provides any benefit to host health. While it has been inferred in the past that treated individuals may benefit from reduced parasite burden after release, there are no studies that empirically demonstrate that parasite loss offers any competitive advantage after translocation. In fact, the intentional removal of parasites may enhance susceptibility to parasite infection (Viggers et al., 1993). Chapter 6 investigates this theory by examining the efficacy of ivermectin treatment and the link between ivermectin treatment, parasite infection and host health in woylies. Chapter 7 is a published case study that investigates the potential aetiology of disease in a resident woylie,

which was captured within Walcott six months after translocation, and found to be in extremely poor body condition with diffuse alopecia, debilitating skin lesions and severe ectoparasite infestation.

Lastly, the effects of ivermectin treatment were also evaluated in Chapter 8; a published paper, that specifically investigates the impact of ivermectin treatment on gastrointestinal parasites and body condition in a subset of woylies. This preliminary study was undertaken following the first fauna translocation in 2014.

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Chapter Two

Materials and Methods

2.1 Study sites

2.1.1 Translocation overview

2.1.1.1 Perup Sanctuary to Walcott and Warrup East

In June 2014, 182 woylies were translocated from Perup Sanctuary into two wild sites, Walcott (*n* = 92; 46 male, 46 female) and Warrup East (*n* = 90; 44 male, 46 female). Perup Sanctuary is a 423 ha predator-proof enclosure located within the Tone-Perup Nature Reserve roughly 50 km east of Manjimup, Western Australia (34.2506 °S, 116.1425 °E) (Figure 5). Walcott and Warrup East are located within the Greater Kingston subregion of the Upper Warren region, approximately 15 km west and 20 km north-west of Perup Sanctuary, respectively (Figure 5). The Greater Kingston indigenous woylie population is genetically distinct from the Perup indigenous woylie population (Pacioni et al., 2011). Perup Sanctuary is located within the Perup subregion of the Upper Warren. With a Mediterranean-style climate, the Upper Warren region is characterised by warm, dry summers and cool, wet winters (average annual rainfall varies across the area from around 650-900 mm; Wayne, 2005). The dry sclerophyll forests are dominated by jarrah (*Eucalyptus marginate*), marri (*Corymbia calophylla*) and wandoo (*Eucalyptus wandoo*) (Wayne et al., 2015). The topography is slightly undulating with low lateritic ridges, wide valleys and highlands (Wayne, 2005).

2.1.1.2 Upper Warren region to Dryandra

In June 2015, 69 woylies (47 male, 22 female) were translocated from six wild sites within the Upper Warren region (Balban *n* = 21, Boyicup *n* = 1, Corbal *n* = 15, Dudijup *n* = 6, Dwalgan *n* = 16 and Winnejup *n* = 10), into an unfenced wild site within Dryandra Woodland. Balban and Boyicup are located within the Perup subregion of the Upper Warren, while the other sites are found within the Greater Kingston subregion of the Upper Warren (Figure 6). Dryandra Woodland, situated within the Lol Gray State Forest, represents the largest remnant of original vegetation within the western wheatbelt and is located roughly 250 km north-east of our study sites within the Upper Warren (Figure 5). The semi-arid woodland of Dryandra experiences a Mediterranean climate, with warm to hot, dry summers and mild, wet winters (McArthur et al., 1977). Rainfall is on average 500-600 mm per annum (DWMP, 2011). The open-canopy woodlands comprise predominantly wandoo, powderbark wandoo (*Eucalyptus accedens*), brown mallet (*Eucalyptus astringens*) and occasional marri. The topography is gently undulating with granite outcrops, lateritic uplands, valley slopes and valley floors (McArthur et al., 1977).

2.2 Study design

2.2.1 Trapping regime

Depending on the site, time of year and focus of each trapping session, the trapping regime varied accordingly (Table 2). Each destination site contained a centrally placed grid and multiple transects within a 3 km radius monitoring area (Figures 7, 8 and 9). Animals were captured using Sheffield cage traps (Sheffield Wire Products, Welshpool, WA), which were set at dusk and baited with universal bait (rolled oats, peanut butter and sardines). With the exception of July 2015 (night trapping), traps were cleared at first light within three hours of sunrise each day. In all cases, traps were covered with a large hessian bag to provide shelter from the weather and prevent exposure.

Prior to translocation I conducted pre-translocation sampling within each destination site, which consisted of grid trapping (7 x 7 traps, 50 m apart) to estimate woylie density and transect trapping to estimate woylie abundance (Table 2). Within Walcott and Warrup East, monitoring was carried out at one (July), three (September), six (December), ten (April 2015) and eleven (May 2015) months after translocation. All post-translocation trapping except May 2015 (grid) consisted of transect trapping (≤106 traps/night, 100-200 m spacing) over three-four consecutive nights. Within Dryandra, monitoring was carried out at one (July), two (August), three (September), six (December), nine (March 2016) and twelve (June 2016) months following translocation. All post-translocation trapping except June 2016 (grid) consisted of transect trapping (100 traps/night, 200 m spacing) over four consecutive nights.

2.2.2 Animal identification

Each individual was identified with two uniquely numbered ear-tags, and in a few cases (e.g. unable to place ear tags due to pinnae injury/malformation) a passive integrated transponder (microchip) was inserted subcutaneously between the shoulder blades.

2.2.3 Sample collection

Samples were collected from woylies at each (re)capture. For resident woylies, this includes all time points prior to, and following, translocation. For translocated woylies, this includes the point of translocation (i.e. prior to release/translocation) and all time points thereafter. For each individual, morphometrics [weight (g), sex, age (adult, sub-adult or juvenile), head length (mm), pes length (mm), reproductive status and subjective body condition score (BCS)] and blood, faecal and ectoparasite samples were collected during the first capture of each trapping session. If a sample

was not obtained on the first capture, the sample was collected upon the first recapture during the same trapping week. Sub-adults (woylies with inferred independence from mother) were those with undeveloped external reproductive organs (i.e. determined by testicle size in males and pouch activity in females). Juveniles were those still dependent on their mother (i.e. pouch young). Subjective BCS was assigned based on palpation of muscle mass/fat over the hindquarters (i.e. 1 = very thin, 2 = thin, 3 = ideal, 4 = overweight, 5 = obese). Within Dryandra, tail base width (mm) and tail circumference (mm) were also measured; and packed cell volume (PCV) and total plasma protein (TPP) measurements were obtained using centrifugation and refractometry in the field, respectively. Morphological measurements and blood/ectoparasite samples were collected using manual restraint in woylies.

Blood collection: Up to 1 ml of blood was collected from the lateral caudal (tail) vein into EDTA and serum MiniCollect tubes (Greiner Bio-One, Germany), and frozen at -20°C before processing.

Faecal samples: Newspaper was placed beneath each trap to collect faeces. Each faecal sample was transported on ice prior to being stored in 10% buffered formalin and 70% ethanol, and refrigerated at 4°C until processing. A subset of samples were frozen at -20°C for faecal cortisol metabolite assays as part of another project (Hing et al., 2017).

Ectoparasites: Each individual was examined in a systematic manner for the presence of ectoparasites (ticks, lice and/or louse eggs, fleas and mites) and graded subjectively for parasite burden (i.e. 0 = none, 1 = light, 2 = moderate, 3 = heavy) based on the number of ectoparasites visible within both ears, and the number of ectoparasites (or eggs) observed during standardised coat combing (3 x coat combs over the rump, performed 3 times). A representative number of ectoparasites (i.e. a subset, not all, of the different ectoparasites observed at the time of sampling) were collected from each animal using forceps for ectoparasites on the ears, and a fine tooth comb for ectoparasites on the body, and stored in 70% ethanol.

2.3 Parasite identification

2.3.1 Haemoparasite detection

Trypanosoma spp. were the main haemoparasite monitored during this study, though piroplasms (*Babesia* and *Theileria* spp.) were also monitored in a subset of translocated woylies during the immediate post-translocation period (June to September).

2.3.1.1 Polymerase chain reaction (PCR)

DNA was extracted from 200 μL aliquots of blood using the QIAamp 96 DNA blood kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, with a final elution volume of 60 μL. For trypanosomes, DNA was initially screened using a nested set of generic trypanosome primers (Table 3) designed by Maslov et al. (1996) and McInnes et al. (2011), which target the second fragment of the variable region of the conserved 18S rDNA gene locus. Samples that tested positive for trypanosomes were subsequently screened for the presence of *T. copemani*, *T. vegrandis*, and *T. noyesi* using specific nested primers designed by McInnes et al. (2011) and Botero et al. (2013). Two microliters of DNA template was added to a 24 μL master mix containing 0.8 μM of each primer (forward and reverse), 2 mM $MgCl_2$, 200 μM dNTPs, and 0.2 units of Taq DNA Polymerase. The thermal profile for all PCR reactions consisted of: an initial denaturation step of 94°C for 5 minutes, 50°C for 2 minutes and 72°C for 4 minutes, followed by 35 cycles of 94°C for 30 seconds (denaturation), the annealing temperature of the primer (Table 3) for 30 seconds, and an extension step of 72°C for either 2 minutes and 20 seconds (generic trypanosome primers and the external *T. copemani* reaction) or 50 seconds (specific trypanosome primers); completing with a final extension step of 72°C for 7 minutes.

For piroplasms, a nested PCR was carried out using primers (Table 3) and conditions described by Jefferies et al. (2007). Briefly, a 24 μL master mix was made up of 0.8 μM of each primer (forward and reverse), 2 mM $MgCl_2$, 200 μM dNTPs and 0.2 units of Taq Pol, with the subsequent addition of 2 μL of DNA template. The cycling conditions consisted of a pre-PCR step of 94°C for 3 minutes, 58°C for 1 minute and 72°C for 2 minutes, followed by 35 cycles of 94°C for 30 seconds, 58°C for 20 seconds, and 72°C for 30 seconds, with a final extension temperature of 72°C for 7 minutes.

For all haemoparasites, negative and positive controls were included in all PCR reactions to rule out contamination, with the positive control derived from a known positive stock. DNA was

amplified using a PT100 thermocycler (MJ-Research) using the same conditions described by Jefferies et al. (2007). PCR products were run on a 1.5% agarose gel stained with SYBR Safe Gel Stain (Invitrogen, USA) and visualised under a dark reader trans-illuminator (Clare Chemical Research, USA). Positive samples were identified based on expected band size for each species (Table 3).

2.3.1.2 DNA Sequencing

Samples that tested positive for trypanosomes using the generic primer set, but negative for specific *Trypanosoma* species using clade-specific primers, underwent Sanger sequencing. Sanger sequencing was also used to identify *T. gilletti* and *T.* sp. ANU2, as species specific primers for these haemoparasites do not exist. All piroplasm-positive samples were sequenced. Bands of the expected length (Table 3) were cut out using a scalpel or gel cutting tip, with the resulting gel fragments placed in the freezer until the purification step. PCR products were purified using one of the following three purification steps: (a) the Agencourt AMPure PCR purification system as per the manufacturer's instructions; (b) using an in-house filter tip method described by Yang et al. (2013); or (c) squeezing the amplicon from frozen gel fragments. All purified amplicons were stored at -20°C in Eppendorf tubes until the sequencing PCR. Purified amplicons were sequenced in both directions using an ABI Prism™ Terminator Cycle Sequencing Kit on an Applied Bio-systems 3730 DNA Analyser (Applied Bio-systems, California, USA) at the Western Australian State Agricultural Biotechnology Centre. To control for GC rich regions in the samples, the denaturation stage of the sequencing PCR was extended from 2 minutes to 10 minutes and a 3:1 ratio of BigDye Terminator v3.1 Ready Reaction mix and dGTP BigDye Terminator v3.0 Ready Reaction mix was used (as opposed to just BigDye Terminator v3.1) in order to generate high quality chromatograms. Sequences were edited and analysed using Geneious 7.2. (Kearse et al., 2012). Identification of strains relied on NCBI Blast search (Altschul et al., 1990) followed by alignment using MUSCLE with relevant strains downloaded from GenBank® (Benson et al., 2004).

2.3.2 Gastrointestinal parasite detection

Faeces were examined for the presence of gastrointestinal parasite eggs, oocysts and larvae, using a modified faecal flotation protocol (Zajac and Conboy, 2012) with sodium nitrate ($NaNO_3$). Formalin-fixed faecal samples were centrifuged (2000 rpm for 2 minutes), washed with deionised water and centrifuged again (2000 rpm for 2 minutes). One gram of faeces was placed into a Fecalyzer® container (EVSCO Pharmaceuticals, USA), mixed with 12.5 ml of flotation solution

(616g $NaNO_3$ mixed with 1 L of deionised water; specific gravity 1.37) and a 22 x 22 mm glass cover slip was placed on top. Each sample underwent flotation for fifteen minutes before being examined in a systematic manner at 10 x magnification using an Olympus BX50 microscope. For strongyle and *Strongyloides*-like eggs, all of the eggs observed under the cover slip and around the edges were manually counted, to obtain a semi-quantitative estimate of parasite abundance (e.g. O'Handley et al., 2000; Inpankaew et al., 2014). Non-invasive methods such as this are often used in threatened species where parasite burden cannot be directly quantified (e.g. via post-mortem; Lynsdale et al. 2015), however these estimates have their limitations (see Chapter 8; *Discussion*) and need to be interpreted cautiously. Coccidian oocysts, cestode eggs, *Potoroxyuris* sp. eggs and first stage (metastrongyloid) lungworm larvae were recorded as present or absent. Coccidian oocyst and cestode egg burden was also graded according to a predefined scale [i.e. 0 = none, 0.5 = few (< 10), 1 = light (< 50), 2 = moderate (50-100), 3 = heavy (> 100), 4 = severe (innumerable)].

2.3.3 Ectoparasites

Each woylie was recorded as positive or negative for the presence of ticks, lice and/or louse eggs, fleas and mites, including larval and nymph stages for ticks and mites. A representative number of ectoparasites (as defined above) were collected and morphologically identified using keys developed by Roberts (1970), von Kéler (1971), Dunnet and Mardon (1974), and Domrow (1987).

2.4 Ivermectin treatment trial

To test whether the removal of parasites from translocated hosts provides any benefit to host health, I treated half of the woylies (Dryandra n = 35, Walcott n = 47 and Warrup East n = 46) with an antiparasitic drug prior to translocation. The antiparasitic drug I selected for this project was ivermectin (Ivomec®) administered at a dose rate of 0.2 mg/kg subcutaneously (SC). Ivermectin was selected based on its use in the closely related eastern bettong (*Bettongia gaimardi*) (Portas et al., 2014) and other macropods (Vogelnest and Portas, 2008); though there are no scientific studies that demonstrate the efficacy of ivermectin in macropods, including woylies. Ivermectin was administered SC to ensure each animal received the exact dose (as compared to oral administration in which there is a risk that not all of the dose will be swallowed, thus reducing its efficacy). While the ratio of treated male to female woylies was equal within the Upper Warren (see details below), there were a greater number of treated males (24/35) than females (11/35) within Dryandra, as a greater number of males were translocated compared to females (47 male, 22 female).

The ratio of treated to untreated hosts, respectively, within each destination site:

- Walcott (n = 92): Male 23:23 (n = 46), female 24:22 (n = 46)
- Warrup East (n = 90): Male 22:22 (n = 44), female 24:22 (n = 46)
- Dryandra (n = 69): Male 24:23 (n = 47), female 11:11 (n = 22)

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Table 2: Overview of trapping regime for each site. The two fauna translocations are highlighted in bold. D1-D4 specifies the number of traps set each day for up to four consecutive days. NA indicates that trapping was not undertaken.

Site	Timeframe	Month	Date	Type of trapping	D1	D2	D3	D4
Warrup East	Pre-translocation	April	14.04.14 - 17.04.14	Transect	50	50	50	50
Walcott	Pre-translocation	April	29.04.14 - 02.05.14	Grid	49	49	49	49
Warrup East	Pre-translocation	May	14.05.14 - 17.05.14	Grid	49	49	49	49
Walcott	Pre-translocation	May	20.05.14 - 23.05.14	Transect	100	100	100	100
Warrup East	Pre-translocation	May	27.05.14 - 30.05.15	Transect	100	100	100	100
Translocation 1	**Translocation**	**June**	**09.06.14 - 11.06.14**	**Transect**	**278**	**282**	**280**	**NA**
Walcott	Post-translocation	July	09.07.14 - 11.07.14	Transect	50	50	50	NA
Warrup East	Post-translocation	July	14.07.14 - 16.07.14	Transect	43	51	50	NA
Walcott	Post-translocation	September	01.09.14 - 04.09.14	Roving transect*	90	76	94	97
Warrup East	Post-translocation	September	09.09.14 - 11.09.14	Roving transect*	NA	98	106	105
Walcott	Post-translocation	December	02.12.14 - 05.12.14	Transect	60	60	60	60
Warrup East	Post-translocation	December	09.12.14 - 12.12.14	Transect	60	60	60	60
Warrup East	Post-translocation	April	14.04.15 - 17.04.15	Transect	62	64	61	65
Walcott	Post-translocation	April	21.04.15 - 24.04.15	Transect	64	61	66	63
Walcott	Post-translocation	May	05.05.15 - 08.05.15	Grid	49	49	49	49
Warrup East	Post-translocation	May	26.05.15 - 29.05.15	Grid	49	49	49	49
Dryandra	Pre-translocation	June	02.06.15 - 05.06.15	Grid	49	49	49	49
Dryandra	Pre-translocation	June	09.06.15 - 12.06.15	Transect	100	100	100	100
Translocation 2	**Translocation**	**June**	**16.06.15 - 19.06.15**	**Transect**	**350**	**350**	**350**	**350**
Dryandra	Post-translocation	July	14.07.15 - 17.07.15	Transect	100	100	100	100
Dryandra	Post-translocation	August	11.08.15 - 14.08.15	Transect	100	100	100	100
Dryandra	Post-translocation	September	08.09.15 - 11.09.15	Transect	100	100	100	100
Dryandra	Post-translocation	December	08.12.15 - 11.12.15	Transect	100	100	100	100
Dryandra	Post-translocation	March	21.03.16 - 24.03.16	Transect	100	100	100	100
Dryandra	Post-translocation	June	07.06.16 - 10.06.16	Grid	49	49	49	49
Dryandra	Post-translocation	June	14.06.16 - 17.06.16	Transect	100	100	100	100

* Roving transect refers to the utilisation of different trapping points on different trap nights and/or traps placed at non-designated trapping points (i.e. set at 100m intervals between designated trap points).

Table 3: Details of generic and species-specific primers (and expectant band size) used for the detection of haemoparasites in woylies.

	External primer (5'-3')	Annealing temperature	Internal primer (5'-3')	Annealing temperature	Band size
Nested PCR					
Generic Trypanosome	SLF GCTTGTTTCAAGGACTTAGC	55°C	S825F ACCGTTTCGGCTTTTGTTGG	56°C	959bp
	S762R GACTTTTGCTTCCTCTAATG		SLIR ACATTGTAGTGCGCGTGTC		
Generic Piroplasm	BTF1 GGCTCATTACAACAGTTATAG	58°C	BTF2 CCGTGCTAATTGTAGGGCTAATAC	62°C	800bp
	BTR1 CCCAAAGACTTTGATTTCTCTC		BTR2 GGACTACGACGGTATCTGATCG		
Species-specific PCR					
Trypanosoma copemani	S825F ACCGTTTCGGCTTTTGTTGG	56°C	WOF GTGTTGCTTTTTTGGTCTTCACG	56°C	457bp
	SLIR ACATTGTAGTGCGCGTGTC		WOR CACAAAGGAGGAAAAAAGGGC		
Trypanosoma vegrandis	TVEF GGGGTCCTTTTATTTTATTTG	58°C	TVIF GACCAAAAACGTGCACGTG	58°C	350bp
	TVER TAATTTATTGGCCAGACAAA		TVIR AAATCGTCTCCGCTTTAAC		
Trypanosoma noyesi	H25EF GCCGACAGTGCATTTTGT	58°C	H25IF TTTGAGGCGCAATGGTTTAG	62°C	400bp
	H25ER GAGCGAGATGAACTCGACC		H25IR CGAGTTGAGGGAAGGTGGC		

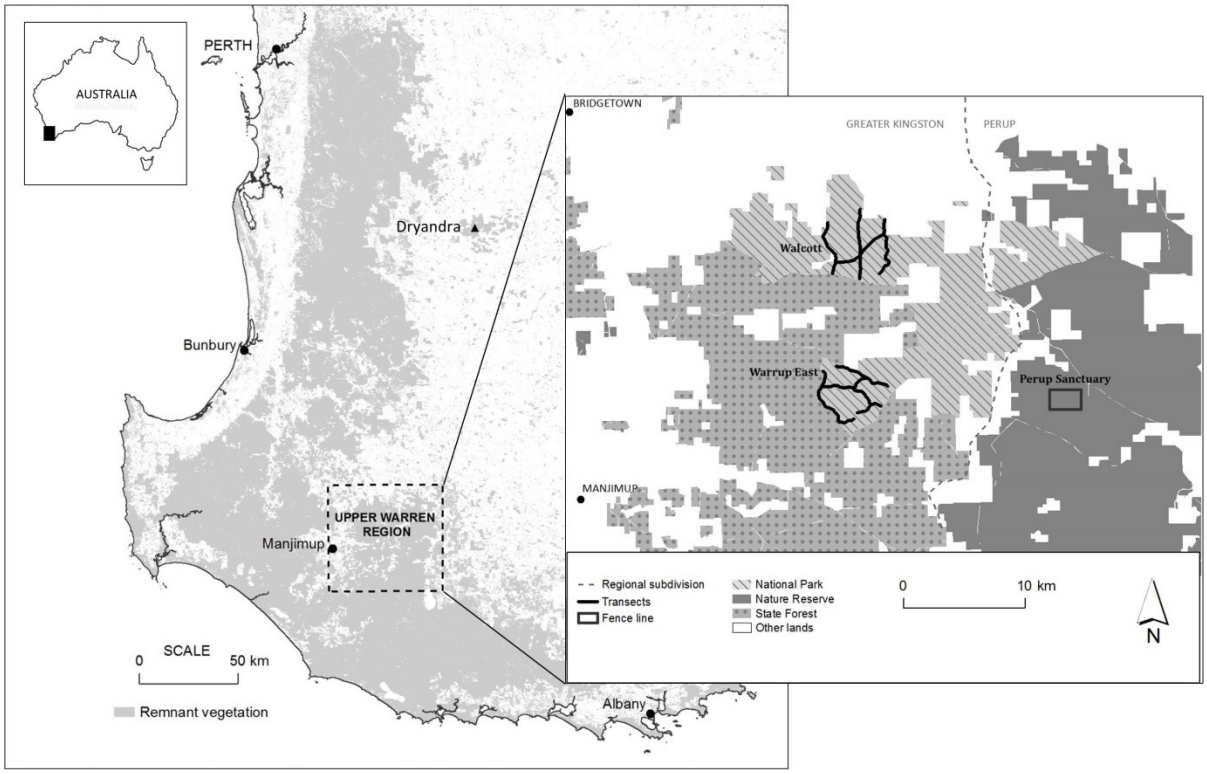

Figure 5: Map illustrating our study sites within south-western Western Australia. The *box* (right) depicts Walcott and Warrup East in relation to Perup Sanctuary. Dryandra is located roughly 250 km north-east of our study sites within the Upper Warren region.

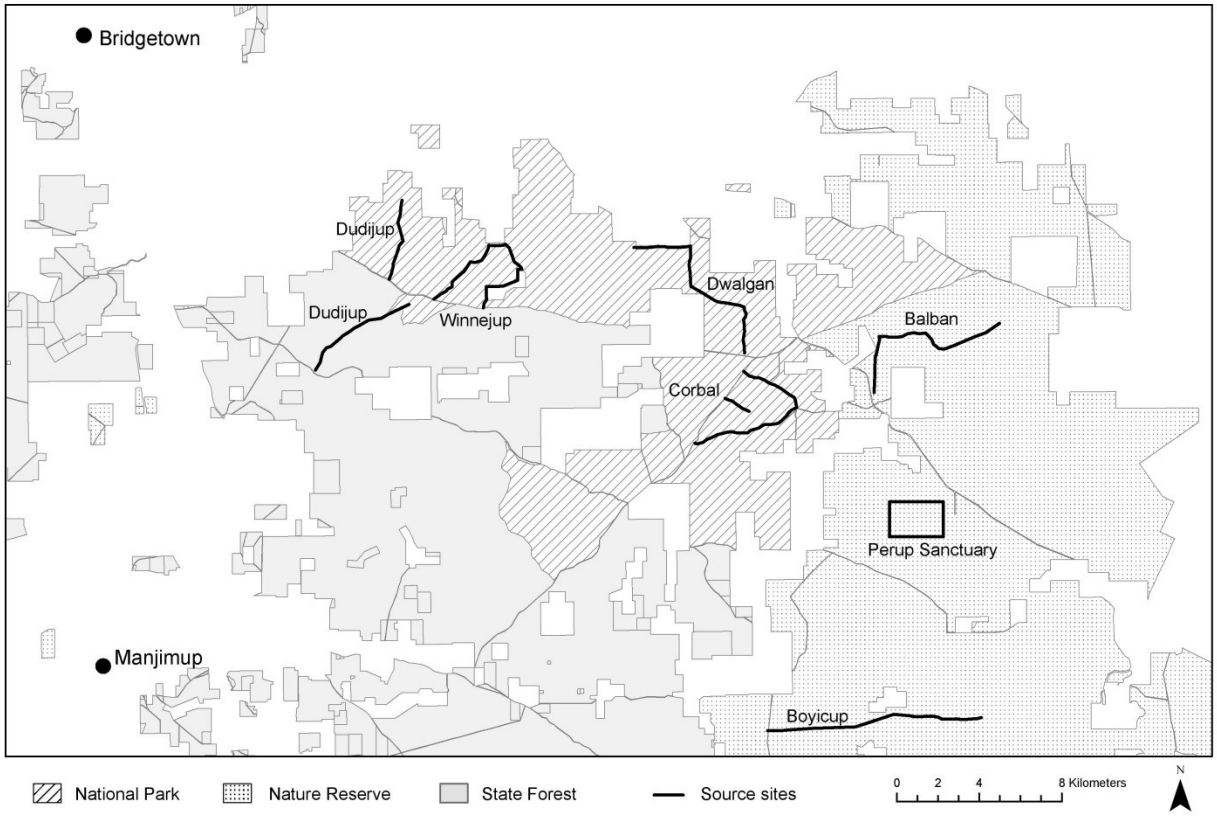

Figure 6: Map depicting the six sites (in relation to Perup Sanctuary) in which woylies were obtained from the Upper Warren region, for translocation into Dryandra.

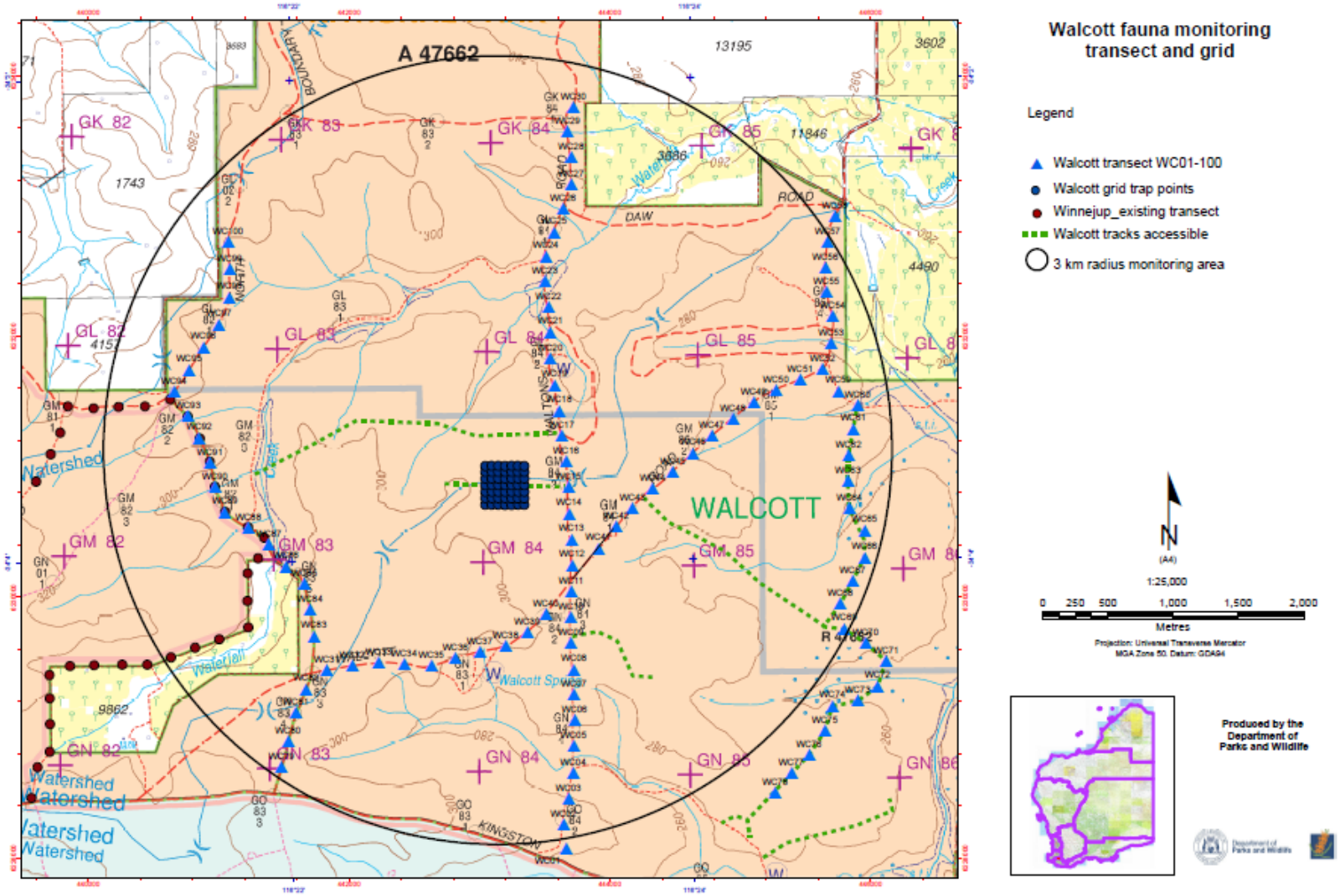

Figure 7: Map showing trap point placement along transects within **Walcott** (blue triangles), with centrally located grid (dark blue circles).

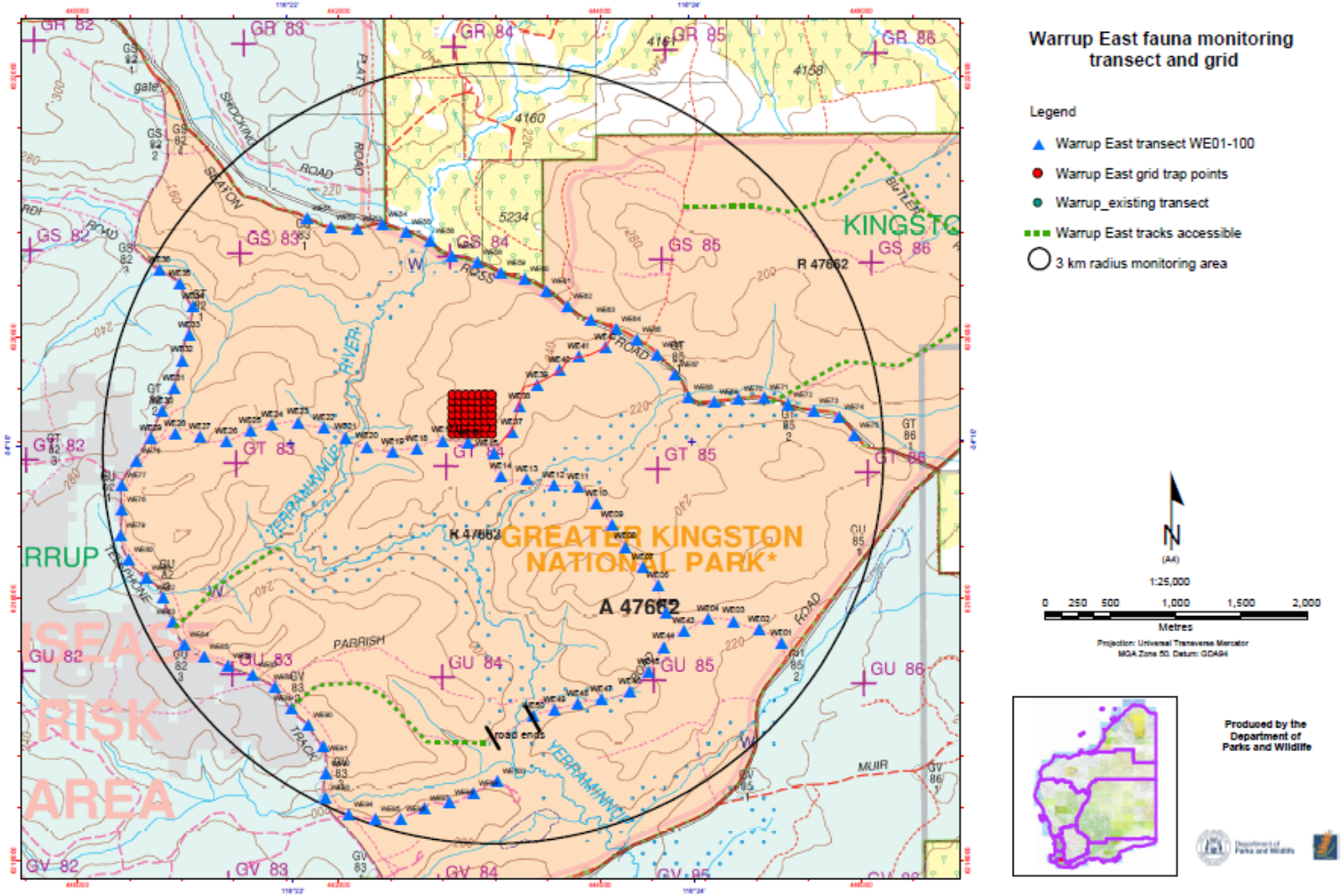

Figure 8: Map showing trap point placement along transects within **Warrup East** (blue triangles) with centrally located grid (red circles).

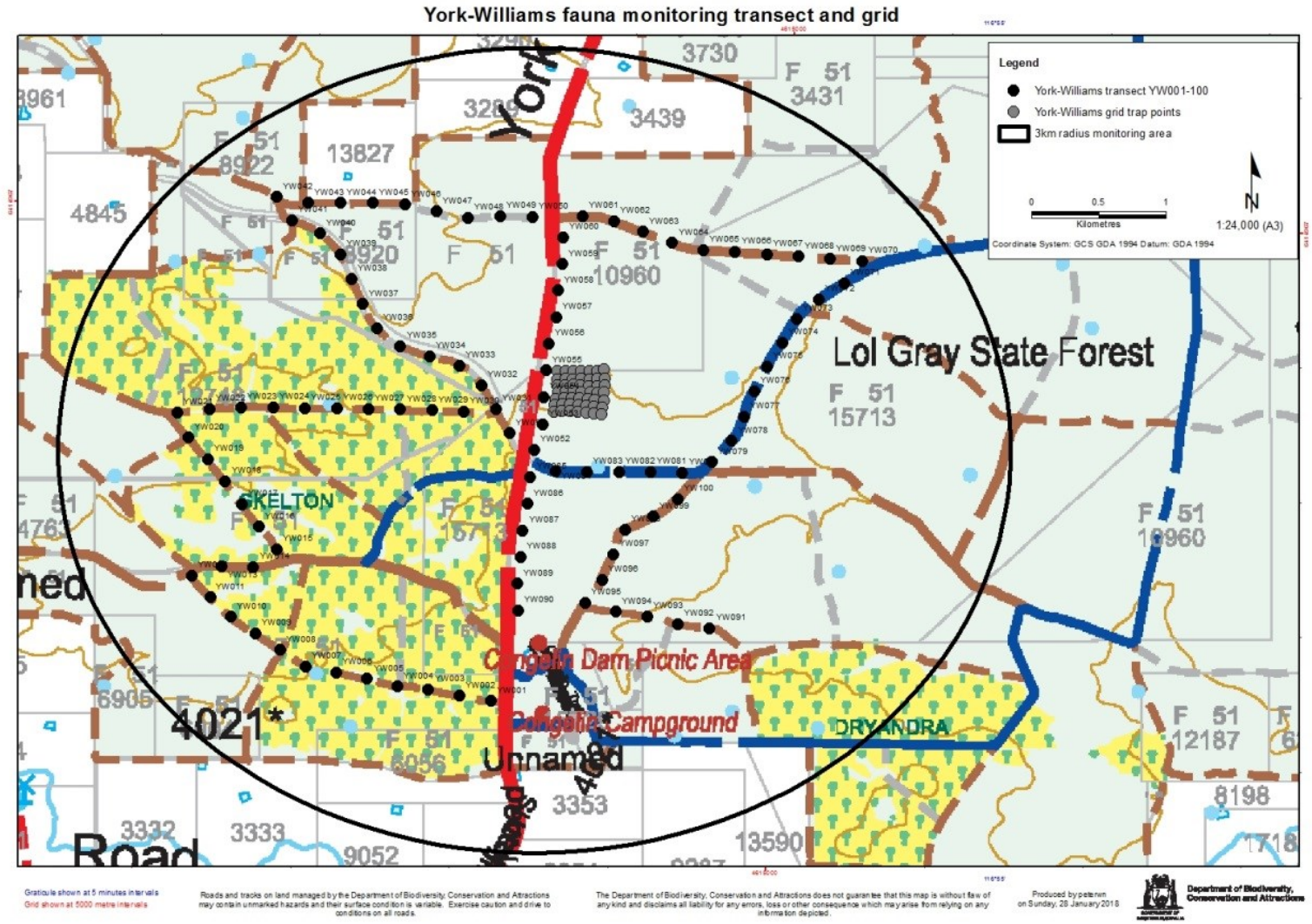

Figure 9: Map showing trap point placement along transects within **Dryandra** (black circles) with centrally located grid (grey Circles).

Chapter Three

Altered parasite community structure in an endangered marsupial following translocation

3.1 Altered parasite community structure in an endangered marsupial following translocation

The following paper has been submitted for publication:

Northover, A.S., Thompson, R.C.A., Lymbery, A.J., Wayne, A.F., Keatley, S., Ash, A., Elliot, A. D., Morris, K., Godfrey, S.S., 2019. Altered parasite community structure in an endangered marsupial following translocation (*submitted for publication*)

Abstract

Fauna translocations play an integral role in the management of threatened wildlife, though we are limited by our understanding of how the host-parasite community changes during translocation. During this longitudinal field-based study, we monitored gastrointestinal, blood-borne and ectoparasite taxa infecting woylies (*Bettongia penicillata*) for up to 12 months following two fauna translocations to supplement existing wild woylie populations in three different sites (Dryandra, Walcott and Warrup East) within the south-west of Western Australia. We aimed to (a) identify changes in parasite community structure of both translocated and resident woylies following translocation; and (b) evaluate the efficacy of ivermectin treatment. Destination site and time since translocation had the strongest effect on parasite prevalence and mean faecal egg counts following translocation. Ivermectin treatment did not significantly reduce parasite prevalence or mean faecal egg counts in treated hosts. Prior to translocation, parasite community composition differed significantly between woylies selected for translocation and resident woylies within each release site. Following translocation, the parasite communities of translocated and resident hosts converged to become more similar over time, with loss of parasite taxa and novel host-parasite associations emerging. This is the first study to examine changes to the broader parasite community in translocated and resident animals following translocation. The dominant site-specific response of parasites following translocation reinforces the importance of incorporating parasite studies to enhance our fundamental understanding of perturbations in host-parasite systems during translocation, in particular the site-level drivers of parasite dynamics.

Introduction

Parasites are an essential component of biodiversity, providing crucial ecosystem services and driving host evolution (Hudson et al., 2006; Hatcher et al., 2012; Gomez et al., 2012). Parasites are also

capable of compromising host health and have been implicated in some species declines (Viggers et al., 1993; Leendertz et al., 2006; Thompson et al., 2014). Despite polyparasitism (co-infection, concomitant infection or multiparasitism) being the norm in wild animal populations (Keusch and Migasena, 1982; Graham, 2008), we often know little about the parasite communities of wildlife or how these communities will be affected by management practices, such as translocation.

Fauna translocations play a pivotal role in sustaining genetic diversity, population health and species survival, but the process of translocating a host from one ecosystem to another will inevitably disrupt pre-existing host-parasite relationships. This will impact both the host and their infracommunity of parasites (Telfer et al., 2010; Moir et al., 2012), with the potential to significantly affect host health and population dynamics (Thompson et al., 2010). Of particular concern during translocations, is the threat of alien or invasive disease, which may impact translocated hosts, resident conspecifics or cohabiting species (e.g. Vadlejch et al., 2017). Fauna translocations may also be a significant stressor capable of inducing immunosuppression (Hing et al., 2017), thus enhancing susceptibility to parasitic disease (Dickens et al., 2009) or stimulating recrudesence of latent infection (Adkesson et al., 2007). For parasites with density-dependent transmission, translocation-induced changes to population density and host connectivity may enhance parasite transfer among hosts and increase disease prevalence within a population (Aiello et al., 2014). For rare host-specific parasites, fauna translocation may promote parasite extinction and loss of biodiversity (Moir et al., 2012; Dougherty et al., 2015; Thompson et al., 2018).

Regrettably, translocation protocols rarely encompass host-parasite studies, thus the impact of fauna translocation on host-parasite dynamics is poorly understood, as are the consequences of such perturbations on translocation outcomes (Northover et al., 2018). In addition, wildlife are often administered antiparasitic drugs prior to translocation, which will disrupt parasite community structure within a host. While this practice may be justified in some species (e.g. McGill et al., 2010), antiparasitic drugs are often used with no clear rationale, or without any effort to determine the efficacy of treatment following translocation (Pedersen and Fenton, 2015).

The woylie or brush-tailed bettong *Bettongia penicillata* is a critically endangered macropodid marsupial confined to three remaining wild indigenous populations within south-western Australia. Following unexpected and abrupt population declines between 1999 and 2006, periodic fauna supplementations continue to play a pertinent role in the conservation management of this species (Wayne et al., 2015). Although the spatio-temporal pattern of population declines suggests the involvement of an

infectious disease agent (Wayne et al., 2015), no study has examined the long-term changes to the broader parasite community (i.e. gastrointestinal, blood-borne and ectoparasite taxa) in translocated and resident woylies following translocation.

In this longitudinal field-based study, we examined the dynamics of the parasite community of woylies for up to 12 months following two translocations to supplement existing wild woylie populations at three different sites. Specifically, we aimed to investigate (a) changes in parasite community structure (i.e. parasite prevalence, infracommunity richness and infracommunity composition) of translocated and resident woylies following translocation; and (b) the efficacy of ivermectin treatment in translocated hosts. Given that parasite loss following fauna reintroductions typically occurs (Torchin et al., 2003; MacLeod et al., 2010) and ivermectin should theoretically reduce the burden of target parasites (nematodes and arthropods), we predicted that parasite infracommunity richness would decrease following translocation and this effect would be most pronounced in treated hosts.

Material and methods

Study sites and trapping regime

Two translocations were undertaken in south-western Australia in collaboration with the Department of Biodiversity, Conservation and Attractions (DBCA) under DBCA Scientific License's (Regulation 4: written notice of lawful authority; and 17: licence to take fauna for scientific purposes) and with approval of the Murdoch University Animal Ethics Committee (RW2659/14). The first translocation was carried out in June 2014, where 182 woylies were translocated from Perup Sanctuary (a fenced reserve; 34.2506°S, 116.1425°E) to supplement two unfenced wild sites, Walcott (n = 92) and Warrup East (n = 90), located within the Upper Warren region approximately 15 km west and 20 km north-west of Perup Sanctuary, respectively (Figure 10). Woylies within the reserve are considered wild as they receive no interventional management (e.g. supplementary food, routine parasite treatment or vaccines). The second translocation was carried out in June 2015, during which 69 woylies were translocated from six unfenced wild sites within the Upper Warren, into an unfenced wild site within Dryandra Woodland (32.8027°S, 116.8854°E). Dryandra is located about 250 km north-east of the Upper Warren region (Figure 10). Within Dryandra, resident woylie density is estimated to be lower than both Upper Warren sites, especially Walcott (Northover et al., 2019).

Each woylie was identified with two uniquely numbered ear-tags. Prior to translocation, half of the translocated woylies (Dryandra n = 35, Walcott n = 47, Warrup East n = 46) were administered a single subcutaneous injection of ivermectin (Ivomec® 0.2 mg/kg).

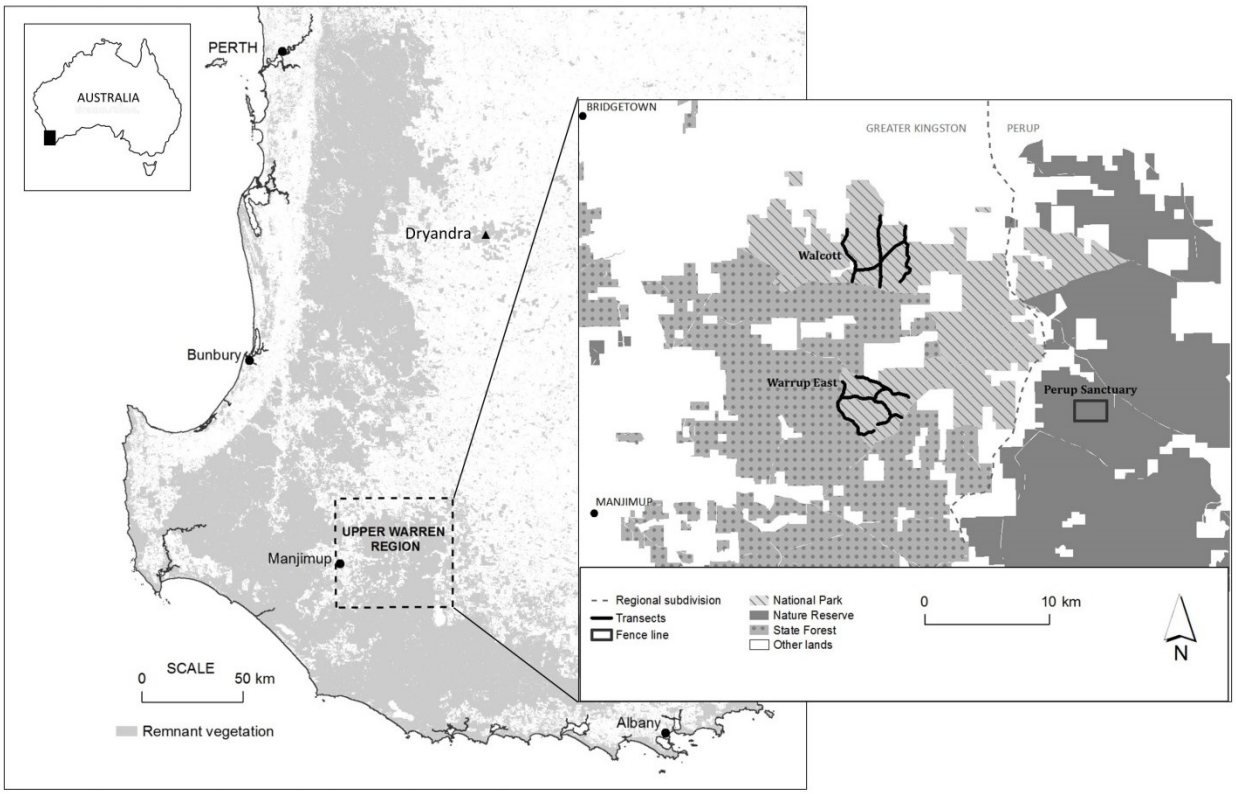

Figure 10: Map (from Northover et al., 2019) illustrating the study sites within south-western Australia, including Walcott and Warrup East in relation to Perup Sanctuary (box, right), and Dryandra, situated roughly 250km north-east of the Upper Warren region.

Parasite sampling

Samples were collected from woylies at each (re)capture. For resident woylies, this includes all time points prior to (April and May 2014, Walcott and Warrup East; June 2015, Dryandra), and following, translocation (one, three, six, ten and eleven months after translocation, Walcott and Warrup East; one, two, three, six, nine and twelve months after translocation, Dryandra). For translocated woylies, this includes the point of translocation (i.e. prior to translocation/release) and all time points thereafter (as for residents); see Northover et al. (2019) for further details of the trapping regimes. Blood and ectoparasite samples were not collected from Warrup East resident woylies in July 2014 due to logistical constraints. Given time limitations and the large number of samples that were collected during the first translocation (June 2014), a randomly selected subset of faecal samples were analysed from translocated hosts destined for translocation into each site.

Newspaper was placed beneath each trap to collect faeces, which were stored in 10 per cent buffered formalin at 4°C until processing. Faecal samples were examined for the presence of gastrointestinal parasite eggs, oocysts and larvae, using faecal flotation with sodium nitrate (specific gravity 1.37), as described by Northover et al. (2017). Strongyle and *Strongyloides*-like nematode eggs were counted (quantified as eggs per gram of faeces) to estimate gastrointestinal nematode burden; non-invasive methods such as this are often used in threatened species where parasite burden cannot be directly quantified (e.g. via post-mortem; Lynsdale et al., 2015). Coccidian oocysts, cestode eggs (undescribed sp.), *Potoroxyuris* sp. eggs and first stage (metastrongyloid) lungworm larvae were recorded as present or absent. Only one faecal sample from each individual per trapping session was included in our analyses.

Each woylie was examined for the presence of ectoparasites in a systematic manner (i.e. visual inspection and standardised coat combing) and was was recorded as positive or negative for the presence of ticks, lice and/or louse eggs, fleas and mites, including larval and nymph stages for ticks and mites.

Blood was collected from the lateral tail vein into EDTA MiniCollect® tubes (Greiner Bio-One, Germany) for the detection of trypanosomes and frozen at -20°C prior to processing. Genomic DNA was extracted from 200 µl aliquots of whole blood using the QIAmp 96 DNA blood kit in accordance with the Qiagen handbook (Qiagen, Hilden, Germany), with a final elution volume of 60 µl. A negative control was included in the extraction process. A nested polymerase chain reaction (PCR), targeting the second fragment of the conserved 18S rDNA gene region, was carried out using generic trypanosome primers as described by Maslov et al. (1996) and McInnes et al. (2011). All PCR reactions were performed as described by Cooper et al. (2018), with the exception that 2 µl of DNA was added to a 24 µl master mix.

Data analysis

For each parasite taxon, prevalence of infection was calculated as the proportion of infected individuals with Jeffrey's 95% confidence intervals calculated assuming a binomial distribution. Where faecal egg counts (FEC) were obtained (i.e. strongyle and *Strongyloides*-like nematodes), means are also reported. Parasite infracommunity richness (polyparasitism) was described by the number of parasite taxa within or on an individual host, without regard to intensity of infection.

To evaluate the effect of destination site (Dryandra, Walcott and Warrup East), time since translocation (TST) and ivermectin treatment (translocated woylies only), and their interactions, on the presence of each parasite taxon, and on parasite infracommunity richness, we used generalised linear mixed-effects models (packages *lme4*, Bates et al., 2015; *glmmADMB*, Skaug et al., 2016) in R (version 6.1.15; R Core Team, 2015). All analyses were conducted separately for translocated and resident woylies. For each of our models, we tested for collinearity between predictor variables using variance inflation factors, and residuals were checked for normality/outliers to ensure model validity. Both strongyle and *Strongyloides*-like egg counts were modelled as a negative binomial distribution with a log link function. Presence/absence data for all other parasites were modelled as binomial distributions with a logit link function. For translocated woylies, Walcott was omitted from the tick model, because tick prevalence was 100% following translocation. We could not run these models for cestode eggs, *Potoroxyuris* sp. eggs or lungworm larvae due to the low prevalence of these parasites (n = 21, n = 3 and n = 13 positive samples, respectively). Parasite community richness was modelled as a Poisson distribution with a log link function and we only included woylies that were sampled for all parasite taxa at each capture. In all models, we included woylie ID as a random effect to account for repeated measures of individuals following translocation.

We evaluated differences in parasite community composition between translocated and resident woylies twice for each site; pre-translocation and six months following translocation. Differences in parasite community composition among translocated and resident hosts were estimated from presence/absence data with the Bray-Curtis coefficient for each site at each time point and visualised with non-metric multidimensional scaling plots. The effect of host group (translocated or resident) and time (pre-translocation or six months after translocation) on community composition were tested using a permutational analysis of variance (PERMANOVA+ for PRIMER v. 6.0; Anderson et al., 2008) in a repeated measures design, with host group and time as fixed factors, and ID nested within host group as a random factor. Pairwise differences in community composition between translocated and resident woylies for each time point in each locality were tested by one-way ANOSIM (implemented in PRIMER v. 6.0; Clarke and Gorley, 2006), with a Bonferroni correction applied for multiple testing. Using the SIMPER procedure in PRIMER, the contribution of individual parasite taxa to differences in composition among host groups was calculated by averaging the Bray-Curtis coefficients for each taxon over all pairwise host combinations.

Results

Overall, we analysed 872 faecal samples, 1211 blood samples and assessed 1277 woylies for the presence of ectoparasites; this included recaptures from 627 individuals (250 translocated, 377 resident).

Effect of site

In translocated woylies, all parasite taxa except for ticks, lice and fleas, varied significantly between sites (Table 4); though Walcott (100% tick prevalence) was excluded from our analyses. Parasite prevalence/mean FEC were on average highest within Walcott and lowest within Dryandra (Figure 11A; Tables A1-3). Trypanosome prevalence, which was highest within Dryandra, was a notable exception to this trend (Figure 11A).

In resident woylies, all parasite taxa except for lice varied significantly between sites (Table 4). Parasite prevalence/mean FEC were highest within the Upper Warren (Walcott and Warrup East) (Figure 11B; Tables A1-3). Strongyle eggs were ubiquitous within all Upper Warren sites but were not detected in Dryandra resident woylies prior to translocation. Cestode eggs were only found in Dryandra resident woylies before translocation, while lungworm larvae and *Potoroxyuris* sp. eggs were only identified in woylies originating from Perup Sanctuary (Tables A1-3).

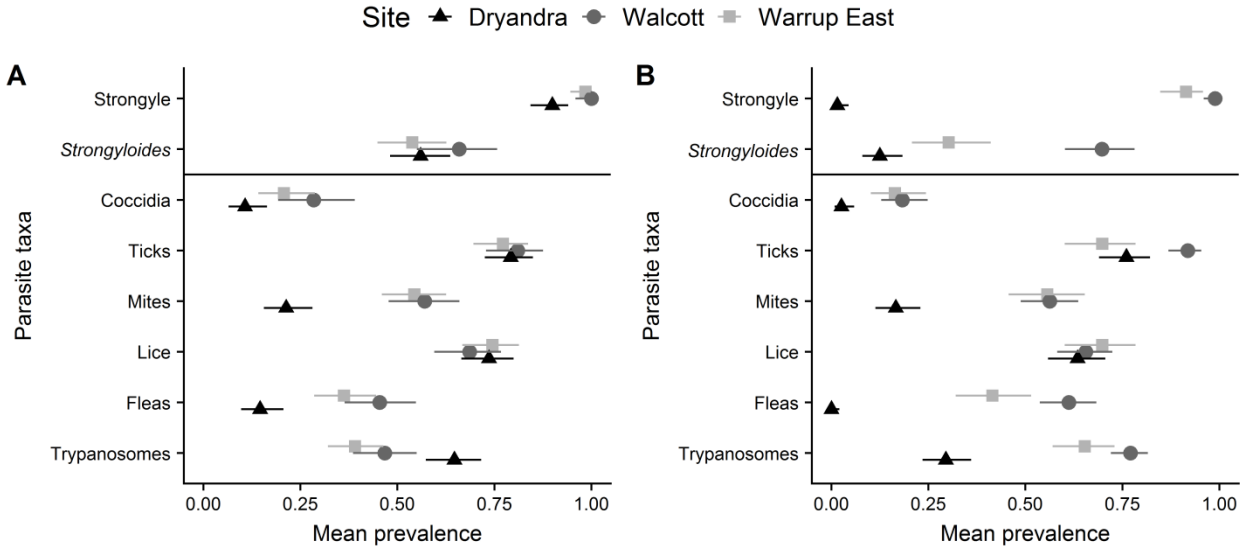

Figure 11: The overall effect of site on mean faecal egg counts (above solid line) and parasite prevalence (below solid horizontal line) for each parasite taxon in (A) translocated and (B) resident woylies. Error bars represent 95% CI.

Table 4: Results from Generalised Linear Mixed Model analysis of factors influencing parasite faecal egg counts (strongyle and *Strongyloides*-like eggs), prevalence (all other parasite taxa) and parasite infracommunity richness in woylies. Significant ($P < 0.05$) results are highlighted in bold; TST: time since translocation.

	Strongyle eggs			***Strongyloides*-like eggs**			**Coccidia**		
	X^2	*df*	*P*	X^2	*df*	*P*	X^2	*df*	*P*
Translocated									
Site	7.322	2	**0.026**	21.441	2	**< 0.001**	11.188	2	**0.004**
TST	75.086	1	**< 0.001**	4.716	1	**0.030**	15.713	1	**< 0.001**
Ivermectin	2.665	1	0.103	0.130	1	0.719	0.000	1	0.990
Site:TST	26.433	2	**< 0.001**	4.993	2	0.082	2.519	2	0.284
Site:Ivermectin	0.246	2	0.884	1.977	2	0.372	4.113	2	0.128
TST:Ivermectin	0.224	1	0.636	0.198	1	0.657	0.371	1	0.542
Site:TST:Ivermectin	3.700	2	0.157	1.737	2	0.420	0.190	2	0.910
Resident									
Site	134.372	2	**< 0.001**	73.543	2	**< 0.001**	15.953	2	**< 0.001**
TST	4.797	1	**0.029**	8.226	1	**0.004**	2.645	1	0.104
Site:TST	5.562	2	0.062	9.703	2	0.008	0.895	2	0.639

	Ticks			**Mites**			**Lice**		
	X^2	*df*	*P*	X^2	*df*	*P*	X^2	*df*	*P*
Translocated									
Site	0.157	1	0.692	42.701	2	**< 0.001**	0.416	2	0.812
TST	9.451	1	**0.002**	0.017	1	0.897	4.241	1	**0.039**
Ivermectin	0.351	1	0.553	0.112	1	0.738	0.692	1	0.406
Site:TST	0.799	1	0.372	8.095	2	**0.017**	0.005	2	0.998
Site:Ivermectin	0.010	1	0.922	2.184	2	0.336	0.714	2	0.700
TST:Ivermectin	0.005	1	0.944	0.000	1	0.983	0.410	1	0.522
Site:TST:Ivermectin	0.961	1	0.327	3.762	2	0.152	0.407	2	0.816
Resident									
Site	47.844	2	**< 0.001**	132.786	2	**< 0.001**	0.109	2	0.947
TST	0.826	1	0.364	1.339	1	0.247	0.927	1	0.336
Site:TST	18.002	2	**< 0.001**	24.039	2	**< 0.001**	4.919	2	0.085

	Fleas			**Trypanosomes**			**Parasite richness**		
	X^2	*df*	*P*	X^2	*df*	*P*	X^2	*df*	*P*
Translocated									
Site	3.506	1	0.061	17.447	2	**< 0.001**	17.854	2	**< 0.001**
TST	0.023	1	0.880	10.917	1	**0.001**	4.028	1	**0.045**
Ivermectin	0.186	1	0.666	0.035	1	0.852	0.196	1	0.658
Site:TST	9.187	1	**0.002**	7.540	2	**0.023**	3.674	2	0.159
Site:Ivermectin	0.137	1	0.712	7.500	2	**0.024**	1.564	2	0.457
TST:Ivermectin	1.657	1	0.198	0.207	1	0.649	0.238	1	0.625
Site:TST:Ivermectin	0.299	1	0.585	2.915	2	0.233	0.004	2	0.998
Resident									
Site	88.655	2	**< 0.001**	59.422	2	**< 0.001**	257.096	2	**< 0.001**
TST	2.814	1	0.093	0.298	1	0.585	3.330	1	0.127
Site:TST	4.584	2	0.101	4.909	2	0.086	1.176	2	0.556

Effect of time since translocation

Major changes to the parasite community in translocated woylies occurred within the immediate post-translocation period (i.e. 1-3 months following translocation). Strongyle and *Strongyloides*-like egg counts, and the prevalence of coccidia and ticks (Walcott excluded) declined with TST, while lice and trypanosome prevalence increased with TST (Table 4; Figure 12). We also detected a significant interaction between TST and site for strongyle egg counts, mites, fleas and trypanosomes (Table 4).

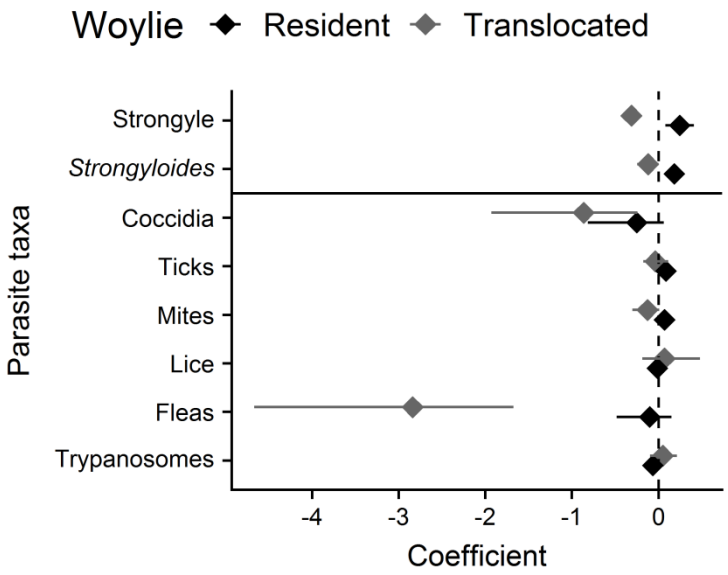

Figure 12: The effect of time since translocation (model coefficients for all sites combined) on mean faecal egg counts (above solid horizontal line) and parasite prevalence (below solid horizontal line) for each parasite taxon in translocated and resident woylies. Left of the dashed vertical line indicates a negative effect, right of the line indicates a positive effect; Error bars represent 95% CI.

Within Dryandra, mean strongyle egg counts decreased considerably between June and September and continued to decrease thereafter (Figure 13A). Flea prevalence also abruptly declined between June (53.6%) and July (0.0%) in Dryandra (Figure 13B), after which we only found fleas (low burden) in a single translocated host in August. In contrast, trypanosome prevalence sharply increased between June and September within Walcott (Figure 13C) and mite prevalence increased after July in Warrup East (Table A3).

In resident woylies, strongyle and *Strongyloides*-like egg counts increased with TST within the Upper Warren, particularly Walcott (Table 4; Figure 12; Tables A1-3). A significant interaction between TST and site was also identified for *Strongyloides*-like egg counts, ticks and mites (Table 4). Within Warrup East, mean *Strongyloides*-like egg counts tripled in July and again in September, and mite prevalence

increased almost three-fold between May and September (Table A3). Tick prevalence markedly decreased between June (95.2%) and July (57.1%) within Dryandra.

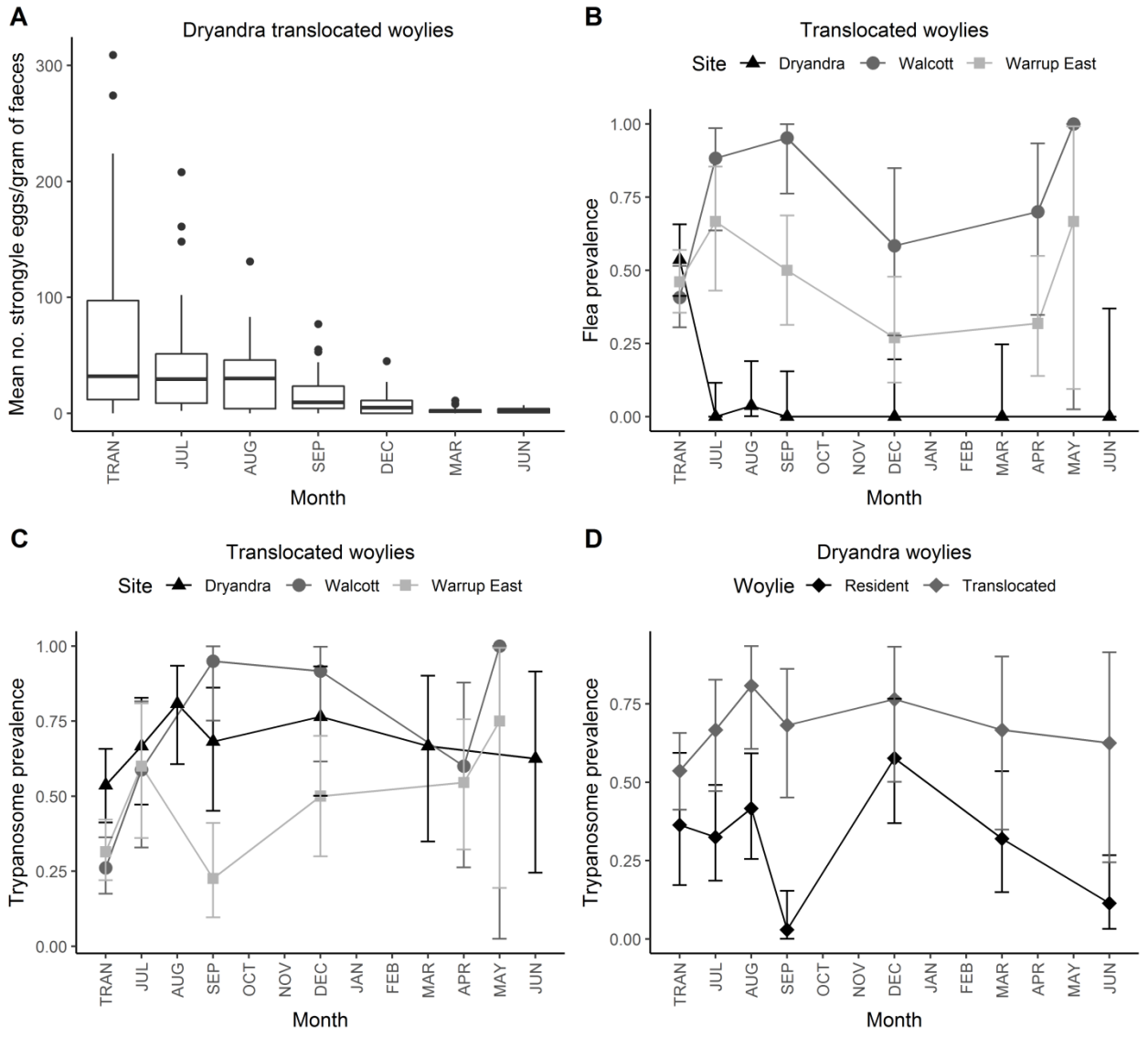

Figure 13: Significant changes to mean strongyle egg counts (A) and parasite prevalence (B, C, D) over time. TRAN: time of translocation; Boxplots (A) are delimited by the first (lower) and third (upper) quartile with the median represented by the thick horizontal line; whiskers represent the 1.5 interquartile range; solid black dots represent outliers; Error bars (B, C, D) represent 95% CI.

Effect of Ivermectin treatment

Ivermectin treatment did not have a significant effect on any parasites in translocated animals, though a significant interaction between ivermectin treatment and site was identified for trypanosomes (Table 4). Trypanosome prevalence was lower in treated compared to untreated

woylies within Dryandra, but higher (on average) in treated woylies within Warrup East (Figure A1). Dead lice were observed on treated woylies following translocation.

Parasite community structure in translocated and resident woylies

In translocated and resident woylies, parasite infracommunity richness differed significantly between sites (Table 4). Overall, parasite richness was highest within Walcott and lowest within Dryandra (Figure 14). The maximum number of parasite taxa identified from a single host was nine (Walcott translocated woylie), with up to eight parasite taxa readily identified in woylies originating from the Upper Warren, compared to a maximum of five in the Dryandra resident woylie population. Dryandra was the only site in which we found woylies without any parasites (n = 4 residents). In translocated woylies, parasite infracommunity richness also decreased (on average) with TST (Table 4; Figure 14A).

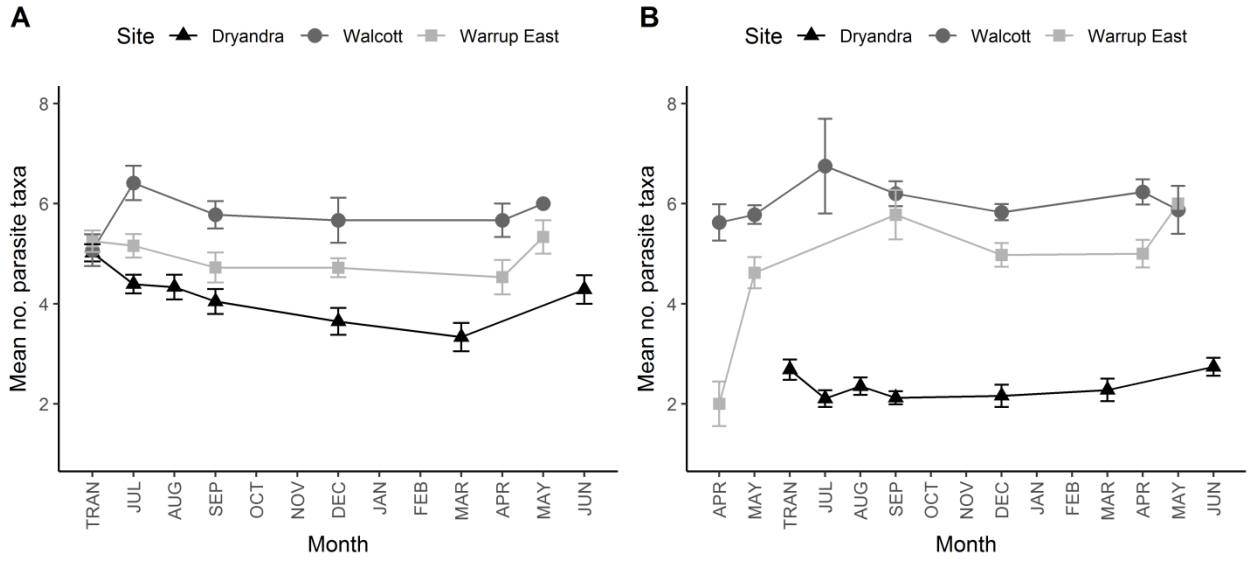

Figure 14: Overall parasite infracommunity richness in (A) translocated and (B) resident woylies over time. TRAN: time of translocation; Error bars represent one standard error.

In all three sites, parasite infracommunity composition was significantly influenced by both host group (Dryandra, Pseudo-F = 22.0, P = 0.001; Walcott, Pseudo-F = 5.7, P = 0.001; Warrup East, Pseudo-F = 4.6, P = 0.008) and time (Dryandra, Pseudo-F = 13.8, P = 0.001; Walcott, Pseudo-F = 3.9, P = 0.02; Warrup East, Pseudo-F = 8.0, P = 0.004), with a significant interaction between these factors (Dryandra, Pseudo-F = 9.4, P = 0.002; Walcott, Pseudo-F = 11.2, P = 0.001; Warrup East, Pseudo-F = 6.5, P = 0.001). The significant interaction was due to increasing similarity in parasite infracommunity composition between translocated and resident hosts over time (Figure 15). Prior to translocation, parasite community composition differed significantly (P < 0.05, with the Bonferroni)

between translocated and resident woylies in all three sites, particularly Dryandra (Dryandra R = 0.658; Walcott R = 0.254; Warrup East R = 0.237); with *Strongyloides*-like nematodes (15.9%), trypanosomes (15.5%), fleas (15.0%) and mites (14.3%) contributing the most towards this dissimilarity. Six months after translocation, there was no significant difference in community composition between translocated and resident woylies in Dryandra (R = 0.131, P > 0.05), Walcott (R = -0.066, P > 0.05) or Warrup East (R = -0.032, P > 0.05) (Figure 15). Within Dryandra, coccidia and fleas were not detected in translocated woylies within a few months of translocation, and novel host-parasite associations were identified. Cestode eggs were identified in two translocated woylies twelve months after translocation when they hadn't been detected in this group previously. Strongyle eggs were also detected in three resident woylies following translocation (n = 1, December 2015; n = 2, March 2016) and they had not been detected within the resident population prior to this. Translocated woylies within Dryandra, however, maintained a significantly higher prevalence of trypanosome infection compared to resident woylies (Figure 13D).

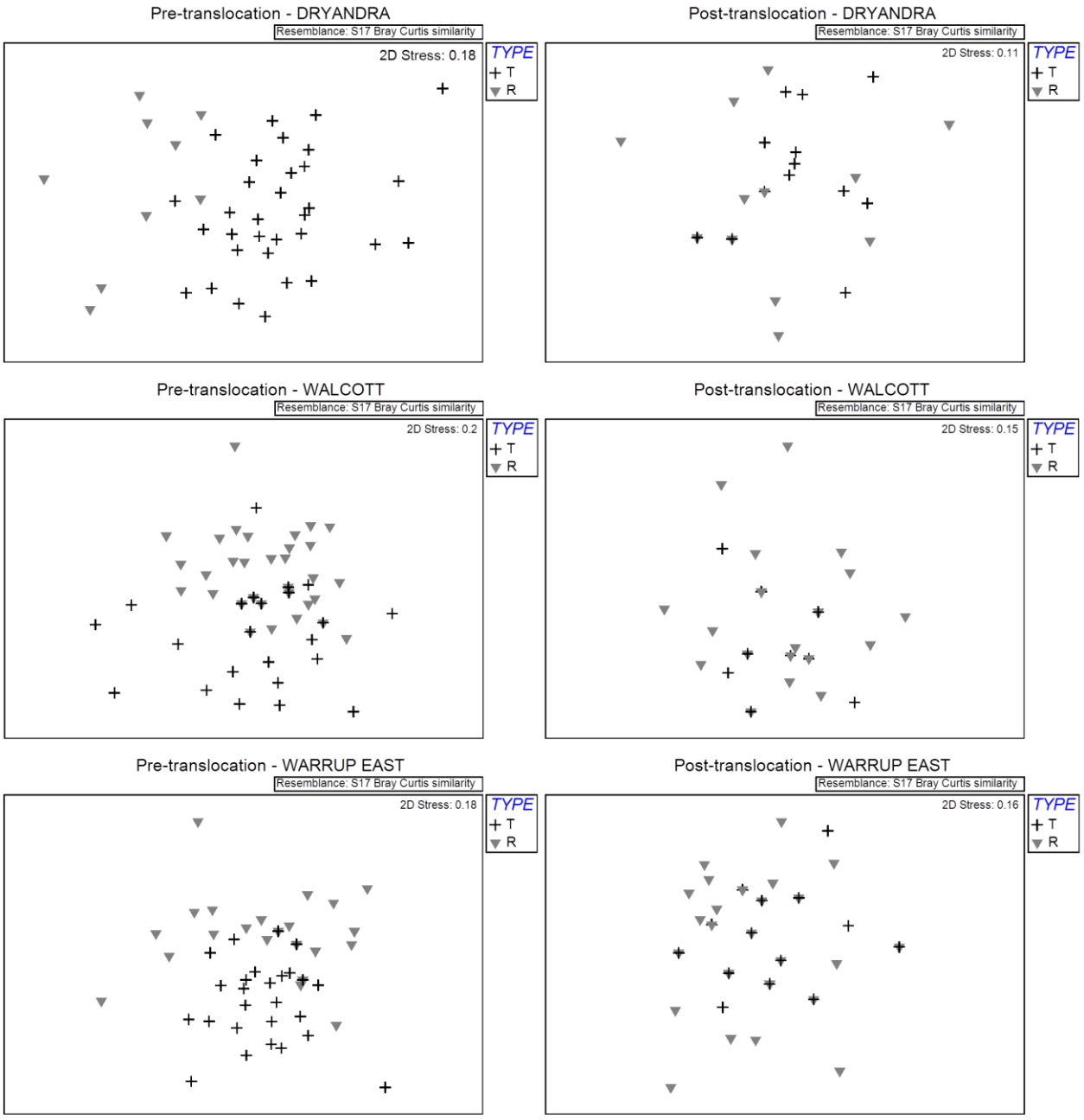

Figure 15: Non-metric multidimensional scaling plots showing convergence of parasite community composition in translocated (TYPE T) and resident (TYPE R) woylie groups following translocation. Boxes on the left depict both groups at all time points prior to and including the point of translocation; boxes on the right depict both groups six months after translocation.

Discussion

One of the notable results from this study was that the response of parasites following translocation differed significantly between sites. Changes in host population size and connectivity are two core concepts of disease ecology that underpin disease dynamics during translocation (Aiello et al., 2014). Host density may explain the particularly high prevalence of parasites observed within Walcott; in

our study, capture rates of woylies were twice as high in Walcott as the other two sites, and capture rates have been found to closely correlate with population density in woylies (Wayne et al., 2013). Low host density may explain the abrupt decline in mean strongyle egg counts and flea prevalence observed within Dryandra (which had the lowest capture rates) following translocation. Alternatively, the spatially independent nature of Dryandra, in which environmental conditions differ from the Upper Warren (McArthur et al., 1977; Wayne, 2005), may be unfavourable for the completion of certain parasite life-cycles or the persistence of eggs within the environment. In addition, the way in which woylies utilse the landscape, or come into contact with cohabiting species that share the same parasites, may also differ between the open-canopy woodland of Dryandra and the comparatively taller and denser forests and woodlands of the Upper Warren. All of these factors may contribute to the site-specific differences we observed in the response of the host-parasite community following translocation.

Parasite species richness and community composition in resident and translocated woylies converged to become more similar over time, and changes to parasite community structure were most pronounced during the first few months following translocation. In Walcott, where resident woylie density and parasite prevalence/mean FEC were highest, parasite infracommunity richness increased in translocated woylies. Tick prevalence in translocated hosts for instance, increased from 70.7% to 100% following translocation, closely resembling the high prevalence observed in resident woylies. In Dryandra, where woylie density and the number of parasites infecting residents were lower, we observed decreasing parasite richness in translocated woylies over time. Our prediction that parasite infracommunity richness would decrease after translocation was therefore site dependent. Variability such as this makes it difficult to predict what will happen to the parasite community within a host following translocation.

In this study, novel host-parasite associations with strongyles and cestodes were detected following translocation. We hypothesise that translocated woylies originating from the Upper Warren, in which strongyle infection was ubiquitous, may have introduced strongylid nematodes into the Dryandra resident woylie population. Similarly, we suspect woylies translocated into Dryandra likely acquired cestode infection within this site. The absence of cestode eggs from over 730 woylie faecal samples collected within the Upper Warren region (Northover et al., unpublished data) suggests that the intermediate host for this parasite is specific to the Dryandra region. The presence of lungworm larvae and *Potoroxyuris* sp. eggs in only translocated hosts originating from Perup Sanctuary also suggests site specificity, although this may be a function of the low prevalence of these parasites. The

apparent occurrence of reciprocal parasite transmission highlights one of the risks associated with translocating wildlife and the importance of host-parasite studies. The rapid response of most parasites following translocation indicates that the immediate post-translocation period is an important time to conduct follow-up sampling. The delayed response of novel host-parasite associations however, also highlights the value of long-term parasite sampling. As for most threatened species, more field studies are needed to build a comprehensive database of the parasite species endemic within each population, and should include cohabiting host species that may impact parasite prevalence within a population.

Given the link between translocation, stress and coccidial disease (Sainsbury and Vaughan-Higgins, 2012) the observation that coccidia prevalence in translocated woylies decreased over time may coincide with woylie acclimation and recovery from translocation-associated stress (e.g. Franceschini et al., 2008). Alternatively, this may reflect the presence of fewer individuals within the destination site (e.g. following dispersal or predation). Seasonal variation may also explain the reduced prevalence of coccidia over summer; however prevalence would be expected to increase again during late autumn when wet conditions are optimum for oocyst development (Barker et al., 1972). For all parasite taxa, it is important to acknowledge that seasonal and annual variation in parasite prevalence/mean FECs were confounded with translocation in our study. The inclusion of a control site, which monitors hosts in the absence of translocation, would be ideal for identifying 'normal' parasite trends over time; although this is not always feasible when working with threatened species such as the woylie, where wild populations are periodically supplemented with translocated animals.

As observed in other fauna reintroduction studies (e.g. Torchin et al., 2003; MacLeod et al., 2010; Fairfield et al., 2016), specific parasite taxa were not detected following translocation, although this effect was site-dependent. The absence of coccidia within Dryandra after September suggests that this parasite may not have survived following translocation. We did not detect coccidia in Dryandra resident woylies prior to translocation, although oocyst shedding is intermittent and the absence of oocysts in faeces does not rule out the presence of infection (Vogelnest and Portas, 2008). For gastrointestinal parasite taxa, limitations associated with the use of faecal flotation for estimating parasite burden (see Bordes and Morand, 2011) must also be considered, particularly when parasite prevalence is low. Likewise, variables such as faecal preservation method and type of flotation solution will impact the ability to recover different parasite taxa (Hu et al., 2016).

Fleas were rare within Dryandra and their absence in translocated hosts after August suggests that they may have failed to persist. Given the increased recent interest in conserving parasites, as well as their hosts, as integral components of biodiversity, the loss of parasite species during translocations represents an important risk that needs consideration in translocation protocols. Thompson et al. (2018) provided a comprehensive list of woylie parasites and identified six species that appear to be host-specific and are in danger of extinction. Given that we identified three of these six species (*Ixodes woyliei*, *Eimeria woyliei* and *Potoroxyuris keninupensis*) plus a novel undescribed species of cestode during this study (Northover et al., unpublished data) and woylies are the most commonly translocated species in Australia (Morris et al., 2015), the consequences of host-specific parasite extinction require careful deliberation. Likewise, the link between parasite presence and host health needs to be investigated. If specific parasite taxa are associated with poor health in woylies, then translocation protocols could potentially be adapted to control for these parasites (e.g. targeted antiparasitic drug treatment or utilizing next generation sequencing to identify potentially harmful *Trypanosoma* genotypes infecting specific populations; see below). On the other hand, if there is no evidence to suggest that known parasite taxa adversely impact host health, parasite conservation should be a key consideration, particularly for rare host-specific parasites.

Within Dryandra, there remained a persistently high prevalence of trypanosome infection in translocated compared to resident woylies, despite a general convergence in parasite infracommunity structure. As detectable parasitaemia associated with the active acute phase of infection is typically short-lived (Campos et al., 2010), trypanosome prevalence would be expected to decrease over time in a site where trypanosome prevalence is low. The fact that trypanosome prevalence remained high suggests that another process is responsible for maintenance of parasitaemia in the absence of reinfection. For example, translocated woylies may be infected with *Trypanosoma copemani* genotype 2, which has the proposed ability to invade tissues, replicate and re-enter the peripheral blood (Botero et al., 2013; Thompson et al., 2013; Botero et al., 2016). Molecular evaluation of specific *Trypanosma* species infecting woylies during the same translocation (Northover et al., 2019) attributed this pattern of divergence to the presence of *T. copemani*, however the specific genotype remains unknown.

Unexpectedly, ivermectin treatment did not significantly reduce parasite prevalence or mean FEC in target parasites, although we did identify a significant interaction between ivermectin treatment and site for trypanosomes. In theory, ivermectin should be ineffective against trypanosomes (Vogelnest and Portas, 2008), though indirect effects of ivermectin treatment have been demonstrated experimentally in other non-target parasites (e.g. *Eimeria* spp., Pedersen and Antonovics, 2013). As

opposing effects were observed in different sites, it is difficult to infer the biological significance of this finding. However, given the link between *T. copemani*, *Trypanosoma* spp. coinfection and woylie population declines (Smith et al., 2008; Botero et al., 2013; Thompson et al., 2014; Godfrey et al., 2018), ivermectin may have the potential to negatively impact host health; particularly when perturbations to the prevalence of specific *Trypanosoma* sp. have been associated with the use of this drug (Northover et al., 2019). Importantly, no clinical studies have evaluated the efficacy of ivermectin in woylies, thus the dose or dosage regime may have been suboptimal. The dose administered to woylies during this study was at the lower end of the suggested reference range for macropods (Vogelnest and Portas, 2008) and was selected based on its apparent safe use in the closely related eastern bettong *Bettongia gaimardi* (Portas et al., 2014). The absence of an effect in target parasites may indicate that woylies require a higher dose or repeat dosing. Furthermore, different parasite species are likely to vary in their susceptibility to ivermectin.

Conclusions

This is the first study to evaluate how the broader parasite community changes following fauna translocation in translocated and resident hosts. The response of most parasites following translocation occurred rapidly but varied significantly among sites. These findings have several important implications for fauna translocations. First, given the innate ability of parasites to impact host health and translocation outcomes, and the large degree of unpredictability associated with translocating wildlife, translocation protocols should incorporate long-term parasite monitoring to better understand (a) how the parasite community within a host changes following translocation; and (b) the biological implications of these changes on individuals (e.g. reproductive fitness, survivorship), host populations (e.g. population health, growth rates) and ultimately translocation success. For woylies, more research is needed to understand the site-level drivers of parasite dynamics. Second, with increasing recognition of the intrinsic biodiversity value of parasites (Colwell et al., 2012; Thompson et al., 2018), the potential loss of host-parasite associations should be a serious consideration when planning fauna translocations. Finally, antiparasitic drugs should be applied prior to translocation only where there is a clear rationale for their use; field studies that examine the response of parasites following experimental manipulation are required to provide such justification.

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3.2 Gastrointestinal parasite taxa

During this study three visually distinctive nematode eggs were identified (strongyle, *Strongyloides*-like and *Potoroxyuris* sp.), a single type of cestode egg (undescribed species), coccidian oocysts (*Eimeria woyliei*), and first stage (metastrongyloid) lungworm larvae (Figure 16A-F). Nematode larvae were also found, and may represent either infective nematode larvae or free-living nematodes; no attempt was made to differentiate between the two groups or perform nematode counts.

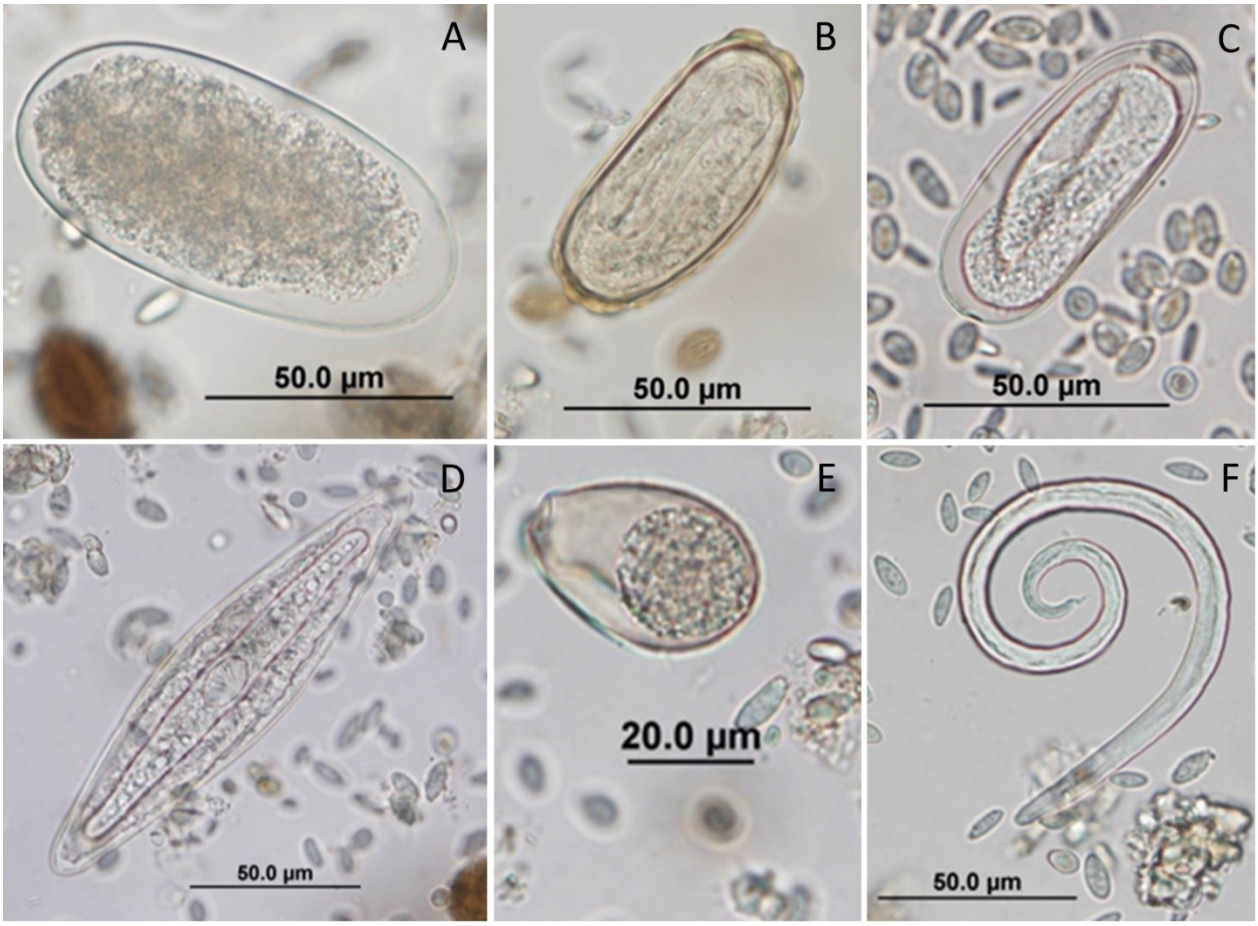

Figure 16: Typical strongyle egg (A), *Potoroxyuris* sp. egg (B), *Strongyloides*-like egg (C), cestode egg (D), unsporulated coccidian oocyst (E), and first stage (metastrongyloid) lungworm larvae (F) found in woylie faeces.

Strongyle eggs (Nematoda: Strongylidae) were typically unsporulated measuring between 80-100 µm in length and most likely represent several strongylid nematode species infecting the woylie. Currently five species of *Potorostrongylus* nematodes have been identified in potoroids including *Potorostrongylus woyliei* (Smales, 2005) and *Paraustrostrongylus bettongia* (Mawson, 1973) in the woylie.

Strongyloides-like eggs (Nematoda: Strongyloididae) were embryonated and noticeably smaller (around 50 μm in length). No *Strongyloides* spp. nematodes have been formally described in woylies. *Potoroxyuris* sp. eggs (Nematoda: Oxiuridae) were embryonated, measuring approximately 60 μm in length and easily identifiable by their morphologically characteristic mammilated outer shell covering (Hobbs and Elliot, 2016). Within Australia, *Potoroxyuris* sp. nematodes have been found in *Potorous tridactylus* from Victoria (*Potoroxyuris potoroo*; Johnson and Mawson, 1939) and the woylie (*Potoroxyuris keninupensis*; Hobbs and Elliot 2016). It is most likely that the *Potoroxyuris* sp. eggs identified in this study are *P. keninupensis* eggs. Cestode eggs (Platyhelminthes: Hymenolepididae) were large (around 140 μm long), marquise-shaped and embryonated. To date, there is only one species of cestode recorded in woylies, *Rodentolepsis fraterna* (Spratt and Beveridge, 2016), and the eggs of *R. fraterna* are morphologically different to those observed here. Coccidian oocysts (Apicomplexa: Eimeriidae) were typically unsporulated, measuring approximately 30 μm long. Sporulated oocysts were formally described as *Eimeria woyliei* during this study (Chapter 4). First stage (metastrongyloid) lungworm larvae (Nematoda: Metastrongylidae) were roughly 250 μm in length with a distinctive curve at the tip of the tail. *Angiostrongylus cantonensis* has been identified in the Rufous bettong (*Aepyprymnus rufescens*) in Eastern Australia (Higgins et al., 1997); however lungworm larvae are yet to be officially described in the woylie.

3.3 Ectoparasite taxa

Five species of tick, two species of lice, seven species of flea and four species of mite were identified on woylies during this study (Table 5).

Table 5: Summary of the number of woylies infected with each of the ectoparasite species identified during this study. Tran: translocated; Res: resident.

Taxa	Species	Dryandra		Walcott		Warrup East		
		Tran	Res	Tran	Res	Tran	Res	Total
Fleas	*Stephanocircus dasyuri*	12	0	35	35	33	8	123
	Pygiopsylla tunneyi	16	0	21	92	21	34	184
	Pygiopsylla hilli	4	0	8	21	4	5	42
	Choristopsylla ochi	1	0	0	0	0	0	1
	Echidnophaga myrmecobii	1	0	0	0	1	0	2
	Echidnophaga perilis	1	0	0	0	0	0	1
	Acidesta chera	1	0	0	0	0	0	1
	Unidentified species	1	4	0	0	0	0	5
Lice	*Paraherterodoxus calcaratus*	129	110	81	118	109	70	617
	Boopia uncinata	0	1	0	0	0	0	1
	Unidentified larval stages	1	0	0	2	1	2	6
	Unknown	1	1	3	0	1	2	8
Mites	*Haemolaelaps hattanae*	21	24	53	95	71	56	320
	Haemolaelaps quartus	16	7	40	36	41	9	149
	Haemolaelaps marsupialis	1	0	0	0	0	0	1
	Androlaelaps fahrenholiz	0	0	0	0	0	1	1
	Unidentified larval stages	4	1	2	1	3	1	12
	Unknown	0	1	2	1	4	0	8
Ticks	*Ixodes woyliei*	26	30	8	24	4	3	95
	Ixodes myrmecobii	2	1	14	11	19	5	52
	Ixodes australiensis	14	0	35	95	48	40	232
	Ixodes tasmani	1	1	0	0	0	0	2
	Amblyomma sp.	17	24	3	25	8	12	89
	Amblyomma triguttatum	1	0	0	0	0	0	1
	Unidentified larval stages	120	107	75	122	82	53	559
	Unknown	0	0	5	1	2	0	8

While most of these species have been previously found on woylies (von Keler, 1971; Dunnet and Mardon, 1974; Domrow, 1987; Kaewmongkol et al., 2011; Ash et al., 2017); this is the first report of *Pygiopsylla tunneyi*, *Choristopsylla ochi*, *Echidnophaga perilis*, *Acidesta chera*, *Boopia uncinata*, *Haemolaelaps marsupialis* and *Androlaelaps fahrenholiz* on woylies. The woylie is likely to be an accidental host of *C.*

ochi, *A. chera* and *H. marsupialis*; bag contamination was plausible in each case as the nominal host was captured beforehand. In contrast, *P. tunneyi* was the most common species of flea identified on translocated woylies, suggesting that the woylie is an alternative host. *Dasyurus* species are the predominant host of *B. uncinata* (von Keler, 1971). *Boopia uncinata* was only detected on a single woylie from Dryandra and was not found on chuditch (Western quoll, *Dasyurus geoffroii*) from this site. It is difficult to infer whether the woylie is an accidental or alternate sympatric host for *B. uncinata*; while bag contamination is possible, only woylies were captured on the day *B. uncinata* was found. Likewise, a possum (*Trichosurus vulpecula hypoleucus*), which is not the nominal host of *E. perilis* (Dunnet and Mardon, 1974), was the only other species captured on the day *E. perilis* was found on a woylie. According to Domrow (1987), birds from the south-east of Australia are the predominant host of *A. fahrenholizi*; its presence in a single woylie is strange.

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Chapter Four

Identification of a novel *Eimeria* species from the woylie (*Bettongia penicillata*) and the genetic characterisation of *Eimeria gaimardi*, *Eimeria potoroi* and *Eimeria mundayi* from other potoroid marsupials.

4.1 Identification of a novel *Eimeria* species from the woylie (*Bettongia penicillata*) and the genetic characterisation of *Eimeria gaimardi*, *Eimeria potoroi* and *Eimeria mundayi* from other potoroid marsupials.

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Abstract

Faecal samples (*n* = 1093) collected from the woylie *Bettongia penicillata* Gray, in south-western Australia were examined for the presence of coccidian parasites. *Eimeria* sp. oocysts were detected in 15.2% of samples. Faecal samples obtained from the eastern bettong *Bettongia gaimardi* (Desmarest) (*n* = 4) and long-nosed potoroo *Potorous tridactylus* (Kerr) (*n* = 12) in Tasmania, were also screened for the presence of *Eimeria* spp. (prevalence 50% and 41.7%, respectively). Morphological and genetic comparison with other known species of *Eimeria* indicates that the *Eimeria* species identified in woylies is novel. This study aimed to (a) morphologically describe and genetically characterise *Eimeria woyliei* n. sp. found in woylies; and (b) genetically characterise *Eimeria gaimardi* Barker, O'Callaghan and Beveridge, 1988, *Eimeria potoroi* Barker, O'Callaghan and Beveridge, 1988, and *Eimeria mundayi* Barker, O'Callaghan and Beveridge, 1988, from other potoroid marsupials. Molecular phylogenetic analyses conducted at the 18S rDNA and mitochondrial cytochrome oxidase (COI) loci revealed that *E. woyliei* n. sp. was most closely related to *Eimeria setonicis* Barker, O'Callaghan and Beveridge, 1988, at the 18S rDNA locus, and *Eimeria trichosuri* O'Callaghan and O'Donoghue, 2001, at the COI locus. *Eimeria woyliei* n. sp. is the sixth species of *Eimeria* to be formally described from potoroid marsupials.

Introduction

Coccidian parasites are known to infect potoroid marsupials, including the critically endangered woylie or brush-tailed bettong *Bettongia penicillata* Gray. Although morbidity and mortality associated with coccidial infection in marsupials is uncommon (Vogelnest and Portas, 2008), disease has been

documented in macropods under stress (e.g. eastern grey kangaroo *Macropus giganteus* Shaw; Barker et al., 1972). Given the reliance of many threatened species on interventional management practices such as translocation, a process which has been identified as a significant stressor (Hing et al., 2017), it is imperative that we gain a greater understanding of the parasite species infecting wildlife, particularly those with the potential to cause disease in their host (e.g. coccidian parasites).

To date, no coccidian parasites (Apicomplexa: Eimeriidae) have been formally described in woylies (Duszynski, 2016), though *Eimeria* sp. oocysts have been detected in faecal samples (Northover et al., 2017). Five *Eimeria* spp. including *Eimeria potoroi* Barker, O'Callaghan and Beveridge, 1988, *Eimeria mundayi* Barker, O'Callaghan and Beveridge, 1988, *Eimeria gaimardi* Barker, O'Callaghan and Beveridge, 1988*, Eimeria aepyprymni* Barker, O'Callaghan and Beveridge, 1988, and *Eimeria burdi* Hulst, Kemp and Slapeta, 2016, have been morphologically described from potoroid marsupials (Marsupialia: Potoroidae). *Eimeria gaimardi* from the eastern bettong *Bettongia gaimardi* (Desmarest), most closely morphologically resembles *Eimeria* sp. oocysts found in woylies. Unfortunately, genetic characterisation of potoroid *Eimeria* spp. has not been undertaken. While *Eimeria* spp. tend to be host-specific (Barker et al., 1988), members of the genus *Macropus* have been known to harbor the same *Eimeria* spp. (e.g. *Eimeria macropodis* Wenyon and Scott, 1925; Barker et al., 1989), thus it is important that additional data (e.g. genetic characterisation) is used to support the description of new species.

During this study we aimed to (a) morphologically describe and genetically characterise *E. woyliei* n. sp. from woylies; and (b) genetically characterise *E. gaimardi* from the eastern bettong; and *E. potoroi* and *E. munda*yi from the long-nosed potoroo *Potorous tridactylus* (Kerr).

Materials and methods

Sample collection

Between 2014 and 2018, woylie faecal samples (n = 1093) were collected from various sites within south-western Australia (Table 6) as part of a collaborative project with the Department of Biodiversity, Conservation and Attractions (DBCA) conducted under DBCA Scientific Licenses (Regulation 4: written notice of lawful authority; and 17: licence to take fauna for scientific purposes) and with approval from the Murdoch University Animal Ethics Committee (RW2659/14). Newspaper was placed beneath each trap to collect faeces, which were stored in 70% ethanol, 10% buffered formalin and/or 2% potassium dichromate until processing.

Table 6: The number of woylies screened for coccidian parasites within each sampling site.

Site	Coordinates	Number screened	Number positive	Prevalence (%)
Balban	34.10°S, 116.58°E	21	2	9.5
Boyicup	34.28°S, 116.58°E	1	0	0.0
Corbal	34.10°S, 116.48°E	15	6	40.0
Dryandra	32.80°S, 116.89°E	357	10	2.8
Dudijup	34.07°S, 116.30°E	4	1	25.0
Dwalgan	34.07°S, 116.46°E	14	8	57.1
Perup Sanctuary	34.16°S, 116.56°E	151	32	21.2
Walcott	34.06°S, 116.39°E	266	56	21.1
Warrup East	34.16°S, 116.39°E	255	50	19.6
Winnejup	34.07°S, 116.35°E	9	1	11.1
		1093	**166**	**15.2**

In 2018, faecal samples collected from the eastern bettong (n = 4) were obtained from a captive population at Bonorong Wildlife Sanctuary in Brighton, Tasmania (42.71°S, 147.27°E). Samples from the long-nosed potoroo (n = 12) were acquired from wild-caught animals within the Peter Murrell reserves, south of Hobart, Tasmania (43.00°S,147.18°E); these samples were collected under authorities and permits issued to the Department of Primary Industries, Parks, Water and Environment (DPIPWE) staff to live-trap wildlife on reserved land in Tasmania. This study followed the Standard Operating Procedures for *Live-trapping and Handling of Wild Tasmanian Mammals 2013* by the DPIPWE. Faecal samples from the eastern bettong and long-nosed potoroo were collected directly from traps and samples were stored in 2% potassium dichromate prior to analysis.

Identification of coccidian oocysts in faecal samples

For woylies, the majority of faecal samples (n = 1073) were examined for the presence of coccidian oocysts using simple faecal flotation with sodium nitrate ($NaNO_3$) as described by Northover et al. (2017). These samples were formalin-fixed. To describe the morphology of *E. woyliei* n. sp., an additional 20 samples were sporulated (using potassium dichromate) and underwent faecal flotation using zinc sulphate ($ZnS0_4$) in distilled water (SG 1.20). Briefly, faeces were placed into a 10ml centrifuge tube (up to the 1.5-2ml mark), emulsified in distilled water (tube filled to the 10ml mark) and centrifuged (2000 rpm for 2 minutes). The supernatant was removed before filling the tube with zinc sulphate solution and re-emulsifying, before final centrifugation (2000 rpm for 2 minutes). A sterile wire loop was used to transfer 2-3 loops from the surface of the tube to a glass slide, and a 22mm x 22mm coverslip was placed on top. Each sample was examined at 100x magnification using

an Olympus BX50 microscope. All other potoroid samples were examined using zinc sulphate. To calculate prevalence of infection, each faecal sample was scored as present or absent for coccidian oocysts.

Morphological description of *E. woyliei* n. sp.

Eighty-six sporulated oocysts from a single woylie originating from Perup Sanctuary were examined at 400x to 1000x magnification, using an Olympus BX50 microscope with a Olympus DP71 Universal Camera with Cellsens software. Photographs of sporulated oocysts were taken using bright field microscopy. Measurements of oocyst and sporocyst length and width, and oocyst wall thickness were acquired using ImageJ software (US National Institute of Health, Bethesda, Maryland). All measurements are recorded in micrometers (μm) with the range given in parentheses following the mean. We measured a single laterally positioned sporocyst within each oocyst; if we could not identify a sporocyst in the correct position, we did not measure sporocyst length or width.

Morphological identification of other potoroid *Eimeria* spp.

Three distinct morphotypes of sporulated oocysts were identified from the faeces of the eastern bettong and long-nosed potoroo. Based on their size and unique oocyst and sporocyst characters as described by Barker et al. (1988) we identified *E. gaimardi* from the eastern bettong, and *E. mundayi* and *E. potoroi* from the long-nosed potoroo.

Genetic characterisation of *Eimeria* spp. in potoroid marsupials

For the woylie, nine faecal samples (eight ethanol- and one potassium dichromate-preserved) were used to genetically characterise *E. woyliei* n. sp. at the 18S rDNA and mitochondrial cytochrome oxidase (COI) loci. *Eimeria gaimardi, E. mundayi* and *E. potoroi* were genetically characterised using a single faecal sample (potassium dichromate-preserved) for each *Eimeria* species; as outlined above, morphological identification of sporulated oocysts confirmed the identity of each species prior to genetic characterisation.

DNA extraction

Samples stored in 70% ethanol and/or 2% potassium dichromate were exposed to four freeze/thaw cycles as described by Yang et al. (2016a) in order to achieve oocyst lysis. Following the freeze/thaw step, faecal samples stored in 2% potassium dichromate were subjected to a wash step prior to lysis in order to remove the fixative, by centrifuging at 3000rpm for 10 min and resuspending in phosphate buffered solution. DNA was isolated from 0.25g of faecal sample using the PowerFecal

DNA Isolation Kit (MolBio, Carlsbad, California) as per the manufacturer's instructions. A negative control was included to omit contamination.

PCR amplification

18S gene region

Faecal samples from the woylie were screened at the 18S rDNA locus using a nested PCR with the external *Eimeria* spp. primers EiF1 (5' GCTTGTCTCAAAGATTAAGCC 3') and EiR3 (5' ATGCATACTCAAAAGATTACC 3'), and the internal *Eimeria* spp. primers EiR4 (5' ACTCAAAAGATTACCTAGAC 3') and EiF4 (5' CTATGGCTAATACATGCGCAAT 3') (Yang et al., 2016a). PCR reactions were carried out in a total volume of 25 μL containing 12.5 μL of 2X KAPA HiFi Hotstart ReadyMix (Millennium Science Pty. Ltd), 0.75 μL primer (10 μM) and 2 μL DNA template. All PCR reactions were performed as described by Yang et al. (2016a) consisting of a pre-PCR step of 94°C for 3 minutes, followed by 45 cycles of 94°C for 30 seconds, 55°C annealing temperature for 30 seconds and 72°C for 2 minutes, and a final extension step of 72°C for 5 minutes.

Faeces from the eastern bettong and long-nosed potoroo were screened using the external primers EiGTF1 (5' TTCACTGGTCCCTCCGATC 3') and EiGTR1 (5' AACCATGGTAATTCTATGG 3') (Yang et al., 2016b), and the internal primers EiGTF2 (5' TTACGCCTACTAGGCATTCC 3') and EiTR2 (5' TGACCTATCAGCTTTCGACG 3') (Yang et al., 2015). The PCR reaction contained 10 μL 2 x GoTaq PCR master mix (Promega, Alexandria NSW, Australia), 1 μL DNA (50ng), 10 μL of each primer (10 μM stock) and 7 μL distilled water. PCR cycling conditions for the external PCR were 1 cycle of 94°C for 3 minutes, followed by 35 cycles of 94°C for 30 seconds, 58°C for 30 seconds and 72°C for 2 minutes, and a final extension step of 72°C for 5 minutes. The conditions for the secondary PCR were the same except for 45 cycles instead of 35. All amplicons were visualised on a 1.5% agarose gel and bands were cut and purified using an in-house filter tip method defined by Yang et al. (2013).

COI gene region

For all potoroid species, amplicons were generated at the COI locus using the external primers COIF1 (5' GGTTCAGGTGTTGGTTGGAC 3') and COIF2 (5' TAAGTACATCCCTAATGTC 3') and the internal primers COIbR1 (5' CCAAGAGATAATACRAARTGGAA 3') and COIbR2 (5' ATAGTATGTATCATGTARWGCAA 3') (Yang et al., 2016a). The PCR reactions and conditions were the same as per the 18S PCR carried out for woylie samples.

Sequencing and phylogenetic analysis

Purified amplicons were sequenced using an ABI Prism™ Dye Terminator Cycle Sequencing Kit (Applied Biosystems, California) according to the manufacturer's instructions. Samples were sequenced in the forward and reverse direction and the denaturation step was extended to 10 minutes to allow efficient primer binding. The sequences were analysed in Geneious v.8.1 (Kearse et al., 2012) and aligned using reference libraries with the MUSCLE (Edgar, 2004) plugin for Geneious v.8.1. All novel sequences were deposited in GenBank®.

Using Bayesian methods, phylogenetic analyses were conducted for both gene regions (18S and COI, respectively), to determine the evolutionary lineage for *E. woyliei* n. sp. (MK182524; MK202806), *E. gaimardi* (MK182525; MK202809), *E. mundayi* (MK182526; MK202808) and *E. potoroi* (MK182527; MK202807). JModelTest (Posada, 2008) was used to determine the most appropriate nucleotide substitution method for the Bayesian analyses for each gene region, which was the GTR+I+G (general time reversible gamma proportion of invariant sites) method. The Bayesian posterior probabilities were generated using Mr Bayes v.3.1.2 (10,000,000 generations, sampling frequency of 1000, burn in 3000).

Results

The prevalence of coccidial infection in woylies is summarised by site in Table 6. It is important to note that Dryandra contains both resident woylies (endemic to the region), and translocated woylies originating from the Upper Warren region (specifically Balban, Boyicup, Corbal, Dudijup, Dwalgan and Winnejup). Likewise Walcott and Warrup East contain both resident and translocated (originating from Perup Sanctuary) woylies. In the eastern bettong 2/4 (50%) samples were positive for *E. gaimardi*. In the long-nosed potoroo, 3/12 (25.0%) samples were positive for *E. potoroi*, while 4/12 (33.3%) samples were positive for *E. mundayi*; mixed infections with both coccidian parasites were identified in two (16.7%) samples.

***Eimeria woyliei* n. sp.**

Type-host: Bettongia penicillata Gray (Mammalia: Marsupialia: Potoroidae), woylie or brush-tailed bettong

Type-known locality: Perup Sanctuary (34.16°S, 116.56°E) in the Upper Warren region, Western Australia, Australia

Type-other known localities: Corbal (34.10°S, 116.48°E), Dwalgan (34.07°S, 116.46°E) and Winnejup (34.07°S, 116.35°E) in the Upper Warren region, Western Australia, Australia; Dryandra Woodland

(32.80°S, 116.89°E) in the wheatbelt region, Western Australia, Australia (*translocated host, origin Dwalgan).

Type-material: DNA sequences have been deposited in the GenBank sequence database under accession numbers MK182524-MK182527 and MK202806-MK202809 for the 18S rDNA and COI genes, respectively.

Prevalence: In this study, 32/151 (21.2%) specimens of the type host (origin Perup Sanctuary) were infected with *E. woyliei*.

Sporulation: Unknown

Site of infection: Unknown, oocysts recovered from faeces

Prepatent and patent periods: Unknown

Endogenous stages: Unknown

Etymology: The name *woyliei* reflects the host species local name 'woylie', which is the Aboriginal name given to this animal by the Noongar people of south-western Western Australia (Abbott, 2001).

Morphological description (Figures 17 and 18A-C)

Sporulated oocyst

Oocysts (n = 86) are pyriform-shaped [length 36.7 (31.6-40.8), width 26.3 (22.6-31.0), length/width (L/W) ratio 1.4 (1.3-1.6)] with a smooth to slightly mammilate, bilayered oocyst wall [1.6 (1.3-1.7)]. Oocyst wall thins at the apex with a visible micropyle. An irregular dome-like structure (referred to here as a submicropyle body) is visible beneath the micropyle. An irregular-shaped polar granule is also apparent, although not detected in all sporulated oocysts. Oocyst residuum is absent.

Sporocyst and sporozoite

Sporocysts (n = 76) are ellipsoidal [length 15.6 (13.3-17.8), width 10.3 (9.0-12.3), L/W ratio 1.5 (1.2-1.7)]. Each sporocyst contains an indistinct domelike Stieda body/Substieda body. Sporocyst residuum is present between two broadly elongate sporozoites. Sporozoites contain two distinct refractile bodies, one large (5-7 wide) and one small (up to 3 wide). Parastieda body is absent.

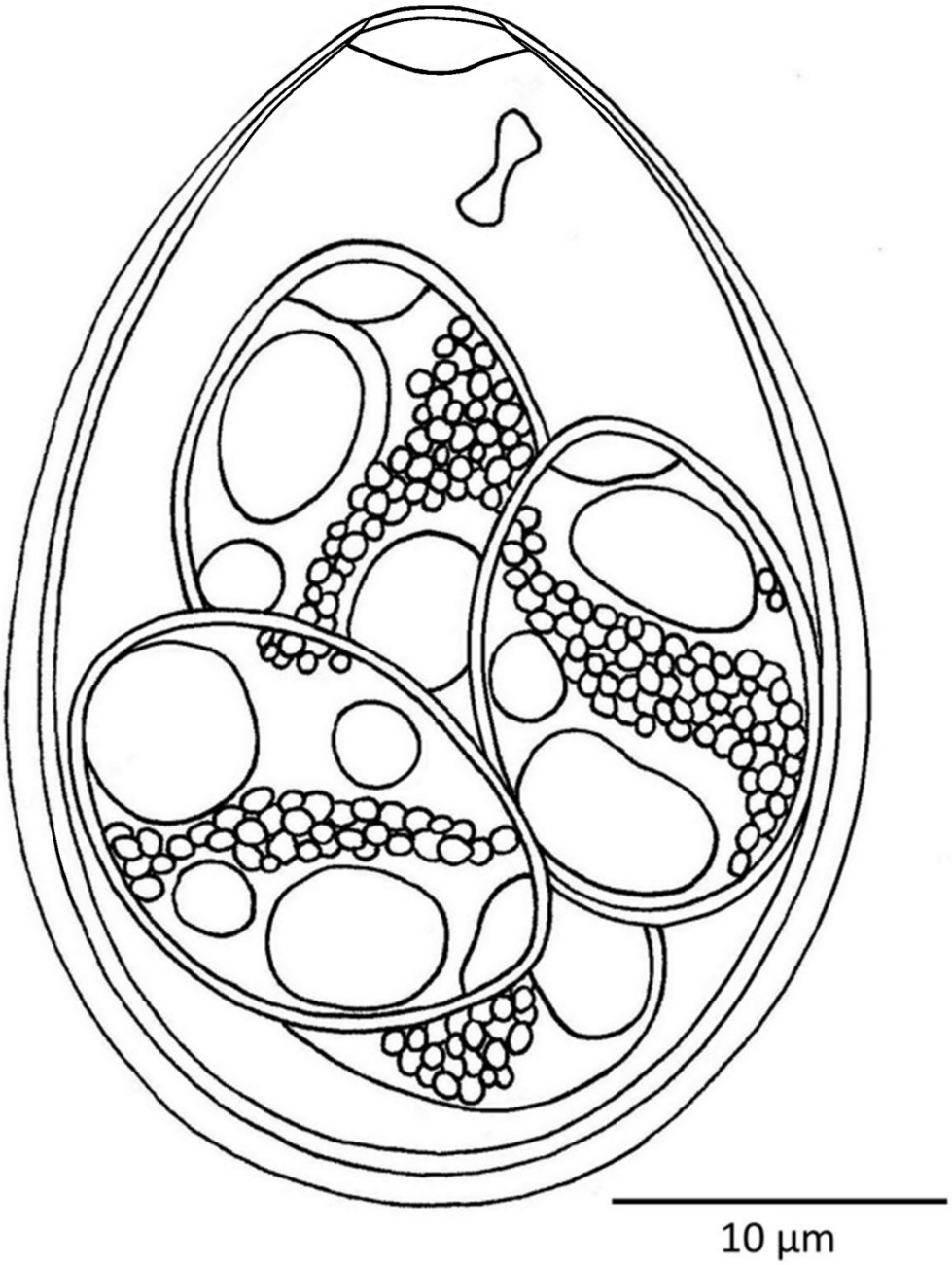

Figure 17: Composite line drawing of a sporulated *Eimeria woyliei* n. sp. oocyst.

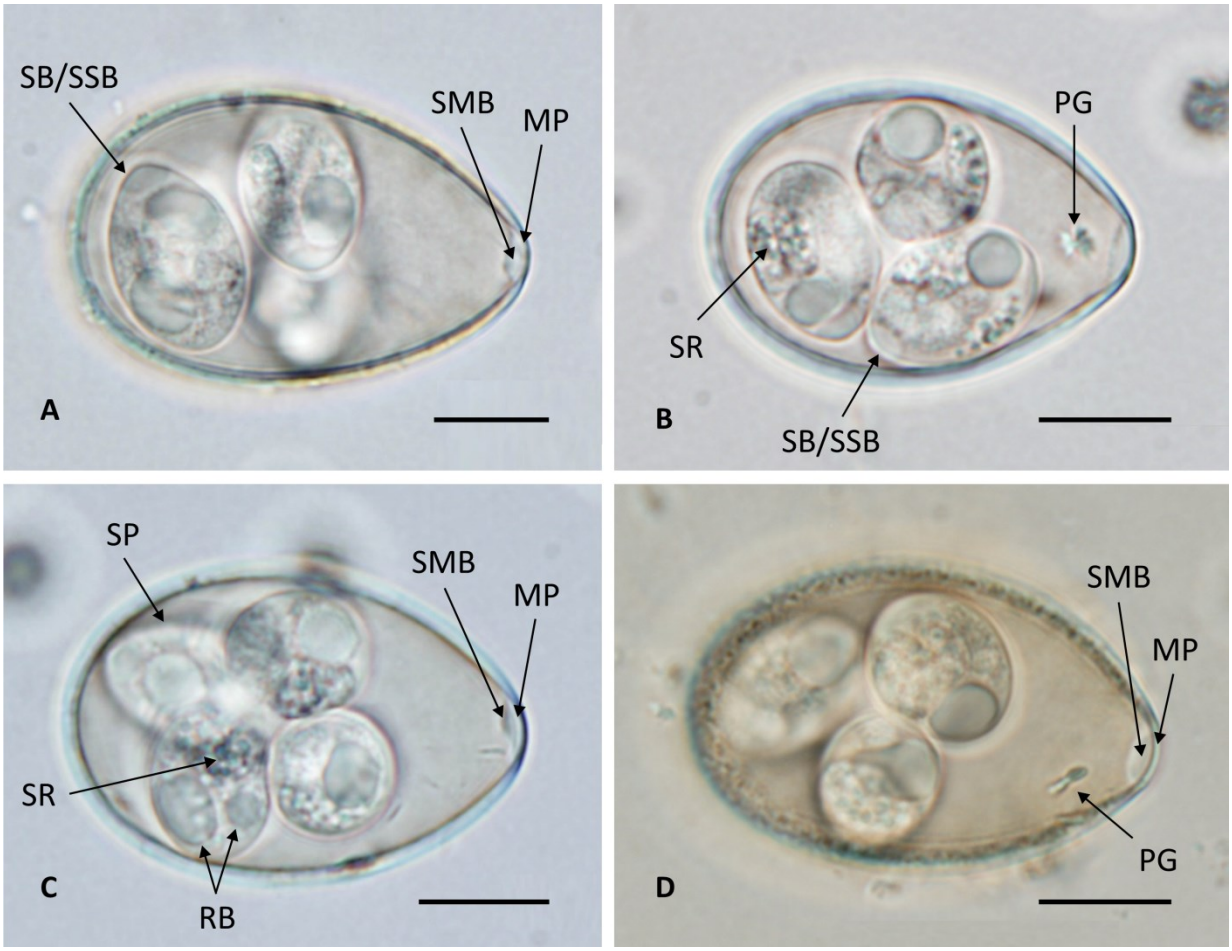

Figure 18: Photomicrographs of sporulated *Eimeria woyliei* n. sp. oocysts (A-C) and a sporulated *Eimeria gaimardi* oocyst (D). *Abbreviations*: PG, polar granule; MP, micropyle; RB, refractile body; SB/SSB, Stieda body/Substieda body; SMB, submicropyle body; SP, sporocyst; SR, sporocyst residuum. *Scale bars*: 10μm.

Genetic characterisation of *Eimeria* spp. in potoroids

The phylogeny of the four potoroid *Eimeria* spp. was investigated using Bayesian analyses at two gene loci (18S and COI). An alignment was generated for the 18S rDNA locus (1158bp, Figure 19) and two alignments for the COI locus (686bp, Figure 20; 211bp, Figure 21). All alignments contained the novel sequences belonging to the four potoroid species as well as reference sequences downloaded from GenBank® including an outgroup (*Toxoplasma gondii*). Similar phylogenetic relationships were observed between both loci, although some species were not observed in the COI tree due to lower availability of genetic data. A second COI alignment was generated (211bp) in order to incorporate relevant marsupial species of smaller fragment size.

18S rDNA locus

Eimeria woyliei shared 99.2% genetic similarity with *Eimeria setonicis* Barker, O'Callaghan and Beveridge, 1988, isolated from the quokka *Setonix brachyurus* (Quoy and Gaimard) (KF225639;

Austen et al., 2014), and 99.1% genetic similarity with *Eimeria trichosuri* O'Callaghan and O'Donoghue, 2001, from the mountain brushtail possum *Trichosurus cunninghami* Lindenmayer, Dubach and Viggers (FJ8292320; Power et al., 2009). Of the potoroid *Eimeri*a species, *E. woyliei* shared 98.8% genetic similarity with *E. mundayi* and *E. potoroi*, and 98.4% similarity with *E. gaimardi*.

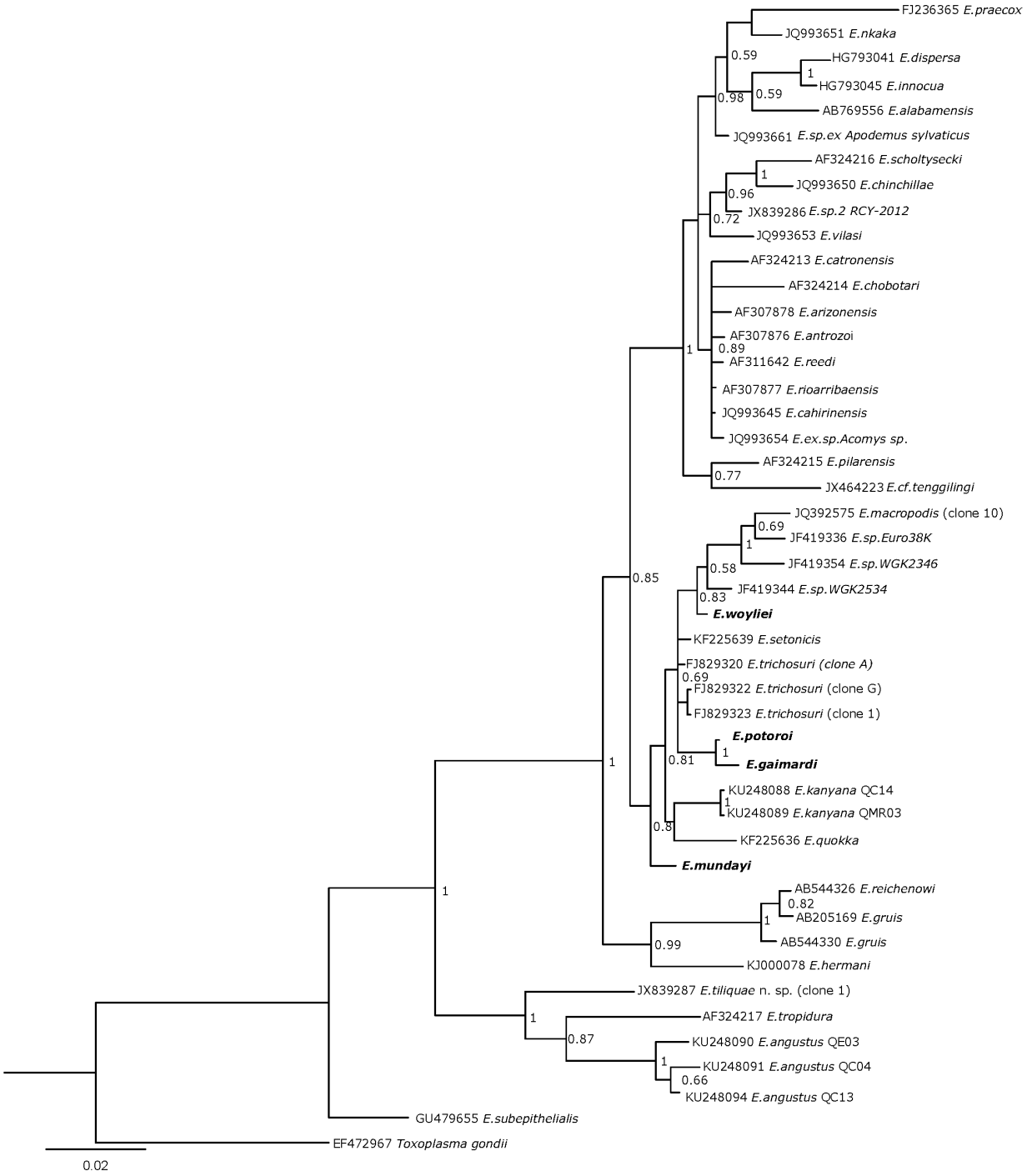

Figure 19: Phylogenetic relationship of *E. woyliei* n. sp. compared to other *Eimeria* spp. using Bayesian analysis of an 1158bp fragment of the 18S rDNA gene.

Figure 20: Phylogenetic relationship of *E. woyliei* n. sp. compared to other *Eimeria* spp. using Bayesian analysis of the large 686bp fragment of the COI gene.

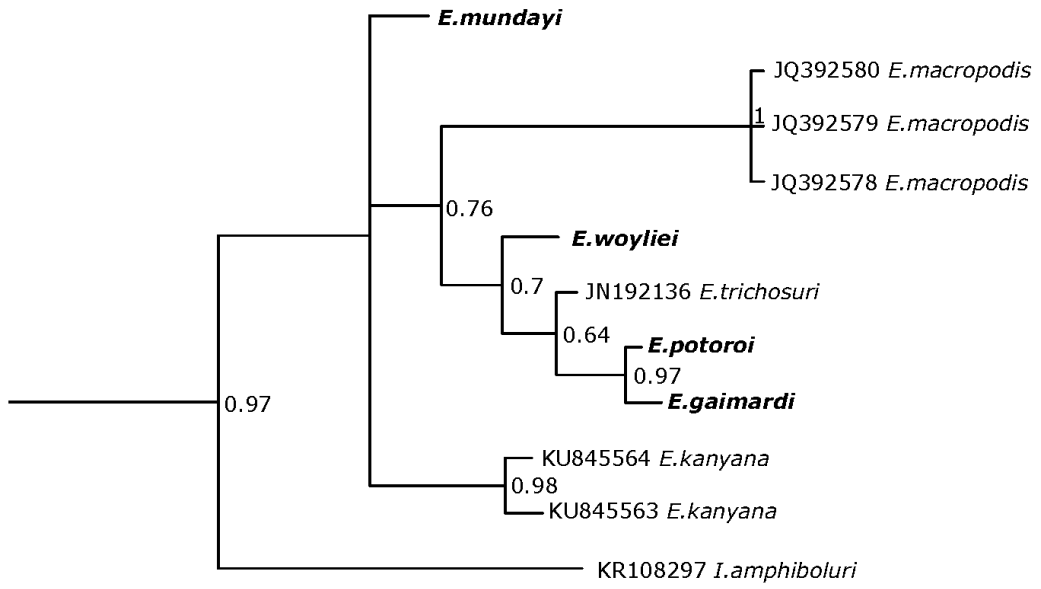

Figure 21: Phylogenetic relationship of *E. woyliei* n. sp. compared to other *Eimeria* spp. using Bayesian analysis of the smaller 211bp fragment of the COI gene.

COI locus

Using the longer (686bp) COI locus, *E. woyliei* shared 97.1% similarity with *E. trichosuri* (JN192136), 95.7% similarity with *E. potoroi*, 95.3% similarity with *E. gaimardi* and 95.0% similarity with *E. mundayi*. Using the shorter (211 bp) COI locus, *E. woyliei* shared 94.8% similarity with *Eimeria kanyana* Bennett, Woolford, O'Hara, Nicholls, Warren and Hobbs, 2006, from the quenda *Isoodon obesulus* (Shaw) (KU845563/64; Hillman, 2016), and 97.2%, 96.7% and 95.3% similarity with *E. potoroi*, *E. gaimardi* and *E. mundayi*, respectively.

Remarks

Five species of *Eimeria* have been previously described in potoroid marsupials (Barker et al., 1988; Hulst et al., 2016). Of these, *E. woyliei* oocysts [36.7 x 26.3 (31.6-40.8 x 22.6-31.0)] most closely resemble *E. gaimardi* oocysts [34.6 x 24.3 (32.0-39.2 x 20.8-26.4), Barker et al., 1988] from the eastern bettong in shape and size. Both oocyst (see above) and sporocyst size of *E. woyliei* and *E. gaimardi* [15.6 x 10.3 (13.3-17.8 x 9.0-12.3) *versus* 15.0 x 9.6 (13.6-16.0 x 9.0-10.4), respectively] are similar, though *E. woyliei* oocysts/sporocysts are slightly larger on average. *Eimeria woyliei* oocysts also contain a micropyle, submicropyle body and a polar granule; *E. gaimardi* oocysts do not according to the original morphological description by Barker et al. (1988). Based on the sporulated *E. gaimardi* oocysts examined during this study however, we propose that *E. gaimardi* oocysts do possess a micropyle, submicropyle body and polar granule (Figure 18D). Thus, the major distinguishing

morphological characteristic between *E. woyliei* and *E. gaimardi* is the appearance of the oocyst wall, which for *E. gaimardi* appears mammilated and more robust (Figure 18D). The oocyst wall of *E. woyliei* in comparison is smooth to slightly mammilate.

Eimeria woyliei oocysts are noticeably larger than *E. potoroi* oocysts [26.2 x 18.5 (24.0-29.6 x 16.8-22.4), Barker et al., 1988] and *E. mundayi* oocysts [16.9 x 16.2 (13.6-20.8 x 13.6-19.2), Barker et al., 1988] from the long-nosed potoroo. *Eimeria woyliei* oocysts are comparable in size to *E. aepyprymni* oocysts [36.7 x 21.9 (32.0-42.8 x 18.4-25.2), Barker et al., 1988] from the rufous bettong *Aepyprymnus rufescens* (Gray), however *E. aepyprymni* oocysts are ovoid rather than pyriform. While similar in shape (pyriform), *E. woyliei* oocysts are considerably larger than *E. burdi* oocysts [22.6 x 14.9 (21.0-24.0 x 14.0-16.0), Hulst et al., 2016] from the burrowing bettong *Bettongia lesueur* (Quoy and Gaimard).

Using the 18s rDNA locus, *E. woyliei* was grouped within the marsupial clade and was most similar to *E. setonicis* from the quokka. *Eimeria woyliei* oocysts can be morphologically distinguished from oocysts of *E. setonicis* by shape (*E. setonicis* oocysts are ellipsoidal; Austen et al., 2014). Despite the morphological similarity between oocysts of *E. woyliei* and *E. gaimardi*, *E. woyliei* was more genetically similar to *E. mundayi* and *E. potoroi*, rather than *E. gaimardi*, though this difference was minimal.

Using the COI locus, *E. woyliei* was most similar to *E. trichosuri* from the mountain brushtail possum and grouped within the same clade as *E. macropodis* from the tammar wallaby *Macropus eugenii* (Desmarest) (Hill et al., 2012), and *E. kanyana* from the quenda (Hillman et al., 2016). *Eimeria woyliei* oocysts can be differentiated from oocysts of *E. trichosuri* by shape (*E. trichosuri* oocysts are ellipsoidal; Power et al., 2009); *E. trichosuri* oocysts are also larger [41.4 x 22.7 (34.4-49.2 x 18.4-27.8); O'Callaghan and O'Donoghue, 2001]. Unexpectedly, *Isospora amphiboluri* Canon, 1967, from the central bearded dragon *Pogona vitticeps* (Ahl) was also present within the same phylogenetic clade (KR108297). Although COI sequences are deemed superior to nuclear 18S rDNA sequences for delimiting species (Ogedengbe et al., 2011), COI sequences in GenBank® are limited. Nonetheless the supposed relationship between *E. woyliei* and *I. amphiboluri* remains unclear.

Within Australia various coccidial species (*Eimeria* and less commonly *Isospora*) have been recorded in captive and free-ranging macropods (Mykytowycz, 1964; Barker et al., 1989; Yang et al., 2012; Duszynski, 2016). Traditional morphological criteria have been useful for identifying coccidial species infecting wildlife. However, the implementation of genetic characterisation combining both 18S rDNA and COI loci helps to discriminate between morphologically similar species and provides

an accurate measure of the evolutionary relationship between coccidial species (Power et al., 2009; Yang et al., 2012). As some *Eimeria* spp. are capable of infecting more than one marsupial host (Barker et al., 1989) and marsupials tend to harbour multiple *Eimeria* spp. (Power et al., 2009), this knowledge may be useful for predicting potential avenues of disease spread during the management of threatened populations (e.g. during fauna translocation). This study contributes toward our knowledge of *Eimeria* spp. infecting potoroid marsupials. *Eimeria woyliei* parasitising woylies is the sixth *Eimeria* spp. to be formally described from potoroid marsupials and we have genetically characterised four of the six known potoroid *Eimeria* species.

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Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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Chapter Five

Increased *Trypanosoma* spp. richness and prevalence of haemoparasite coinfection following translocation

5.1 Increased *Trypanosoma* spp. richness and prevalence of haemoparasite coinfection following translocation

The following is a published paper:

Northover, A.S., Godfrey, S.S., Keatley, S., Lymbery, A.J., Wayne, A.F., Cooper, C., Pallant, L., Morris, K., Thompson, R.C.A., 2019. Increased *Trypanosoma* spp. richness and prevalence of haemoparasite coinfection following translocation. Parasites & Vectors **12**: 126. https://doi.org/10.1186/s13071-019-3370-6.

Abstract

Background: Understanding how fauna translocation and antiparasitic drug treatment impact parasite community structure within a host is vital for optimising translocation outcomes. *Trypanosoma* spp. and piroplasms (*Babesia* and *Theileria* spp.) are known to infect Australian marsupials, including the woylie (*Bettongia penicillata*). However relatively little is known about these haemoparasites, or how they respond to management practices such as translocation. We monitored haemoparasites infecting woylies for up to 12 months during two fauna translocations to supplement existing woylie populations in three different sites (Dryandra, Walcott and Warrup East) within south-western Australia between 2014 and 2016, with the aim of investigating (i) how haemoparasite prevalence, *Trypanosoma* spp. richness and *Trypanosoma* spp. community composition varied over time and between different sites following translocation; and (ii) whether ivermectin treatment indirectly impacts haemoparasite prevalence. Using molecular methods, 1211 blood samples were screened for the presence of trypanosomes, and a subset of these samples (n = 264) were also tested for piroplasms.

Results: Trypanosomes and piroplasms were identified in 55% and 94% of blood samples, respectively. We identified five *Trypanosoma* species, two *Theileria* species, a single species of *Babesia* and a novel *Bodo* species. *Trypanosoma* spp. richness and the prevalence of haemoparasite co-infection increased after translocation. Prior to translocation, *Trypanosoma* spp. community composition differed significantly between translocated and resident woylies within Walcott and Warrup East, but not Dryandra. Six months later, there was a significant difference between translocated and resident woylies within Dryandra, but not Walcott or Warrup East. The response of haemoparasites following translocation was highly site-specific, with predominant changes to the haemoparasite

community in translocated woylies occurring within the first few months after translocation. Ivermectin treatment had no significant effect on haemoparasite prevalence.

Conclusions: This study contributes to our understanding of haemoparasite dynamics in woylies following translocation. The highly site-specific and rapid response of haemoparasites following translocation highlights the need to better understand what drives these effects. Given that haemoparasite prevalence and composition of translocated and resident animals changed significantly following translocation, we propose that parasite monitoring should form an essential component of translocation protocols, and such protocols should endeavour to monitor translocated hosts and cohabiting species.

Introduction

Suitably termed a "biological package", a host and its suite of parasites are unique, coexisting but continually adapting entities, enhancing the competitive fitness and ultimately affecting the survival of one another (Strickberger, 2000; Corn and Nettles, 2001). While it is now recognised that polyparasitism (co-infection, concomitant infection or multiparasitism) is the rule rather than the exception in wildlife (Keusch and Migasena, 1982; Graham, 2008), deciphering the manner in which parasites interact with each other and their host is complex; even more so in situations where perturbations to the host-parasite community are likely to occur.

Fauna translocations play a pivotal role in the management of threatened species worldwide. However, the act of moving a host and its infracommunity of parasites from one ecosystem to another will inevitably disrupt host-parasite associations (Corn and Nettles, 2001; Telfer et al., 2010; Moir et al., 2012). Changes in the composition of the parasite community may significantly impact host health and population dynamics (Thompson et al., 2010). Likewise, translocation-associated stress may enhance susceptibility to parasite infection (Dickens et al., 2009), or promote recrudescence of latent disease (Adkesson et al., 2007). Unfortunately, translocation protocols rarely incorporate parasite monitoring and field studies that examine parasite prevalence pre- and post-translocation (e.g. Fairfield et al., 2016) are rare; thus we lack an understanding of how translocation impacts the host-parasite community (Northover et al., 2018). Additionally, antiparasitic drugs may be administered to a host, often without any attempt to evaluate treatment efficacy (Pedersen and Fenton, 2015). With demonstrated effects of antiparasitic drugs in target and non-target parasites, (e.g. Pedersen and Antonovics, 2013), there is the potential to negatively impact translocation outcomes.

The critically endangered woylie (syn. brush-tailed bettong *Bettongia penicillata*) is a small macropodid marsupial, which once occupied most of southern Australia. Adult woylies weigh between 1.0–1.6 kg, measure roughly 600 mm in length (nose to tail tip) and live for approximately 4–6 years in the wild (Richardson, 2012). Over the past decade, woylies have undergone greater than 90% population declines and are now restricted to three remaining wild indigenous populations (Kingston, Perup and Dryandra) in south-western Australia (Wayne et al., 2015). Both the Kingston and Perup woylie populations are located within the Upper Warren region, where woylie population declines were most pronounced (Wayne et al., 2013b). While various hypotheses have been proposed to explain the declines, their spatio-temporal pattern suggests the potential role of an infectious disease agent (Wayne et al., 2015). Woylie monitoring carried out immediately prior to, and during the declines, detected a high prevalence of skin disease, but despite investigation, a causative disease agent could not be found (Wayne et al., 2013b). Since then, the focus of investigation has shifted toward the potential role of other disease agents and *Trypanosoma* spp. have been of particular interest (Smith et al., 2008; Botero et al., 2013; Thompson et al., 2014; Godfrey et al., 2018).

Five *Trypanosoma* species have been detected in woylies: *T. copemani*, *T. vegrandis* (Botero et al., 2013), *T. noyesi* (Botero et al., 2016a), *Trypanosoma* sp. ANU2 and *T. gilletti* (Cooper et al., 2018a). Two distinct genotypes of *T. copemani* are formally recognised, *T. copemani* genotype 1 (G1) and *T. copemani* genotype 2 (G2) (Botero et al., 2013). *Trypanosoma copemani* G2 has been detected in woylie tissues by PCR, associated with tissue pathology, and is capable of invading cells *in vitro* (Botero et al., 2013; 2016b). Molecular studies have identified a higher prevalence of *T. copemani* and *Trypanosoma* spp. co-infection in a declining woylie population compared to a stable population (Smith et al., 2008; Botero et al., 2013; Thompson et al., 2014), and the extent of *Trypanosoma* co-infection was found to increase during the decline (Godfrey et al., 2018).

Various piroplasms (*Theileria* and *Babesia* spp.) have also been identified in Australian wildlife, including *Theileria penicillata* (Clark and Spencer, 2007) and a *Babesia* sp. (Paparini et al., 2012) in woylies. While piroplasms have been associated with significant clinical disease in domestic livestock and companion animals (Irwin, 2010), relatively little is known about their biology, transmission and clinical impact within native Australian wildlife (Clark et al., 2004). To date, *Th. penicillata* has been detected at high prevalence (> 80%) in several woylie populations (Rong et al., 2012), while *Babesia* sp. infection has been found at moderate prevalence (47%) in a single wild woylie population (Paparini et al., 2012).

As part of ongoing management of the woylie, remaining wild populations are periodically supplemented by translocations (Wayne et al., 2015). Like many threatened wildlife species, parasite monitoring is not routinely undertaken and there is little understanding of how translocations impact parasite community structure in woylies. In this longitudinal field-based study, we examined *Trypanosoma* spp. and piroplasms (*Theileria* and *Babesia* spp.) infecting woylies during two translocations to supplement existing woylie populations. Specifically, we aimed to investigate (i) how haemoparasite prevalence, *Trypanosoma* spp. richness and *Trypanosoma* spp. community composition varied over time and between different sites following translocation; and (ii) whether ivermectin treatment indirectly affects haemoparasite prevalence. We predicted that haemoparasite prevalence would decrease following translocation, in a similar manner to fauna reintroductions, where parasite loss commonly occurs (Torchin et al., 2003; MacLeod et al., 2010). As ivermectin targets nematodes and arthropods, we did not expect ivermectin to directly affect *Trypanosoma* spp. prevalence in woylies, but we predicted that the impact of ivermectin on arthropod vectors may indirectly reduce trypanosome transmission.

Methods

Study sites and trapping regime

During the first translocation (June 2014), 182 woylies were translocated from Perup Sanctuary, a 423-ha fenced reserve situated within the Tone-Perup Nature Reserve near Manjimup in Western Australia (34.2506°S, 116.1425°E), to supplement two unfenced wild populations [Walcott (n = 92) and Warrup East (n = 90)] within the Upper Warren region (Figure 22). Much of the native forests within this region are dominated by jarrah (*Eucalyptus marginata*), marri (*Corymbia callophyla*), and occasionally wandoo (*Eucalyptus wandoo*) (Wayne et al., 2015). During the translocation, woylies (equal ratio of male to female) were captured over three consecutive nights using Sheffield cage traps (Sheffield Wire Products, Welshpool, Western Australia) set along multiple transects (≤ 280 traps/night, 100 m spacing) at dusk, baited with universal bait (rolled oats, peanut butter and sardines), and cleared within three hours of sunrise the following day. Monitoring was carried out over three-four consecutive nights at one (July), three (September), six (December), ten (April 2015) and eleven (May 2015) months after translocation. All post-translocation trapping except May 2015 (grid trapping; 7 × 7 traps, 50 m apart) consisted of transect trapping (≤ 106 traps/night, 100-200 m spacing). Resident woylies within each destination site were also sampled prior to translocation (April and May 2014), using a grid (as above) and transect trapping (≤ 100 traps/night, 100-200 m spacing).

The second translocation (June 2015) involved the relocation of 69 woylies (47 male, 22 female) from various wild sites within the Upper Warren, into an unfenced site within Dryandra Woodland (32.8027°S, 116.8854°E). Dryandra is situated in the Western Australian wheatbelt, roughly 250 km north-east of the Upper Warren (Figure 22). The open-canopy woodlands in this region are dominated by wandoo, powderbark wandoo (*Eucalyptus accedens*), brown mallet (*Eucalyptus astringens*) and to a lesser extent marri (McArthur et al., 1977). During the translocation, woylies were captured (as above) over four consecutive nights. Monitoring was carried out over four consecutive nights at one (July), two (August), three (September), six (December), nine (March 2016) and twelve (June 2016) months following translocation. All post-release trapping except June 2016 (grid trapping, as above) consisted of transect trapping (100 traps/night, 200 m spacing). Resident woylies within the release site were also sampled prior to translocation (June 2015) using a grid and transects (as above).

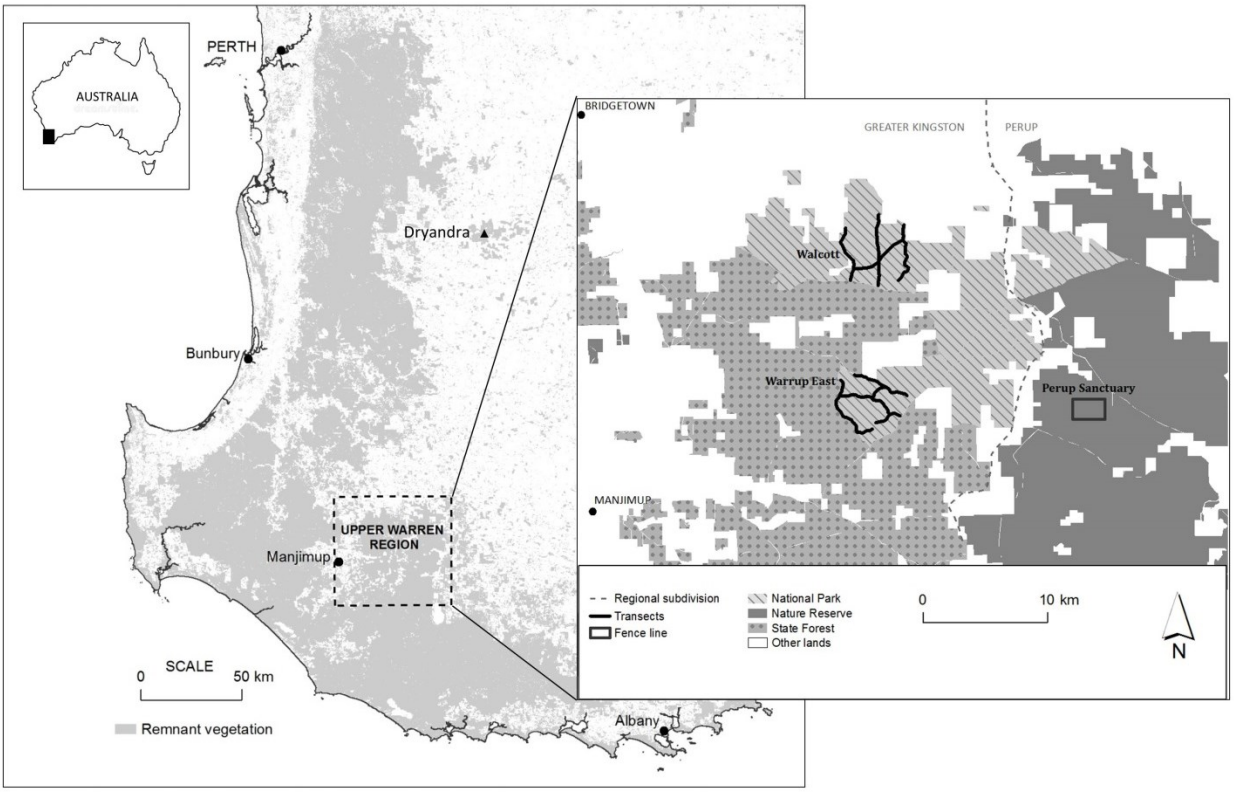

Figure 22: Map depicting the study sites within the south-west of Western Australia. The box (right), shows Walcott and Warrup East in relation to Perup Sanctuary. Dryandra is located approximately 250 km north-east of the Upper Warren region

Woylie identification and ivermectin treatment

Each woylie was identified with two uniquely numbered ear-tags. Prior to translocation, half of the woylies [Dryandra (n = 35); Walcott (n = 47); and Warrup East (n = 46)] were administered a single

subcutaneous dose of ivermectin (Ivomec® 0.2 mg/kg). While the ratio of treated male to female woylies was equal within the Upper Warren, there were a greater number of treated males (24/35) than females (11/35) within Dryandra.

Haemoparasite detection

Blood was collected from the lateral tail vein into MiniCollect® EDTA tubes (Greiner Bio-One, Frickenhausen, Germany) and frozen at -20 °C prior to processing. DNA was extracted from 200 µl aliquots of whole blood using the QIAmp 96 DNA blood kit as per the manufacturer's instructions (Qiagen, Hilden, Germany), with a final elution volume of 60 µl. A negative control was included in the extraction process.

DNA was screened for the presence of trypanosomes using a nested set of generic trypanosome primers (Table A4) designed by Maslov et al. (1996) and McInnes et al. (2011), which target the second fragment of the conserved *18S* rDNA gene region. Samples that tested positive for trypanosomes were subsequently screened for the presence of *T. copemani*, *T. vegrandis* and *T. noyesi* using specific nested primers designed by Botero et al. (2013) and McInnes et al. (2011) (Table A4). Although *T. gilletti* is described as a distinct species (McInnes et al., 2011), it is very closely related to *T. vegrandis* (Cooper et al., 2018a), and species-specific PCR primers for *T. vegrandis* are unable to discriminate between the two species; thus *T. gilletti* could only be identified by DNA sequencing (see below). All PCR reactions were performed as described by Cooper et al. (2018a), with the exception that 2 µl of DNA was added to a 24 µl master mix.

A nested PCR was used to screen for the presence of piroplasms using primers (Table A4) and conditions (Table A5) modified from Jefferies et al. (2007). Negative and positive controls were included in all PCR reactions, with the positive control derived from a known positive stock. Haemoparasites were identified based on expected band size for each species (Table A4). PCR products were purified using either the Agencourt AMPure PCR purification system, or by using an in-house filter tip method described by Yang et al. (2013).

Samples that tested positive for trypanosomes using the generic primer set, but negative for specific *Trypanosoma* species using clade-specific primers underwent Sanger sequencing at the *18S* rDNA locus (Table A5). All piroplasm-positive samples were sequenced. Phylogenetic analyses (Table A5) were conducted for putative new haemoparasites (see Results; Figures A2, A3 and A4).

Data analysis

For each haemoparasite species, prevalence of infection was calculated as the proportion of infected individuals with Jeffrey's 95% confidence intervals (CI) calculated assuming a binomial distribution. We estimated trypanosome infracommunity richness (polyparasitism) in terms of the number of *Trypanosoma* spp. (*T. copemani*, *T. vegrandis*, *T. noyesi*, *Trypanosoma* sp. ANU2 and *T. gilletti*) infecting a host. Piroplasms were excluded from our analyses of parasite infracommunity richness and community composition (see below) as piroplasm infection was only assessed in a subset of translocated woylies (n = 264) between June and September. To evaluate the impact of site (Dryandra, Walcott and Warrup East), time since translocation (TST) and ivermectin treatment (translocated woylies only), and their interactions, on the presence of each haemoparasite species, and on *Trypanosoma* spp. richness, we used generalised linear mixed-effects models (package *lme4*; Bates et al., 2015) in R (version 6.1.15; R Core Team, 2015). We could not run these models for *T. noyesi*, *Trypanosoma* sp. ANU2, *T. gilletti*, *Th. apogeana* genotype ANO2 or *Babesia* sp., as too few individuals were infected (prevalence 7.5%, 2.3%, 0.6%, 16.3% and 4.5%, respectively). All analyses were conducted separately for translocated and resident woylies. For each of our models, we tested for collinearity using variance inflation factors, and residuals were checked for normality/outliers to ensure model validity. Presence/absence data for all haemoparasites were modelled as binomial variables with a logit link function. Measures of *Trypanosoma* spp. richness were modelled as Poisson variables with a log link function. To account for repeated measures of individuals after translocation, woylie ID was included as a random effect.

Differences in *Trypanosoma* spp. community composition (presence/absence data only) between translocated and resident woylies were evaluated twice for each site; pre-translocation and six months after translocation. For each site at each time point, dissimilarities in community composition among hosts were estimated from presence/absence data with the Bray-Curtis coefficient. Differences in community composition between groups were visualised with non-metric multidimensional scaling plots, with the variance in mean rank order of similarity values within and between groups (R) tested for significance by a permutation technique applied to the pairwise dissimilarity matrix (one-way ANOSIM, implemented in PRIMER v. 6.0; Clarke and Gorley, 2006). The contribution of individual *Trypanosoma* spp. to differences in composition among host groups was assessed by averaging the Bray-Curtis coefficients for each species over all pairwise host combinations, using the SIMPER procedure in PRIMER.

Results

We analysed 1211 blood samples collected from 631 individual woylies (380 residents, 251 translocated) for the presence of haemoparasites (Tables A6, A7 and A8). Of these, 49 samples could not be identified to the species level for any of the haemoparasites, so they were excluded from our analyses and prevalence estimates. Pre-translocation woylie capture rates (total number of captures divided by the total trap effort; Wayne et al., 2013a) were much higher within Walcott (0.11) compared to Warrup East (0.05) or Dryandra (0.04). DNA sequencing revealed the presence of three novel haemoparasites, which are referred to here as *Trypanosoma* sp. ANU2 (MF459652; a putative new species genetically characterised by Cooper et al., 2018a), *Theileria apogeana* genotype ANO2 (MK182522), and *Bodo* sp. ANO4 (MK182523). For each novel haemoparasite, genetic similarity was compared to other previously described species (Table A9). *Trypanosoma gilletti* was also identified in woylies for the first time (Cooper et al., 2018a).

Overall, trypanosomes were detected in 55.3% of samples. Of the 1162 samples that could be identified to species (or putative species), *T. vegrandis* was the most common (32.5%), followed by *T. copemani* (23.9%), *T. noyesi* (7.5%), *Trypanosoma* sp. ANU2 (2.3%) and *T. gilletti* (0.6%). *Trypanosoma* sp. ANU2 and *T. gilletti* were only found in woylies originating from, or trapped within, the Upper Warren region (Tables A6, A7 and A8). Piroplasms were highly prevalent (93.6%) in the subset of samples we tested (translocated woylies only). *Theileria penicillata* was identified in 73.5% of samples, while *Th. apogeana* genotype ANO2 (16.3%) and *Babesia* sp. (4.5%) were comparatively rare. *Theileria apogeana* genotype ANO2 was common in translocated woylies originating from Perup Sanctuary, but rare outside of the reserve (Tables A6, A7 and A8). *Babesia* sp. was infrequently detected in woylies originating from Perup Sanctuary (n = 1, Walcott) and was predominantly isolated in woylies obtained from wild Upper Warren sites (Table A6). A *Bodo* species (*Bodo* sp. ANO4) was detected in a single woylie from Perup Sanctuary.

Effect of site on haemoparasite prevalence

In translocated and resident woylies, both *T. copemani* and *T. vegrandis* prevalence differed significantly between sites (Table 7). In translocated woylies, *T. copemani* prevalence was markedly higher in Dryandra than in Walcott or Warrup East, whereas *T. vegrandis* prevalence was much lower in Warrup East than the other sites (Figure 23A). In resident woylies, both *T. copemani* and *T. vegrandis* prevalence were on average highest within Walcott (particularly *T. vegrandis*) and lowest within Dryandra (Figure 23B). There were no differences in *Th. penicillata* prevalence in translocated hosts among sites (Table 7).

Table 7: Results from Generalised Linear Mixed Model analysis of factors influencing haemoparasite prevalence and *Trypanosoma* spp. polyparasitism in translocated and resident woylies. Significant ($P < 0.05$) results are highlighted in bold.

	Trypanosoma copemani			***Trypanosoma vegrandis***			***Trypanosoma* spp. polyparasitism**			***Theileria penicillata***		
	X^2	*df*	*P*	X^2	*df*	*P*	X^2	*df*	*P*	X^2	*df*	*P*
Translocated woylies												
Site	16.621	2	**< 0.001**	14.842	2	**0.001**	10.435	2	**0.005**	2.923	2	0.232
TST (time since translocation)	4.934	1	**0.026**	28.180	1	**< 0.001**	17.020	1	**< 0.001**	2.462	1	0.117
Ivermectin	0.186	1	0.666	0.632	1	0.426	0.061	1	0.804	1.595	1	0.207
Site:TST	2.685	2	0.261	16.827	2	**< 0.001**	3.921	2	0.141	9.611	2	**0.008**
Site:Ivermectin	3.557	2	0.169	5.340	2	0.069	8.927	2	**0.012**	1.225	2	0.542
TST:Ivermectin	0.005	1	0.946	3.944	1	**0.047**	1.146	1	0.284	0.006	1	0.938
Site:TST:Ivermectin	0.657	2	0.720	1.924	2	0.382	–	–	–	–	–	–
Resident woylies												
Site	29.158	2	**< 0.001**	43.527	2	**< 0.001**	65.988	2	**< 0.001**	–	–	–
TST	6.487	1	**0.011**	0.933	1	0.334	0.006	1	0.940	–	–	–
Site:TST	2.744	2	0.254	2.757	2	0.252	1.740	2	0.419	–	–	–

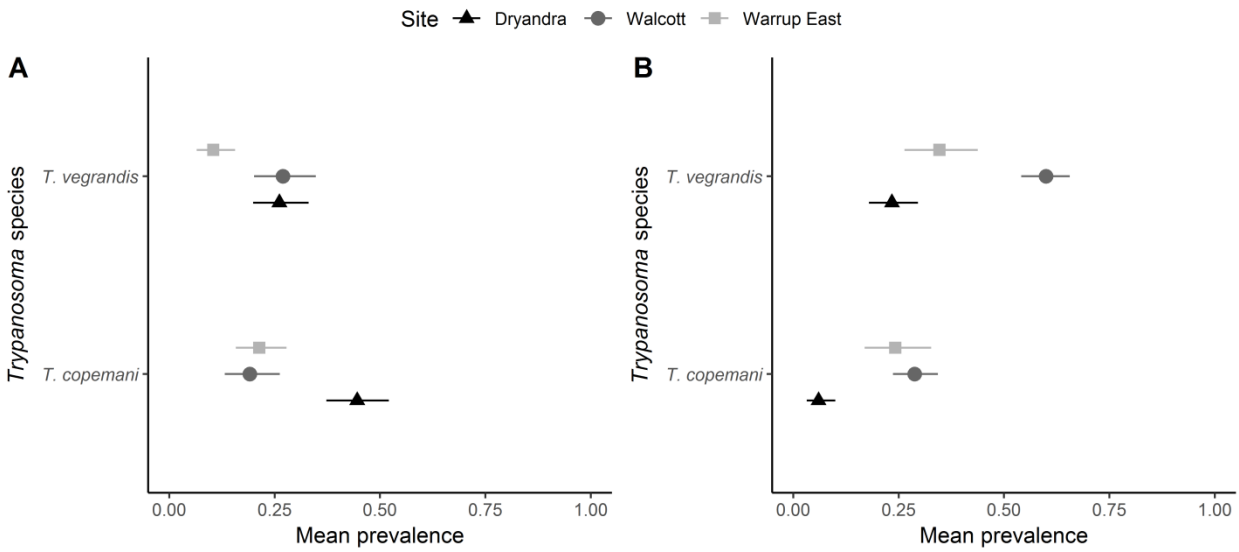

Figure 23: The overall impact of site on *Trypanosoma* spp. prevalence (with 95% CI) in (A) translocated and (B) resident woylies

Effect of time since translocation on haemoparasite prevalence

In translocated woylies, *T. copemani* prevalence increased in all sites with TST, while *T. vegrandis* prevalence increased in all sites except Warrup East, leading to a significant interaction between TST and site for this species (Table 7, Figure 24A-B). In resident woylies, the prevalence of *T. copemani*, but not *T. vegrandis*, decreased with TST (Table 7, Figure 24C-D).

In translocated woylies, there was a significant interaction between TST and site for *Th. penicillata* (Table 7), with prevalence increasing over time in Walcott and Warrup East, while remaining high within Dryandra (Figure 25).

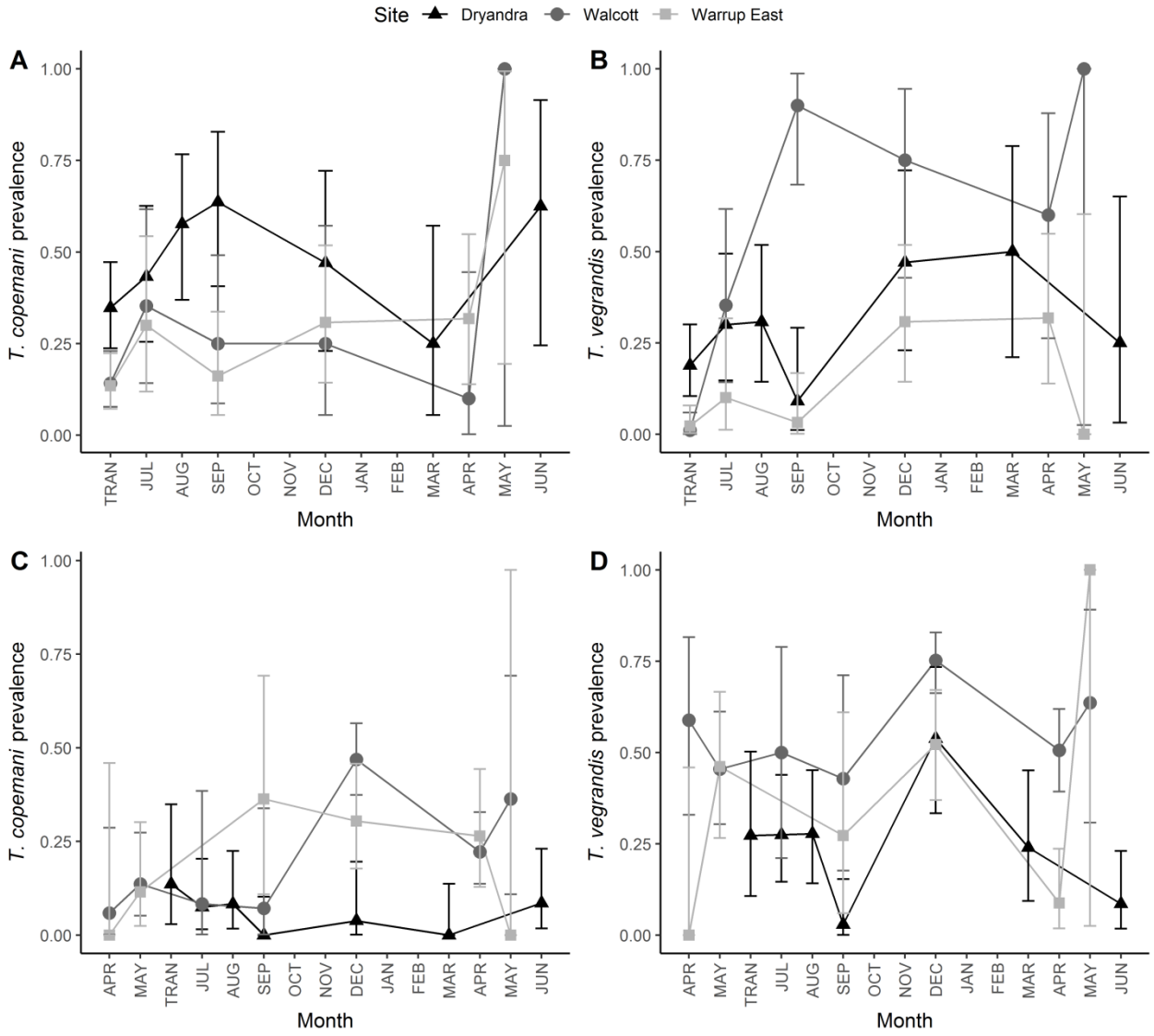

Figure 24: *Trypanosoma* spp. prevalence over time (with 95% CI) in translocated (A, B) and resident (C, D) woylies. TRAN: time of translocation.

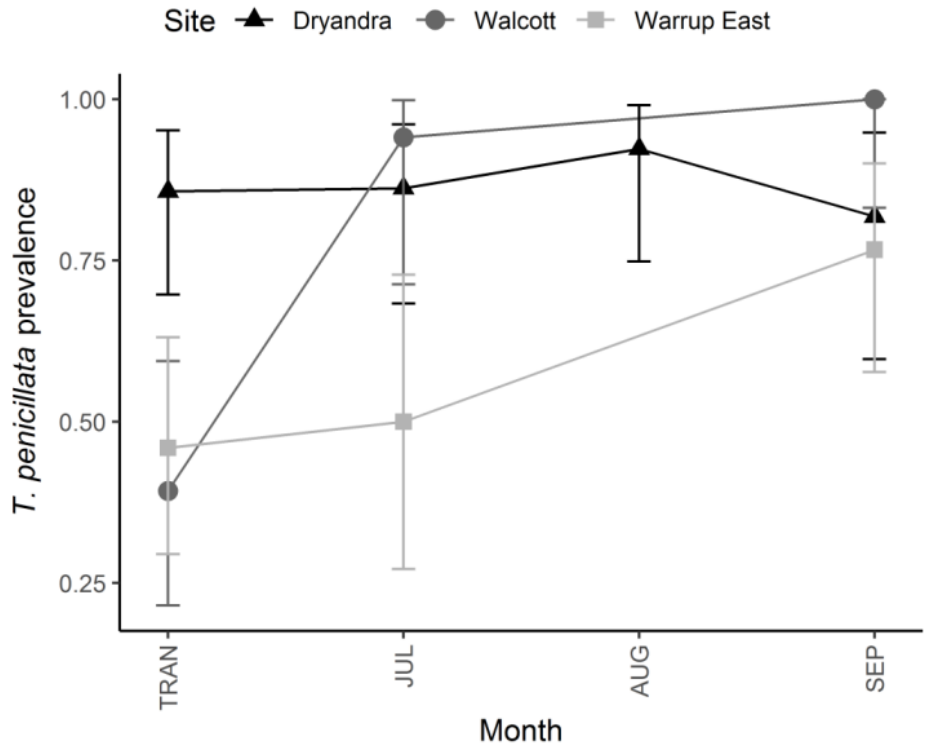

Figure 25: *Theileria penicillata* prevalence over time (with 95% CI) in translocated woylies. TRAN: time of translocation.

Haemoparasite community structure in translocated and resident woylies

In translocated and resident woylies, *Trypanosoma* spp. richness differed significantly between sites (Table 7, Figure 26); being highest (on average) within Walcott. In translocated woylies, *Trypanosoma* spp. richness was higher in Dryandra compared to Warrup East, while in resident woylies, Warrup East had higher species richness than Dryandra. In *Trypanosoma*-positive woylies, *Trypanosoma* spp. polyparasitism was identified in 24% of cases. The maximum number of haemoparasites (including piroplasms) identified in a single host was four (Walcott translocated group; Table 8). In translocated woylies, *Trypanosoma* spp. richness increased with TST (Table 7, Figure 26A) and the prevalence of haemoparasite co-infection increased from 40% to 63% following translocation. There was no effect of TST on *Trypanosoma* spp. richness in resident woylies (Table 7).

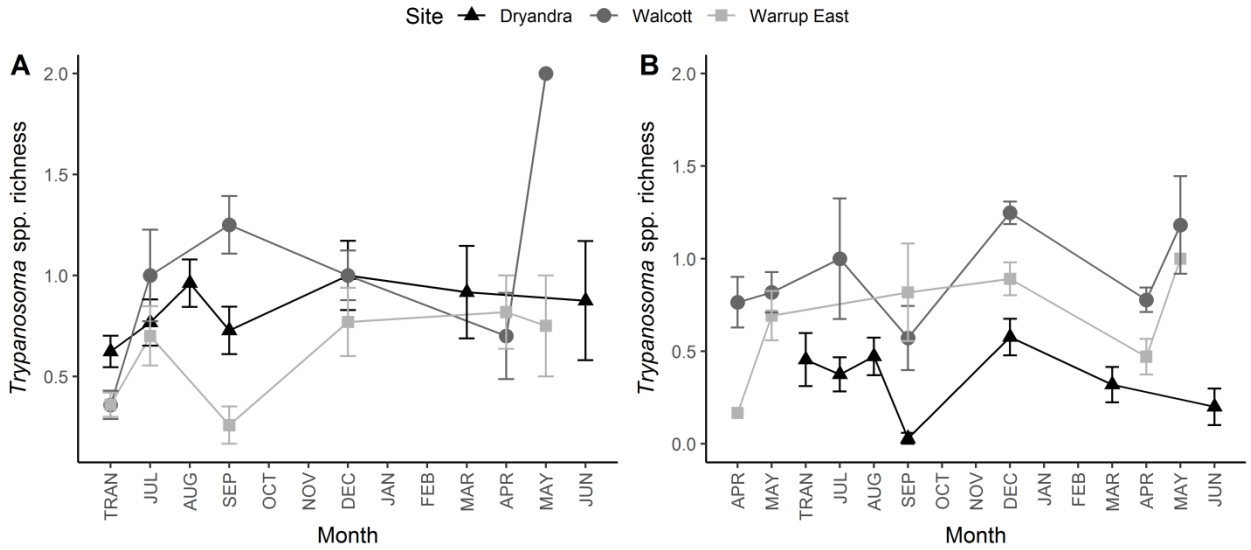

Figure 26: *Trypanosoma* spp. infracommunity richness (polyparasitism) over time (with standard error bars) in translocated (A) and resident (B) woylies. Error bars represent one standard error. TRAN: time of translocation

Prior to translocation, *Trypanosoma* spp. community composition differed significantly between translocated and resident woylies in Walcott and Warrup East, but not Dryandra; this dissimilarity (84%) was mainly associated with differences in *T. vegrandis* prevalence (Tables 6A, 7A and 8A). Six months after translocation, there was a significant difference between translocated and resident woylies within Dryandra, but not the Upper Warren sites (Figure 27). This dissimilarity (61%) was largely attributed to the prevalence of *T. copemani*, which was significantly higher in translocated compared to resident woylies at all time points within Dryandra (Figure 28).

Table 8: The type and overall number of cases of haemoparasite coinfection identified in translocated and resident woylies from each site.

	Dryandra	Walcott	Warrup East	Total
Translocated woylies				
T. copemani/*T. vegrandis*/*T. noyesi*	0	2	0	2
T. copemani/*T. vegrandis*	22	11	8	41
T. copemani/*T. noyesi*	0	5	3	8
T. copemani/*T.* ANU2	0	3	3	6
T. vegrandis / *T. gilletti*	0	0	5	5
T. vegrandis/*T. noyesi*	1	2	0	3
T. noyesi/*T.* ANU2	0	0	1	1
Th. penicillata/*T. copemani*/*T. vegrandis*/*T. noyesi*	0	1	0	1
Th. ANO2/*T. copemani*/*T. vegrandis*/*T. noyesi*	0	1	0	1
Th. penicillata/*T. copemani*/*T. vegrandis*	9	8	0	17
Th. penicillata/*T. copemani*/*T. noyesi*	0	3	2	5
Th. penicillata/*T. vegrandis*/*T. noyesi*	0	2	0	2
Th. ANO2/*T. copemani*/*T.* ANU2	0	1	1	2
Th. penicillata/*T. copemani*	41	3	8	52
Th. penicillata/*T. vegrandis*	13	13	0	26
Th. penicillata/*T. noyesi*	4	3	7	14
Th. penicillata/*T.* ANU2	1	1	1	3
Babesia/*T. copemani*/*T. vegrandis*	1	0	0	1
Babesia/*T. copemani*	5	0	0	5
Babesia/*T. noyesi*	1	0	0	1
Th. ANO2/*T. copemani*	1	1	2	4
Th. ANO2/*T. vegrandis*	0	0	1	1
Th. ANO2/*T. noyesi*	0	1	3	4
Th. ANO2/*T.* ANU2	0	0	1	1
Total	99	61	46	189
Resident woylies				
T. copemani/*T. vegrandis*/*T. noyesi*	0	3	0	3
T. vegrandis/*T. noyesi*/*T.* ANU2	0	1	0	1
T. copemani/*T. vegrandis*	9	50	6	65
T. copemani/*T. noyesi*	0	1	2	3
T. copemani/*T.* ANU2	0	1	0	1
T. vegrandis/*T. gilletti*	0	1	1	2
T. vegrandis/*T. noyesi*	0	5	2	7
T. vegrandis/*T.* ANU2	0	0	1	1
Total	9	62	12	83

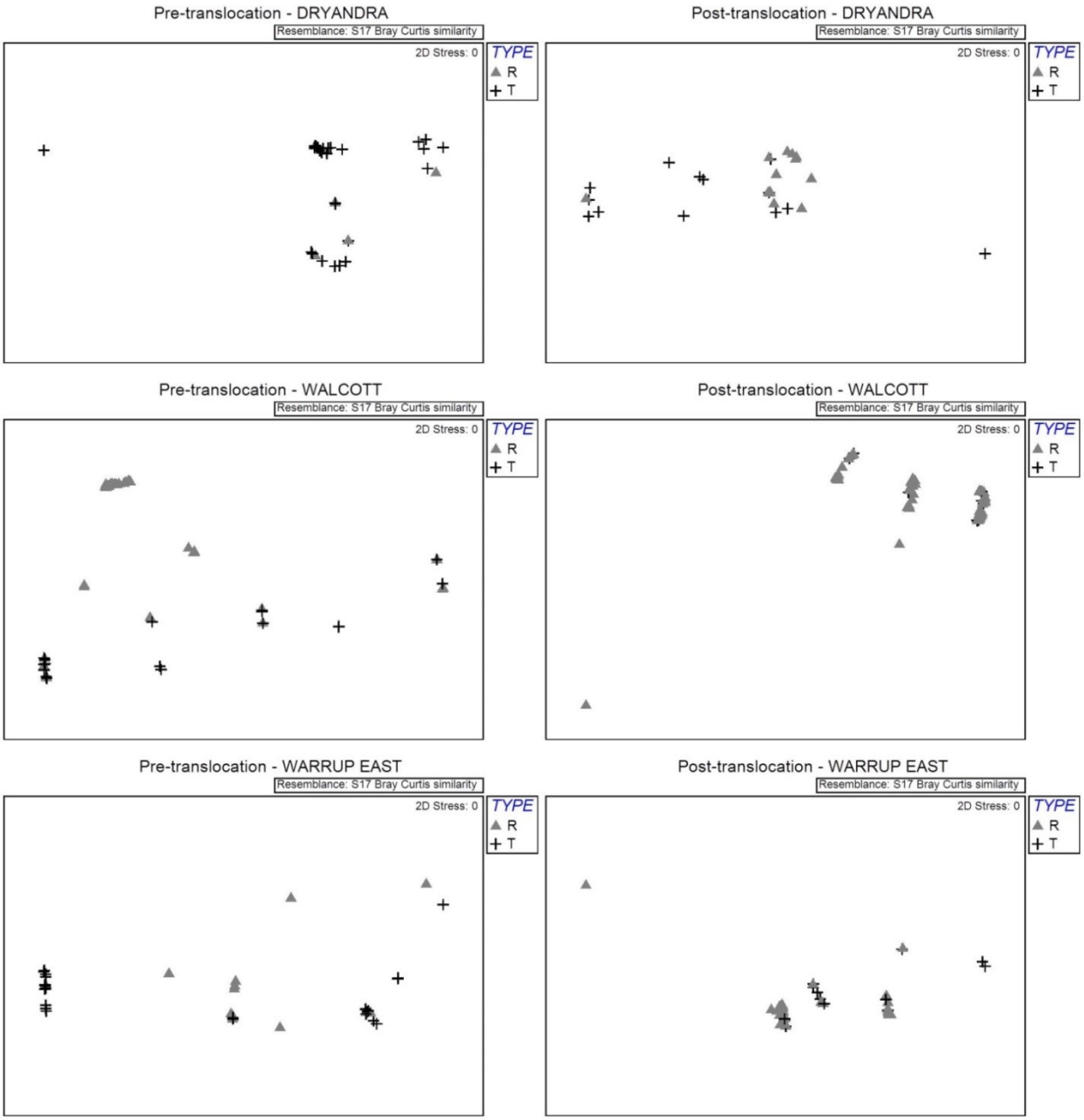

Figure 27: Non-metric multidimensional scaling plots of parasite communities in translocated (TYPE T) and resident (TYPE R) woylies pre-translocation (left; all time points prior to and including the point of translocation) and six months post-translocation (right).

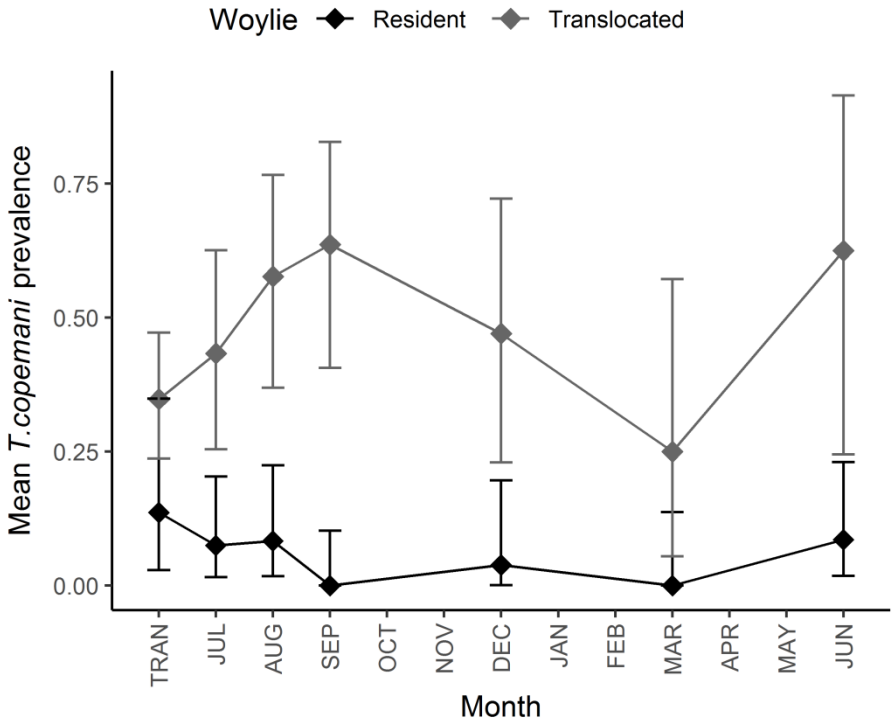

Figure 28: *Trypanosoma copemani* prevalence over time (with 95% CI) in translocated *versus* resident woylies within Dryandra. TRAN: time of translocation

Effect of ivermectin treatment on haemoparasite prevalence and community composition

We did not detect a significant effect of ivermectin treatment on the prevalence of any haemoparasite species in translocated woylies, although there was a marginally significant interaction (P = 0.047) between ivermectin treatment and TST for *T. vegrandis* (Table 7), with *T. vegrandis* prevalence being lower in treated compared to untreated hosts from September onwards (Figure A5). A significant interaction between ivermectin treatment and site was also found for *Trypanosoma* spp. polyparasitism (Table 7). *Trypanosoma* species richness was lower in treated compared to untreated woylies at all time points within Dryandra, whereas species richness was on average higher in treated woylies within Warrup East (Figure A6).

Discussion

During this study, the response of haemoparasites following translocation was highly site-specific, with major changes in haemoparasite prevalence occurring within the first few months after translocation. *Trypanosoma* spp. richness and the prevalence of haemoparasite co-infection increased following translocation. *Trypanosoma* spp. community composition became more similar over time within Walcott and Warrup East, but not Dryandra, where translocated woylies maintained a significantly higher prevalence of *T. copemani* infection compared to resident woylies. Ivermectin

treatment had no significant effect on haemoparasite prevalence. We identified several new haemoparasites infecting woylies from the Upper Warren and Dryandra region. To our knowledge, this is the first published report of a *Bodo* sp. isolated from a marsupial blood sample; however *Bodo* sp. have been found previously in mammalian blood and tissue (Botero et al., unpublished data).

Haemoparasite differences among sites

An important observation from this study is that haemoparasite prevalence, *Trypanosoma* spp. richness and *Trypanosoma* spp. community composition differed significantly between sites. Overall, Walcott was the most parasite-rich site, whilst Dryandra was comparatively parasite-poor; though translocated hosts within Dryandra maintained a high prevalence of haemoparasite infection (particularly *T. copemani*) following translocation. Differences in environmental conditions, woylie population density, contact with cohabiting species and the presence/absence of appropriate vectors may all have contributed towards the differences in haemoparasite prevalence and community composition and the site specific response of haemoparasites following translocation. As the translocations to the Upper Warren and Dryandra occurred 12 months apart, we may also be observing temporal fluctuations in parasite prevalence.

We also identified apparent site specificity of certain haemoparasites during this study. The absence of *Trypanosoma* sp. ANU2 in Dryandra resident woylies (and translocated hosts within Dryandra post-release) suggests that this trypanosome is specific to the Upper Warren region only. The absence of *Trypanosoma* sp. ANU2 in cohabiting marsupials (the brushtail possum *Trichosurus vulpecula hypoleucus* and the chuditch *Dasyurus geoffroii*) within Dryandra, but not the Upper Warren (Northover et al., unpublished data) supports this theory. Site differences in the prevalence of *T. gilletti*, *Th. apogeana* genotype ANO2 and *Babesia* were also observed, but difficult to interpret given the low prevalence of these parasites. Site differences in the type and prevalence of parasites (and their vectors) highlights the importance of parasite monitoring, particularly with regard to the inclusion of cohabiting host species within the release site, to gain a greater understanding of the parasites a translocated host might acquire (or potentially lose) following translocation. Likewise, incorporation of a control site that monitors host-parasite dynamics in the absence of translocation would be useful for identifying normal fluctuations in parasite prevalence, however may not be realistic when working with threatened host species.

Changes in haemoparasite structure over time

In translocated woylies, significant changes in parasite prevalence occurred within the first few months after translocation, with *Trypanosoma* spp. richness and the prevalence of haemoparasite co-infection increasing. As follow-up monitoring of reintroduced populations commonly report parasite loss during translocation (Torchin et al., 2003; MacLeod et al., 2010; Fairfield et al., 2016; Portas et al., 2016), this outcome was unexpected; though supplementing pre-existing populations might increase the likelihood of parasite persistence particularly when resident conspecifics are infected with the same parasites. For haemoparasites infecting a host, it would seem inevitable that they will be translocated along with their host given their location in the blood stream; however, their persistence following translocation is usually dependent upon the presence of an appropriate arthropod vector and sufficient host density to enable parasite transmission. In the case of Walcott and Warrup East, it would appear that resident woylie density and the prevalence of infection (and vectors) within each site were sufficient to maintain, and even enhance, parasitaemia in translocated woylies after translocation; which resulted in *Trypanosoma* spp. community composition in translocated and resident woylie groups becoming more similar over time. Alternatively, enhanced parasitaemia may be the result of recrudescence of latent infections (e.g. secondary to stress), as has been reported for piroplasms in other species (Alvarado-Ryback et al., 2016). With the exception of *T. copemani* G2 (see below), however, there is no evidence to suggest that recrudescence of latent trypanosome infection occurs in woylies.

Within Dryandra, *Trypanosoma* spp. community composition diverged after translocation as translocated woylies maintained a significantly higher prevalence of *T. copemani* infection compared to residents. The introduction of translocated woylies with a much higher prevalence of *T. copemani* did not appear to affect infection prevalence in residents, which suggests that the prevalence of trypanosome vectors within Dryandra is low and/or contact with trypanosome-infected vectors/conspecifics occurs infrequently. Differences in *T. copemani* prevalence may be associated with the specific genotype of *T. copemani* infecting translocated and resident woylies. A high number of woylies infected with *T. copemani* G2 have been identified from the Upper Warren region (Cooper et al., 2018a), the origin of the translocated animals. Measurable parasitaemia associated with active *Trypanosoma* infection is typically transitory (Campos et al., 2010), but it has been proposed that *T. copemani* G2 is capable of invading tissues, replicating and sporadically re-entering the blood stream (Botero et al., 2013; Thompson et al., 2013; Botero et al., 2016b) in a similar manner to *Trypanosoma cruzi*. If this is the case and if *T. copemani* G2 was present in translocated woylies, this could explain the consistently high detection in these animals. Alternatively, translocation-associated stress may

have promoted reactivation of infection, thereby increasing the number of trypomastigotes entering the blood (and the likelihood of detecting infection in the present study). Reactivation of chronic *T. cruzi* infection has been linked with immunosuppression in humans (Perez et al., 2015). While a similarity to *T. cruzi* is a plausible explanation, a recent study looking into the proposed intracellular life-cycle of *T. copemani* showed no evidence that *T. copemani* G2 can actually replicate in cells (Cooper et al., 2018b). However, there is clearly an interaction between *T. copemani* G2 and the host that is not entirely understood. *Trypanosoma copemani* infection seems to be more persistent than other *Trypanosoma* infections (Botero et al., 2013; Thompson et al., 2014; Godfrey et al., 2018) and ultimately leads to a decrease in cell health *in vitro* (Cooper et al., 2018b). In animals that are stressed this interaction could make parasite infection more difficult to eliminate.

The efficacy of ivermectin treatment

The significant interaction between ivermectin treatment and site for *Trypanosoma* spp. polyparasitism was surprising. However, contrasting effects in different sites limits our ability to interpret the biological significance of this result. Likewise, the importance of the interaction between ivermectin treatment and TST for *T. vegrandis* is unclear, given the small effect we observed. If treatment-induced changes in *Trypanosoma* spp. polyparasitism and *T. vegrandis* prevalence are biologically significant however, this could have important consequences for host health given the association between *T. copemani*, *Trypanosoma* spp. co-infection, and declining woylie populations (Smith et al., 2008; Botero et al., 2013; Thompson et al., 2014; Godfrey et al., 2018); particularly if *T. vegrandis* imposes some form of competitive dominance over *T. copemani*, as previous studies have suggested (Lymbery and Thompson, 2012; Thompson et al., 2014). Further manipulative experiments that evaluate the effect of ivermectin on trypanosomes, and arthropod vectors, may be warranted if ivermectin is to be used in future woylie translocations. Likewise, studies that examine changes in the haemoparasite community in conjunction with measures of host health would assist with determining the biological significance of haemoparasite infection in woylies.

Novel haemoparasite species

Lastly, we identified three novel haemoparasites infecting woylies. *Trypanosoma* sp. ANU2 was first isolated from woylies during this study, and has been phylogenetically characterised by Cooper et al. (2018a). The *Babesia* sp. we identified (*Babesia* sp. 28, JQ682873) has been formerly described in woylies (Paparini et al., 2012), but was reported to be most similar (98.4%; using a 527 bp alignment) to *Babesia occultans*, the causative agent of cattle babesiosis (Gray and de Vos, 1981). Our results (using a larger 693 bp alignment) suggest that this *Babesia* sp. is most similar to other marsupial-

derived species. This is the first report of *Th. apogeana* genotype ANO2 in woylies, and this piroplasm is most similar to the recently described *Th. apogeana* from *Ixodes tasmani* on a dog in Tasmania (Greay et al., 2018). Previous phylogenetic analysis (Greay et al., 2018) clustered *Th. apogeana* within a clade of *Theileria* spp. found in Australian marsupials, which was also the case here. While the vector of *Th. apogeana* genotype ANO2 in woylies is unknown, *Ixodes australiensis* and *Ixodes myrmecobii* were the only species of tick identified on woylies infected with *Th. apogeana* genotype ANO2 at the time of, or in the month preceding sample collection (Northover et al., unpublished data).

The detection of *Bodo* sp. DNA in the blood of a woylie was surprising given that the group is comprised of free-living species. However, environmental contamination cannot be ruled out, in a similar way to how *Bodo* sp. DNA was discovered in bats (Szoke et al., 2017). Woylies are ground dwelling herbivores feeding mostly on underground fungi (Richardson, 2012), an environment in which *Bodo* sp. thrive. Thus, the potential transfer of DNA through the intestinal mucosa into the host's blood circulation could be a likely consequence. However, the theory of trypanosomes evolving from free-living *Bodo* spp. (Szoke et al., 2017) also needs to be considered.

Future considerations for evaluating co-infection

It is worth noting that our molecular screening technique may have limited our ability to accurately detect mixed infections, leading to an underestimate of the extent of polyparasitism in our study. For example, species-specific trypanosome primers may identify the *Trypanosoma* species with the greatest parasitaemia. During this study, intermittent detection of haemoparasites was common and we often observed changes in the predominant species of haemoparasite and the presence/absence of co-infection. For parasites that can only be identified *via* DNA sequencing (e.g. *T. gilletti*) it is likely that their true prevalence is under-estimated. The use of alternative screening techniques such as targeted amplicon next generation sequencing (NGS), which can be superior for detecting co-infection (Cooper et al., 2018a) could be used to examine polyparasitism within a host and benefit translocation planning through the identification of potentially harmful *Trypanosoma* genotypes (e.g. *T. copemani* G2) within certain populations. Given the potential role of trypanosomes in the recent decline of the woylie, which resulted in the conservation status of this species being elevated to critically endangered, NGS technology may be more adept for monitoring perturbations to the parasite community following translocation in greater detail, thus enabling more informed decisions for the management of woylies and their parasite taxa during future translocations. Despite this limitation, we still detected a relatively high (40–63%) instance of polyparasitism in our study.

Conclusions

As fauna translocations form an integral part of fauna conservation and management, consideration needs to be given to the biological implications of altering the parasite community within a host. To our knowledge, this is the first study to evaluate haemoparasite prevalence and composition in translocated and resident animals following translocation. The results from our study suggest that major changes in the host-parasite community happen relatively quickly (within the first three months). The unexpected observation that *Trypanosoma* spp. richness and the prevalence of haemoparasite co-infection increased after translocation, suggests that the outcome of fauna supplementations may differ from reintroductions where parasite loss typically occurs. Our study adds further weight to the idea that the response of haemoparasites following translocation can be highly site-specific and further understanding of what site characteristics drive these responses would improve our ability to predict how parasites may respond to translocation. Future studies that examine haemoparasites infecting subsequent generations of woylies would be useful for identifying population level haemoparasite changes, and separating within-individual effects (e.g. prevalence fluctuating with factors such as age, stress, condition, breeding status, season) from translocation-associated effects, which we were unable to do here. Importantly, the value of long-term parasite monitoring and undertaking well-designed scientific studies that examine parasite dynamics following experimental manipulation cannot be over-emphasised, particularly with regard to the collection of comprehensive mark-recapture data that can detect changes in the parasite community over time. Lastly, consideration needs to be given to the effects of antiparasitic drug treatment, which as observed here may be indirect, with potentially adverse consequences for host health. Studies that utilise next generation sequencing rather than traditional PCR assays and Sanger sequencing will be more adept at accurately quantifying changes in the parasite infracommunity during translocation.

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Availability of data and materials

Sequences have been submitted to the GenBank sequence database under the accession numbers MK182522 and MK182523. The raw datasets used and/or analysed during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

ASN, KM, RCAT, AJL, SSG and AFW played an instrumental role in the design and implementation of this study. ASN, SK, AJL, SSG, AFW and RCAT undertook field work and sample collection. SK, CC and LP performed all molecular laboratory work. ASN, SSG and AJL carried out data analysis. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This research was undertaken in collaboration with the Department of Biodiversity, Conservation and Attractions (DBCA) under DBCA Scientific Licenses (Regulation 4: written notice of lawful authority; and 17: licence to take fauna for scientific purposes) and with approval from the Murdoch University Animal Ethics Committee (RW2659/14).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Chapter Six

Parasite infection and ivermectin treatment have minimal impact on woylie health during fauna translocation

6.1 Parasite infection and ivermectin treatment have minimal impact on woylie health during fauna translocation

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Abstract

Parasites of threatened wildlife such as the woylie (*Bettongia penicillata*) are of interest during translocation given their potential to impact host health and survival. Assessment of host health in response to parasite infection and antiparasitic drug treatment during translocation is thus vital for optimising translocation outcomes. During this longitudinal field-based study, we aimed to (a) explore how different parasite taxa impact the health of translocated and resident woylies [as measured using body condition, packed cell volume and total plasma protein (TPP)] during two fauna translocations to supplement existing woylie populations at three different sites (Dryandra, Walcott and Warrup East) in south-western Australia; and (b) determine whether ivermectin treatment provides any benefit to the health of translocated hosts. Prior to translocation, and at each recapture following translocation (≤12 months), we monitored gastrointestinal, ectoparasite and *Trypanosoma* spp. infection in translocated and resident woylies. Destination site and time since translocation had the strongest effect on body condition in translocated and resident woylies following translocation. Body condition increased over time in both groups but varied between sites. The presence of coccidia during the first three months following translocation and higher *Strongyloides*-like egg counts were associated with lower body condition in translocated woylies, while strongyle infection and higher parasite infracommunity richness were associated with lower body condition in resident woylies. Ivermectin treatment had no significant effect on body condition in translocated woylies during this study.

Introduction

The term parasite often has negative connotations. Parasites are capable of adversely influencing host health, and the disease risks associated with translocating wildlife are widely recognised (Viggers et al., 1993; Cunningham, 1996; Kock et al., 2010; Sainsbury and Vaughan-Higgins, 2012). Conversely, there is an emergent body of evidence to suggest that parasites constitute a vital component of biodiversity (Hudson et al., 2006), and their conservation during translocation may enhance host immunity (Pizzi, 2009; McGill et al., 2010; Boyce et al., 2011) and improve translocation outcomes (Rideout et al., 2016). Unfortunately, we know little about the parasite communities infecting wildlife, or the way in which parasites affect host health in response to management practices such as translocation and antiparasitic drug treatment.

Translocating a host from one ecosystem to another will inevitably disrupt parasite community structure with variable outcomes on host health (Northover et al., 2018a). Stress associated with translocation may induce immunosuppression (Hing et al., 2017) and increase susceptibility to parasite infection (Dickens et al., 2009) or stimulate recrudescence of latent disease (Adkesson et al., 2007). In some species, certain parasites (e.g. coccidia) are known to cause disease in their host during the stress of translocation and are often considered a high risk hazard (Sainsbury and Vaughan-Higgins, 2012). The administration of antiparasitic drugs will likewise disrupt the host-parasite balance. Although prophylactic drug treatment may be required for the successful translocation of some species (e.g. McGill et al., 2010), antiparasitic drugs are often administered without clear purpose, or any attempt to determine the outcome of treatment (Pedersen and Fenton, 2015). In studies that do evaluate treatment efficacy, effects of treatment in target parasites may be transitory (e.g. Almberg et al., 2012) or provide no benefit to host health (e.g. Northover et al., 2017). Of particular concern, is that indirect effects of antiparasitic drugs have been identified (e.g. Pedersen and Antonovics, 2013), which reduces our ability to predict how treatment will impact the parasite community, and hence host health.

The critically endangered woylie or brush-tailed bettong *Bettongia penicillata* (Gray), which was once abundant across southern Australia, has suffered profound population declines over the past decade and is now extant within only three wild populations in the south-western Australia (Wayne et al., 2015). With the exception of trypanosomes, where *Trypanosoma copemani* has been associated with tissue pathology and woylie population declines (Smith et al., 2008; Botero et al., 2013; Thompson et al., 2014; Godfrey et al., 2018), we know little about the potential pathogenicity of other parasites infecting woylies (Pacioni et al., 2015). *Paraustrostrongylus* sp.

nematodes have been associated with disease in a woylie (Northover et al., 2018b), and other nematodes (e.g. *Strongyloides* spp.) have been associated with morbidity and mortality in macropods (Winter, 1958; Speare et al., 1982). Coccidian oocysts have been detected in woylie faecal samples in the absence of clinical disease (Northover et al., 2017); while uncommon, coccidial disease has been documented in other free-ranging macropods secondary to environmental stressors (Barker and Harrigan, 1972). Ectoparasites are also frequently found on apparently healthy woylies, though skin disease in association with particularly high ectoparasite numbers was observed during the woylie decline (Wayne et al., 2013b) and has been reported sporadically thereafter (e.g. Northover et al., 2018b).

As for many threatened species, fauna translocations play a pivotal role in woylie population management. Regrettably, translocation protocols do not routinely incorporate assessment of host health in response to parasite infection, thus we lack an understanding of which parasite species are most influential in shaping host health during translocation. In the field, various measures of body condition may be used to infer the health of an individual, and when evaluating condition-infection relationships, it has been recommended that multiple measures of condition are used (Sanchez et al., 2018). Hing et al. (2017) found a significant negative relationship between body condition and physiological stress (measured using faecal cortisol metabolites) in woylies following translocation, and studies on other host species have reported links between translocation, stress and parasitic disease (Ellis et al., 1996; McGill et al., 2010; Sainsbury and Vaughan-Higgins, 2012).

Monitoring wildlife in remote locations can restrict our ability to perform more complex health assessments, however limited haematological analyses [packed cell volume (PCV) and total plasma protein (TPP)] can be performed in the field, and may be used to complement body condition measures to give a greater understanding of the overall effects of parasites on host health. Reduced PCV has been associated with trypanosome infection, strongyle and *Strongyloides* spp. infection and heavy flea/tick infestations in various marsupials (Holz, 2003; Vogelnest and Portas, 2008; McInnes et al., 2011). Reduced TPP may be associated with malnutrition or chronic disease (Reiss et al., 2015).

In this long-term (≤12 months) longitudinal field-based study we aimed to (a) explore how different parasite taxa (gastrointestinal parasites, ectoparasites and *Trypanosoma* spp.) impact the health of translocated and resident woylies (as measured using body condition, PCV and TPP) during translocation; and (b) determine whether ivermectin treatment provides any benefit to the

health of treated hosts. We hypothesised that parasite infection would be negatively associated with host health, particularly in translocated woylies. As translocation-associated stress is reported to be most pronounced within the first few months following translocation (e.g. Franceschini et al., 2008; Patt et al., 2012) we predicted that changes in body condition associated with parasite infection would also manifest within this time frame.

Methods

Study sites and trapping regime

Two translocations were undertaken in south-western Australia. In June 2014, 182 woylies were translocated from a fenced reserve (Perup Sanctuary) to supplement two wild populations (Walcott n = 92; Warrup East n = 90) within the Upper Warren region near Manjimup (34.2506 °S, 116.1425 °E; Figure 29). Native forests within this region are generally dominated by jarrah (*Eucalyptus marginata*), marri (*Corymbia callophyla*) and in some places wandoo (*Eucalyptus wandoo*) (Wayne et al., 2015). During the translocation, woylies (equal ratio of male to female) were captured over three consecutive nights using Sheffield cage traps (Sheffield Wire Products, Welshpool, Western Australia) set along multiple transects (≤280 traps total/night, 100m spacing) at dusk, baited with universal bait (rolled oats, peanut butter and sardines) and cleared within three hours of sunrise each day. Monitoring was carried out over three-four consecutive nights at one (July), three (September), six (December), ten (April 2015) and eleven (May 2015) months following translocation. All post-translocation trapping except May 2015 (grid trapping; 7 x 7 traps, 50 m apart) consisted of transect trapping (≤106 traps/night, 100-200 m spacing). Resident woylie populations were also sampled prior to translocation (April and May 2014), using a grid (as above) and transect trapping (≤100 traps/night, 100-200 m spacing).

In June 2015, 69 woylies (47 male, 22 female) were translocated from various unfenced wild sites within the Upper Warren, into an unfenced wild site within Dryandra (32.8027 °S, 116.8854 °E), which is located roughly 250 km north east of the Upper Warren (Figure 29). The open-canopy woodlands consist of wandoo, powderbark wandoo (*Eucalyptus accedens*), brown mallet (*Eucalyptus astringens*) and to a lesser extent marri (McArthur et al., 1977). During, prior to and following translocation, woylies were captured (as described above) over four consecutive nights. Monitoring was carried out at one (July), two (August), three (September), six (December), nine (March 2016) and twelve (June 2016) months following translocation. All post-translocation trapping except June 2016 (grid trapping, as above) consisted of transect trapping (100 traps/night, 200 m

spacing). Resident woylies were also sampled prior to translocation (June 2015) using a grid and transects. Resident woylie density within Dryandra is estimated to be lower than both Upper Warren sites, particularly Walcott (Northover et al., 2019).

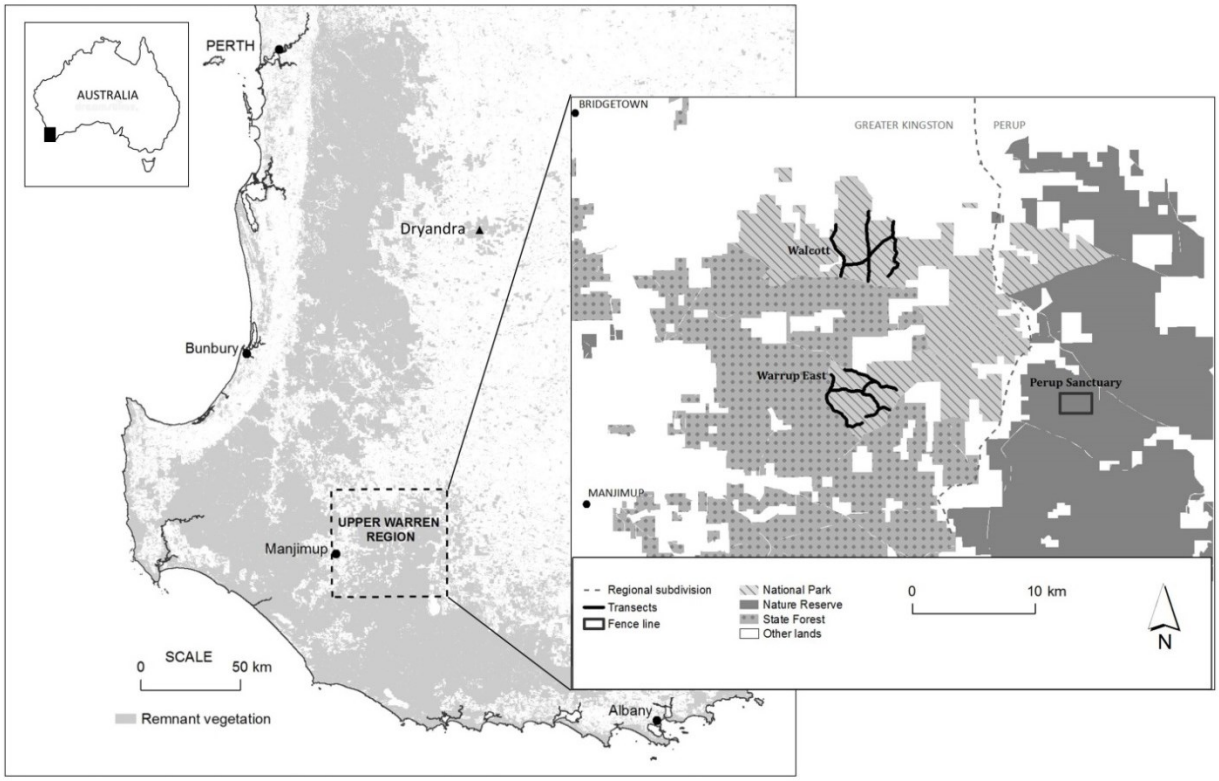

Figure 29: Map illustrating the location our study sites within south-western Western Australia. The box (right), depicts the proximity of Walcott and Warrup East in relation to Perup Sanctuary. Dryandra is located about 250 km north-east of our study sites within the Upper Warren region.

Sample collection and ivermectin treatment

Each woylie was identified with two individually numbered ear-tags, and weight (g), sex, head length (mm), pes length (mm) and reproductive status were recorded at each capture. Packed cell volume (determined via centrifugation of blood in microhaematocrit tubes) and TPP (determined using refractometry) were also measured in a subset of woylies (n = 346; Dryandra only). Half of the translocated woylies (Dryandra n = 35, Walcott n = 47 and Warrup East n = 46) were administered a single subcutaneous dose of ivermectin (Ivomec® 0.2 mg/kg) prior to release. While the sex ratio of treated *versus* untreated woylies was equal within Walcott and Warrup East, there were more treated males (24/35) than females (11/35) within the Dryandra translocated group.

Gastrointestinal parasites

Faeces were collected from newspaper placed beneath each trap, and stored in 10 per cent buffered formalin at 4°C until processing. Using simple faecal flotation with sodium nitrate, each sample was inspected for the presence of nematode eggs and coccidian oocysts, as described by Northover et al. (2017). Coccidian oocysts were recorded as present or absent, while strongyle and *Strongyloides*-like eggs were counted (quantified as eggs per gram of faeces) to estimate nematode burden. We only included one faecal sample from each individual per trapping session in our analyses.

Ectoparasites

Each woylie was examined systematically (i.e. standardised coat combing and visual inspection) for the presence of lice and/or louse eggs, fleas, ticks and mites [including larval and nymph stages for ticks and mites, which were collected into 70% ethanol and morphologically identified to suborder using keys developed by Roberts (1970) and Domrow (1987)].

Trypanosomes

Blood was collected from the lateral tail vein into EDTA MiniCollect tubes (Greiner Bio-One, Germany) and frozen at -20°C prior to analysis. DNA was extracted from 200 μL aliquots of whole blood using the QIAmp 96 DNA blood kit as per the manufacturer's specifications (Qiagen, Hilden, Germany), with a final elution volume of 60 μL. A negative control was included in the extraction process. DNA was screened for trypanosomes using a nested set of generic *Trypanosoma* spp. 18S rDNA PCR primers (Table A4) designed by Maslov et al. (1996) and McInnes et al. (2011). *Trypanosoma*-positive samples were then tested for the presence of *T. copemani* and *Trypanosoma vegrandis* using specific nested primers (Table A4) designed by Botero et al. (2013) and McInnes et al. (2011). PCR reactions were conducted as described by Cooper et al. (2018), with the exception that 2 μl of DNA was added to a 24 μl master mix.

Samples that tested positive for trypanosomes, but negative for specific *Trypanosoma* spp. using clade-specific primers, underwent Sanger sequencing. PCR products were purified using either the in-house filter tip method (Yang et al., 2013) or the Agencourt AMPure PCR purification system. Purified amplicons were sequenced in both directions using an ABI Prism™ Terminator Cycle Sequencing Kit on an Applied Bio-systems 3730 DNA Analyser (Applied Bio-systems, California, USA). The denaturation stage of the sequencing PCR was extended from 2 min to 10 min to control for GC rich regions in the samples, and a 3:1 ratio of BigDye Terminator v3.1 Ready

Reaction mix and dGTP BigDye Terminator v3.0 Ready Reaction mix were used to produce high quality chromatograms.

Data analyses

To investigate the effects of each parasite taxon, and parasite infracommunity richness (polyparasitism) on body condition, we used an Akaike Information Criteria (AIC) model reduction approach. We modelled body condition separately for translocated and resident woylies, using linear mixed effect models (packages *lme4*, Bates et al., 2015; *car*, Fox and Weisberg, 2011) in R (R Core Team, 2015). Body condition index (BCI) was the response variable, and was derived from the residuals of a linear regression between weight and mean pes length; calculated separately for each sex (accounting for pouch young in females), and checked for goodness of fit using adjusted R-squared values (R^2 = 0.198 and 0.379 for translocated; 0.540 and 0.521 for resident, male and female regressions, respectively). Sub-adults (n = 18) were excluded from our analyses. Our predictor variables included parasite taxa (strongyle and *Strongyloides*-like egg counts; coccidia, tick, flea, lice, mite, *T. copemani* and *T. vegrandis* presence), parasite infracommunity richness (the number of parasite taxa within or on an individual host; maximum of 9), time since translocation (TST), destination site (Dryandra, Walcott and Warrup East) and ivermectin treatment (translocated woylies only). Parasite infracommunity richness was evaluated in a separate model to individual parasite taxa. Each of our predictor variables were considered on their own and we also examined two-way interactions between individual parasite taxa (or infracommunity richness) and TST, site and ivermectin treatment. Collinearity between predictor variables was evaluated visually and using variance inflation factors (VIF). None of our predictor variables had a VIF greater than four. Prior to analysis, we scaled and centred our continuous predictor variables (TST, strongyle and *Strongyloides*-like egg counts and infracommunity richness) to ensure effect sizes were comparable (Grueber et al., 2011). We only included results from woylies that were sampled for all parasite taxa at each capture, and woylie ID was included as a random effect to account for repeated measures of individuals throughout the study.

During the second translocation (Dryandra), we also measured PCV (%) and TPP (g/L) to further evaluate the relationship between parasite infection and host health; these values were compared to published reference values for woylies in the Species 360 (2017) database. Both PCV and TPP (response variables) were analysed separately for translocated and resident woylies, using linear mixed effect models (as above), including woylie ID as a random effect.

For all models (BCI, PCV and TPP), a model reduction approach was used. We started with a full model (including all main effects and interactions specified above), and removed them in a stepwise fashion based on minimising AIC (removing the effect that led to the smallest AIC). The smallest minimal model was determined when further reductions to the model led to an increase in AIC, and this model is presented in the results. Model reduction was automated using the bfFixefLMER_F function (package *LMERConvenienceFunctions*, Tremblay and Ransijn, 2015) in R (R Core Team, 2015).

Results

Overall we analysed 763 samples (367 translocated; 396 resident) from 300 individual woylies (133 translocated; 167 resident), for the presence of gastrointestinal parasites, ectoparasites and *Trypanosoma* spp. as described above.

Translocated woylies

Body condition of translocated woylies was significantly impacted by site, TST and *Strongyloides*-like eggs counts; there was also a significant interaction between coccidia presence and TST (Table 9). Body condition index increased following translocation (Figure 30A) and BCI was highest in woylies translocated into Dryandra (Figure 31A). Lower BCI was strongly associated with the presence of coccidia during the first three months following translocation (Figure 32). Lower BCI was also associated with higher *Strongyloides*-like egg counts (Figure 33). Ivermectin had no effect on BCI in treated hosts and was not included in the final model. A significant positive interaction between parasite infracommunity richness and TST was identified for PCV and TPP (Table 9), however this effect was small and could not be interpreted with confidence as translocated woylies were not infected with more than six parasite taxa after July (Figure A7). Total plasma protein was also significantly associated with the presence of fleas, TST, and their interactions (Table 9). Total plasma protein was lower in woylies infected with fleas, though this result was hard to interpret given the large variability in sample size between infected (n = 29) and non-infected (n = 135) groups (Figure A8). Mean TPP values increased one month after translocation before decreasing over time (on average), though this effect was small (Figure A9). The positive interaction between fleas and TST, although significant, could not be interpreted with confidence as we only detected fleas in a single translocated host after translocation (August) and the TPP value from this animal was elevated (Figure A10).

Table 9: Results from Generalised Linear Mixed Model analysis (after AIC model reduction) of factors related to body condition index, total plasma protein and packed cell volume in translocated and resident woylies, with coefficients and 95% profile confidence intervals of standardised and centred model coefficients. Significant results are highlighted in bold; Reference category for site is Dryandra.

	Translocated					
	X^2	*df*	*P*	Coefficient		95% CI
Body condition index					2.5%	97.5%
Strongyloides-like eggs	10.177	1	**0.001**	-24.449	-39.507	-9.540
Coccidia	2.293	1	0.130	13.764	-12.171	39.520
Site Walcott	16.163	2	**< 0.001**	-73.693	-113.371	-34.217
Site Warrup East	16.163	2	**< 0.002**	-57.542	-95.096	-20.164
TST (time since translocation)	34.180	1	**< 0.001**	37.939	22.197	53.598
Coccidia x TST	15.541	1	**< 0.001**	142.069	71.868	212.414
Total plasma protein						
Fleas	14.906	1	**< 0.001**	21.068	2.017	39.998
TST	5.558	1	**0.018**	-2.840	-5.067	-0.647
Fleas x TST	7.542	1	**0.006**	60.743	17.439	103.792
Polyparasitism x TST	9.801	1	**0.002**	8.725	3.300	14.222
Packed cell volume						
Polyparasitism x TST	7.616	1	**0.006**	5.759	1.671	9.819
	Resident					
	X^2	*df*	*P*	Coefficient		95% CI
Body condition index					2.5%	97.5%
Strongyle eggs	5.813	1	**0.016**	-8.566	-29.660	12.395
Site Walcott	19.531	2	**< 0.001**	48.830	16.193	81.425
Site Warrup East	19.531	2	**< 0.001**	78.118	42.855	113.317
TST	14.700	1	**< 0.001**	24.140	10.504	37.731
Strongyle eggs x TST	9.082	1	**0.003**	-57.279	-94.303	-19.860
Polyparasitism x TST	12.305	1	**< 0.001**	-49.922	-77.674	-21.982
Total plasma protein						
T. vegrandis	1.078	1	0.299	-1.809	-3.946	0.303
TST	0.004	1	0.949	1.446	-0.451	3.318
T. vegrandis x TST	14.460	1	**< 0.001**	-9.747	-14.736	-4.730

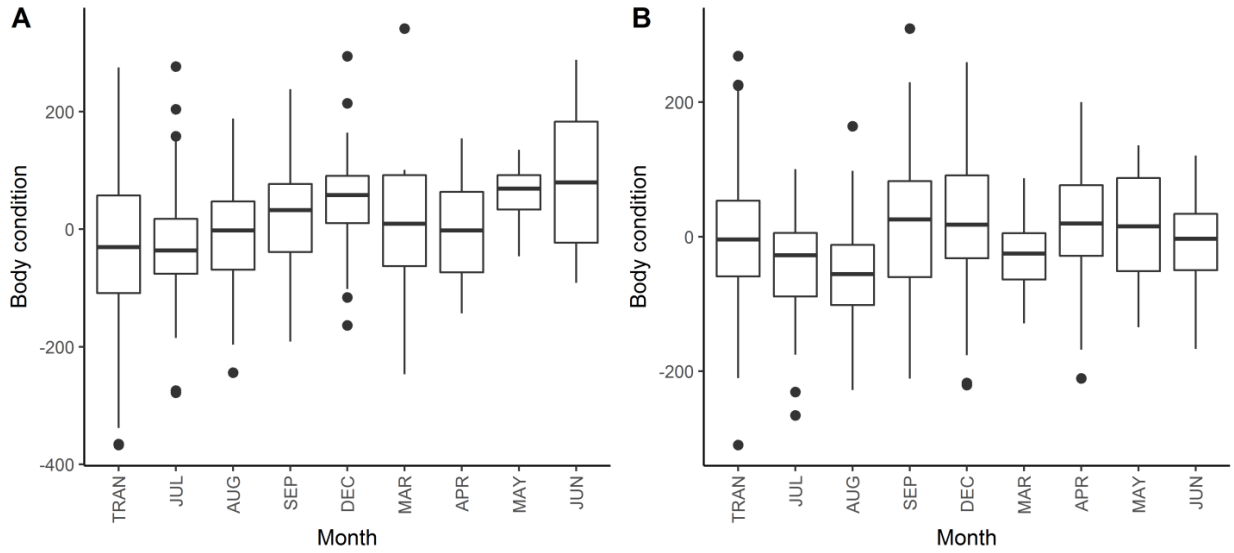

Figure 30: Boxplots depicting mean body condition over time (all three sites combined, sampled across two years) in (A) translocated and (B) resident woylies following translocation. Each box is delimited by the first (lower) and third (upper) quartile with the median represented by the thick horizontal line; whiskers represent the 1.5 interquartile range; solid black dots represent outliers. Data points above zero reflect above average body condition, while those below zero reflect below average body condition. TRAN: time of translocation.

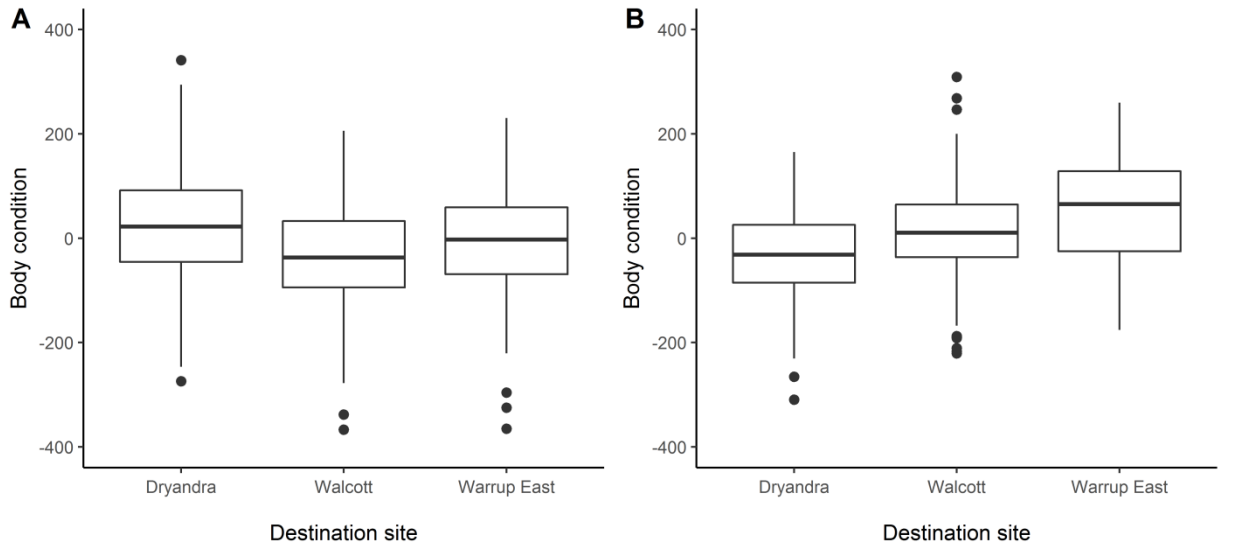

Figure 31: Boxplots showing mean body condition for each site (all time points combined) in (A) translocated and (B) resident woylies. Each box is delimited by the first (lower) and third (upper) quartile with the median represented by the thick horizontal line; whiskers represent the 1.5 interquartile range; solid black dots represent outliers. Data points above zero reflect above average body condition, while those below zero reflect below average body condition.

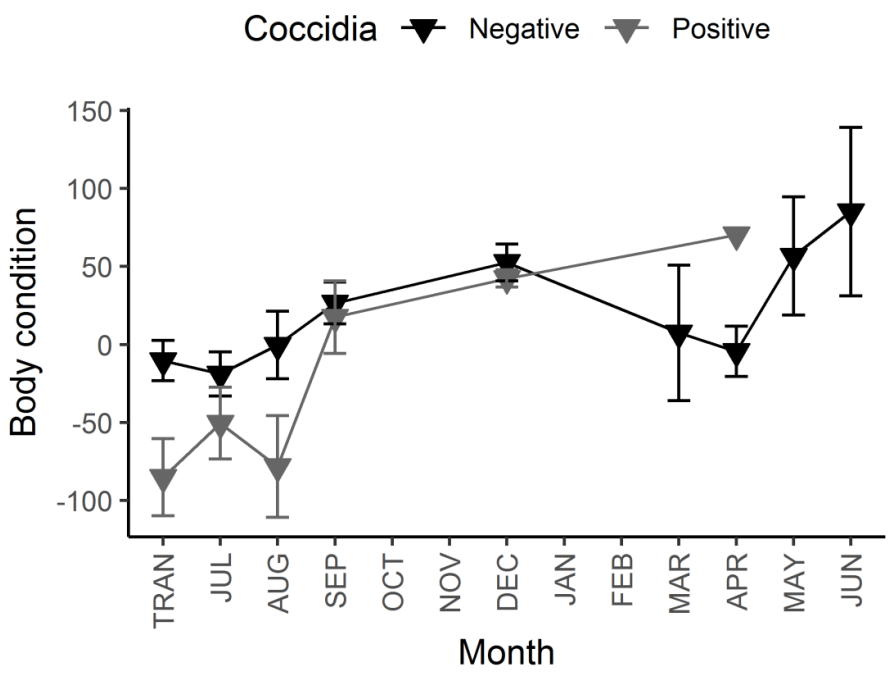

Figure 32: Mean body condition over time in coccidia-positive *versus* coccidia-negative translocated woylies (all three sites combined, sampled across two years). Data points above zero reflect above average body condition, while those below zero reflect below average body condition. TRAN: point of translocation; Error bars represent 95% CI.

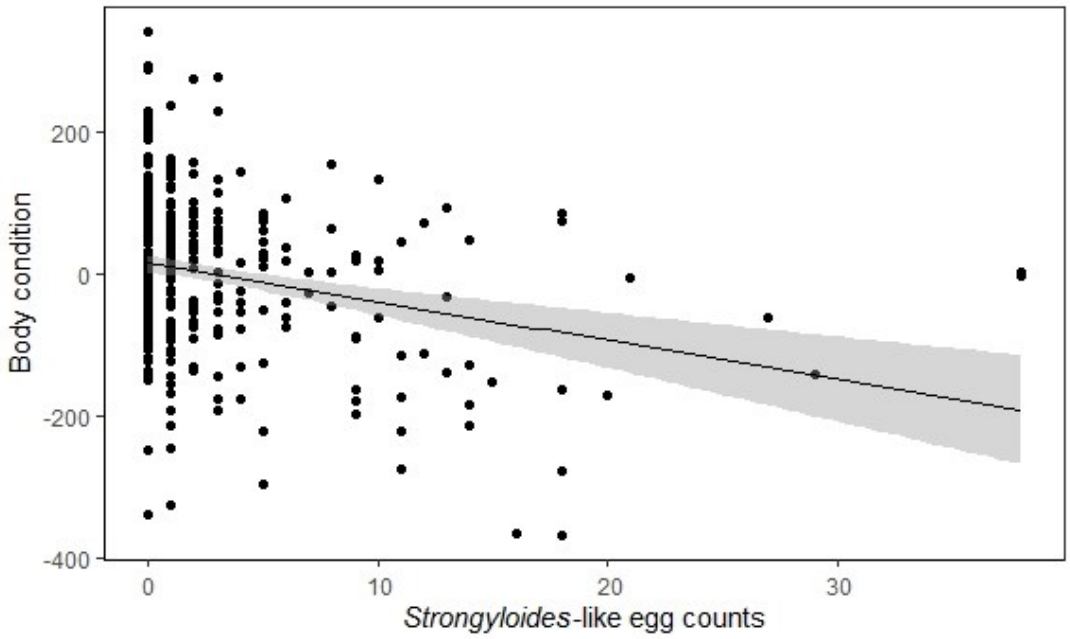

Figure 33: The relationship between body condition and increasing *Strongyloides*-like egg counts (two highest outliers removed) in translocated woylies (all three sites combined, sampled across two years). Data points above zero reflect above average body condition, while those below zero reflect below average body condition.

Resident woylies

Body condition of resident woylies was significantly impacted by site, TST and strongyle eggs. A significant interaction between strongyle eggs and TST, and parasite infracommunity richness and TST was also observed (Table 9). Body condition index increased following translocation (Figure 30B), and BCI was highest in the Upper Warren, particularly Warrup East (Figure 31B). Higher strongyle egg counts were negatively associated with BCI (Figure A11); though this effect was only small and the biological significance deemed weak as the confidence intervals for the model coefficients overlapped zero (Table 9). The significant negative interaction between strongyle eggs and TST reflects a pronounced increase in BCI over time in strongyle-negative hosts, while BCI decreased over time in strongyle-positive hosts (Figure 34). In a similar manner, the BCI of woylies with higher parasite richness declined over time after December, whereas BCI in woylies infected with fewer parasites increased over time (on average) (Figure 35). None of our predictor variables were significantly associated with PCV in resident woylies. For TPP, a significant (though small) interaction between TST and *T. vegrandis* infection was identified where TPP was lower in woylies infected with *T. vegrandis* from September onwards (Table 9; Figure A12).

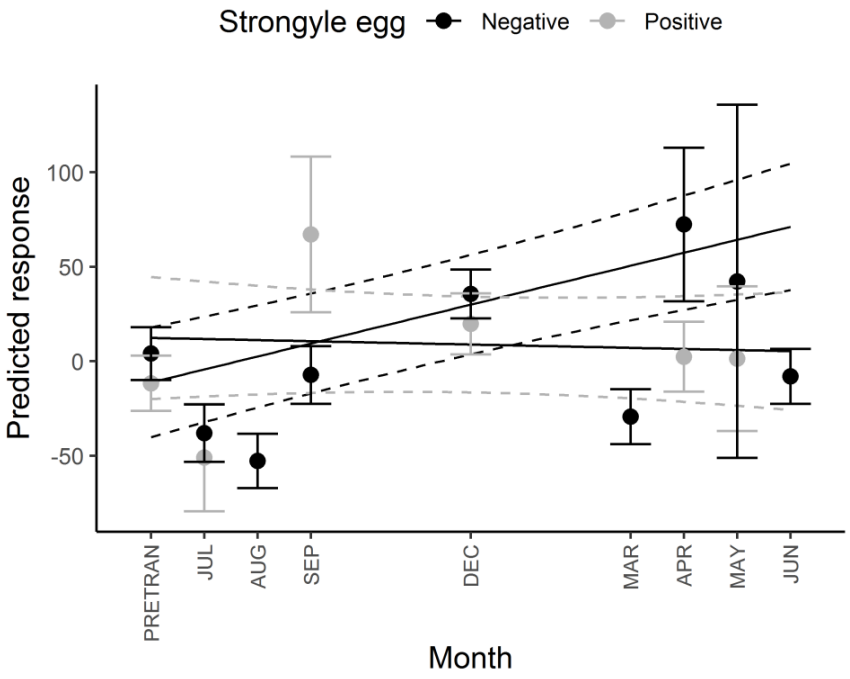

Figure 34: Mean body condition over time (all three sites combined, sampled over two years) in strongyle-positive *versus* strongyle-negative resident woylies. Data points above zero reflect above average body condition, while those below zero reflect below average body condition. PRETRAN: All time points prior to translocation combined; Error bars represent 95% CI; Solid lines represent model predictions with 95% CI (dashed lines).

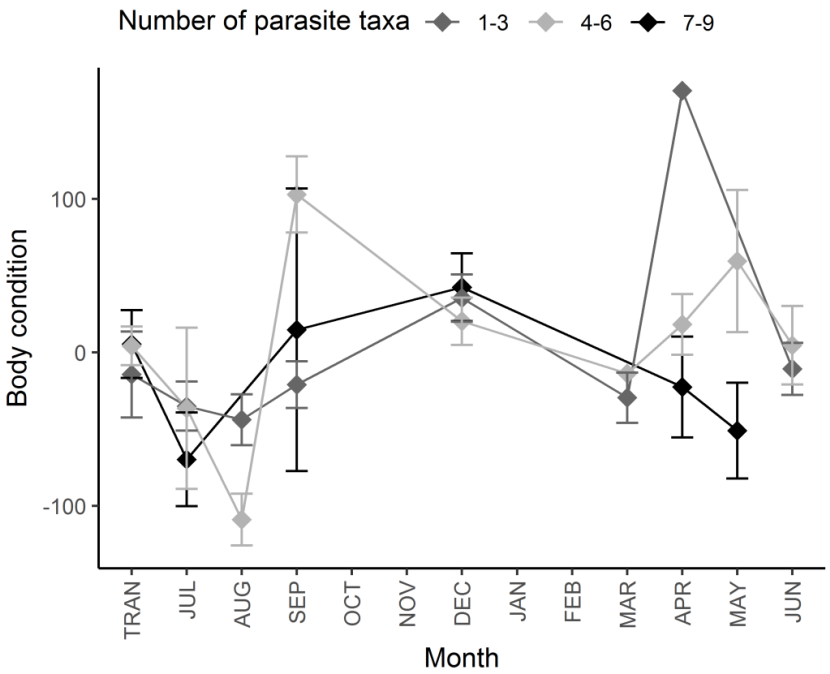

Figure 35: Mean body condition over time (all three sites combined, sampled across two years) in relation to the number of parasite taxa infecting resident woylies. Data points above zero reflect above average body condition, while those below zero reflect below average body condition. TRAN: time of translocation; Error bars represent one standard error.

Discussion

This study enhances our understanding of how woylie health may be impacted by parasite infection and ivermectin treatment during translocation. Changes to BCI in translocated and resident woylies following translocation were strongly impacted by site and TST. In translocated woylies, the presence of coccidia during the first three months following translocation and higher *Strongyloides*-like egg counts were associated with lower BCI. Ivermectin treatment did not provide any benefit to host health in treated compared to untreated (translocated) woylies. In resident woylies a strong time-dependent relationship was identified in hosts infected with strongyle eggs and those with higher parasite species richness.

In both translocated and to a lesser extent resident woylies, we observed increasing BCI over time following translocation. Hypogeous fungi, the predominant food source of woylies (Claridge, 2002), is most abundant during winter, the time of release, (Zosky, 2011) and woylies are reported to consume a more diverse range of nutrient-rich fungi in winter and spring (Zosky et al., 2017). Soil is also less compact and easier to dig following the winter rain (Yeatman and Wayne, 2015). Increasing BCI over time may therefore reflect seasonal variation in food availability, rather than

an effect of translocation. Alternatively, for translocated hosts originating from Perup Sanctuary, less competition and greater availability of food outside of the fenced reserve may have contributed toward this outcome.

Site variation in BCI of woylies may also reflect ecological differences in the type, availability and location of food, the genetically distinct nature of these populations (Pacioni et al., 2013) and/or adaptations to the environment. Within Dryandra for example, woylies are known to consume a greater amount of epigeous (compared to hypogeous) fungi (Murphy, 2009) and invertebrates (Zosky et al., 2017). Dryandra resident woylies are also smaller, weigh less and have a comparably shorter pes than Upper Warren woylies (Northover et al., unpublished data). In contrast to resident woylies, where BCI was highest within the Upper Warren, BCI in translocated woylies was highest within Dryandra. The larger body size of woylies originating from the Upper Warren may have conferred a competitive advantage within Dryandra. This result may have also been associated with a male-biased cohort being released into Dryandra; males are typically larger and more dominant than females.

In translocated woylies, BCI was considerably lower in coccidia-positive hosts during the first few months following translocation. Coccidia are commonly found in macropods in the absence of disease, though coccidiosis may be precipitated by stress, and clinical signs associated with coccidial disease (e.g. abdominal pain, reduced appetite, diarrhea and lethargy) may result in poor body condition (Vogelnest and Portas, 2008). Although we identified an association between lower body condition and the presence of coccidial infection, we do not know whether this relationship is causative. The fact that we observed this change in the translocated (and not resident) woylies, which were subject to the additional stress of transport and release into a novel environment (Dickens et al., 2009) however, indicates that the presence of coccidia may adversely impact host health during periods of stress. This is further supported by the observation that this effect subsides after the first few months following translocation; a time which has been shown to coincide with host acclimation and recovery from translocation-associated stress in other species (e.g. Franceschini et al., 2008; Patt et al., 2012).

Nematode infection was negatively associated with BCI in translocated and resident woylies during this study. Both strongyle and *Strongyloides* spp. nematodes are typically well tolerated by macropods (Vogelnest and Portas, 2008). Depending on the species of nematode, or chronicity of infection however, strongyle and *Strongyloides* spp. nematodes may negatively impact host health;

though this is usually associated with stress (Winter, 1958; Speare et al., 1982). During this study, higher egg counts were associated with lower BCI, and in resident woylies, body condition in strongyle-positive hosts did not increase over time as we observed in strongyle-negative hosts. While interpreting noninvasive estimates of nematode burden via faecal flotation has its limitations (see Bordes and Morand, 2011) and we were unable to accurately quantify nematode burden or visualise disease pathology, our results suggest that nematode infection may negatively affect BCI in woylies; whether this is clinically significant however, remains unclear, particularly when other health parameters (i.e. PCV/TPP) were not affected by the presence of either of these parasite taxa (though this may reflect the species of nematode infecting woylies). In a previous study (Northover et al., 2018b) that isolated *Strongyloides*-like eggs in the faeces from a deceased woylie, the stomach was grossly and histopathologically unremarkable on post-mortem examination, suggesting that *Strongyloides* spp. nematodes were non-pathogenic.

A primary aim of this study was to evaluate whether ivermectin treated (translocated) woylies had improved BCI compared to untreated (translocated) woylies. In the past, parasite control has often been used based on the assumption that fewer parasites may offer a competitive advantage. While it has been suggested that the absence of parasites may be associated with the success of invasive species (e.g. Torchin et al., 2002), it has not been empirically demonstrated that fewer parasites enhance host health or translocation outcomes, despite the observation that parasite loss often occurs during fauna reintroductions (Torchin et al., 2003; MacLeod et al., 2010). Experimental studies often report no benefit of treatment to host health (e.g. Cripps et al., 2014). Transient effects of antiparasitic drug treatment are also common (e.g. Almberg et al., 2012), with treated hosts becoming re-infected with parasites at the same (Marcus et al., 2015), or greater (e.g. Cripps et al., 2014) prevalence after treatment. During this study, ivermectin treatment did not improve BCI in woylies; though we cannot empirically demonstrate that the dosage of ivermectin administered was sufficient to reduce target parasites. Nonetheless, without evidence to suggest that ivermectin treatment benefits host health, we cannot recommend the use of this drug (at 0.2 mg/kg SC) in the future.

Consideration also needs to be given to the broader impacts of ivermectin in non-target parasites such as coccidia, as the use of ivermectin has been shown to cause a reciprocal increase in *Eimeria* spp. prevalence in other species experimentally (Knowles et al., 2013; Pedersen and Antonovics, 2013). During this study, the association between coccidia presence and lower BCI in translocated woylies during the first three months following translocation had the largest effect size (e.g. biggest

biological effect), yet the antiparasitic drug administered does not target this parasite, and could potentially impact (indirectly) coccidia prevalence as above. In other host species where coccidian parasites have been identified as a health hazard during translocation (e.g. Sainsbury and Vaughan-Higgins, 2012) prophylactic treatment to reduce but not eliminate coccidian parasites is undertaken. In woylies, further studies are needed to evaluate host-parasite-immunological associations, particularly for 'high risk' parasites such as coccidia, in order to justify the use of alternative antiparasitic drugs. Experimental studies that investigate potential interactions between strongyloid nematodes and ivermectin treatment on coccidia are also justified.

Parasite infracommunity richness was not significantly associated with host health during this study. An interaction between parasite infracommunity richness and TST however was found for TPP and PCV in translocated woylies and BCI in resident woylies. For translocated woylies, the small effect size and limited data for woylies with high parasite richness restricted our ability to interpret these interactions with any confidence. For resident woylies, declining BCI after December in woylies with higher parasite richness could suggest the presence of an adverse relationship, particularly when food resources, particularly fungi, are limited at this time of year (Zosky et al., 2017). Furthermore, as the highest parasite richness category only contained Upper Warren woylies (data not shown), other site-related factors may be influencing this result. Nonetheless given the large effect size of this interaction, further studies are warranted.

Total plasma protein was lower in translocated woylies infected with fleas, though interpreting the biological significance of this result was challenging given the large difference in sample size between infected and non-infected groups. Poor health, including anaemia, have been associated with heavy flea infestations in macropods (Vogelnest and Portas, 2008), and may be associated with immunosuppression or underlying disease (Reiss et al., 2015). None of the TPP values obtained during this study were below the cut off reference values for woylies (Species 360, 2017) however, and in the absence of particularly high flea burdens (data not shown) this result is suspected to be clinically insignificant. Likewise, given the small effect size of the interaction between *T. vegrandis* and TST for TPP in resident woylies, this result is unlikely to reflect pathological change. Notably, 65% of TPP values obtained from woylies during this study were higher than normal (Species 360, 2017). Woylies from the Upper Warren region are reported to have higher TPP values than those reported in Species 360 (2017), and TPP values are known to differ between sites (Pacioni et al., 2013). A small percentage of PCV values (13%), were also higher than normal, but as TPP was also elevated in most of these cases, dehydration is the most

likely explanation (Clark, 2004). It is important to highlight the difficulty in determining whether changes in host health are the cause or an effect of parasite infection in observational studies such as this. Although challenging when dealing with a threatened host species, experimental manipulation studies that quantify parasite burden in relation to host health may be more adept at evaluating such a relationship.

Lastly, given that we observed increasing BCI in translocated and resident hosts following translocation and the effects of parasites on PCV/TPP are of questionable significance, it is important to recognise that many parasite taxa (and parasite infracommunity richness) had little or no adverse effect on host health during this study; though further studies are required to evaluate the impact of coccidia and nematodes on host health in woylies. In the absence of evidence to suggest that specific parasite taxa negatively impact host health, parasite conservation and preservation of the fundamental host-parasite relationship should be a key consideration in the future planning and implementation of fauna translocations. Most importantly, fauna translocation protocols should endeavor to incorporate parasite monitoring and scientific studies to better understand what drives parasite dynamics during translocation.

Conclusion

During this study we identified significant changes to BCI associated with certain parasite taxa. Changes to body condition were also strongly impacted by site and TST, emphasising the difficulty of predicting how BCI may change following translocation and the value of parasite monitoring. The association between lower BCI and the presence of coccidia and nematodes suggests that these parasites may adversely impact host health in woylies during translocation. Further studies are required to evaluate whether this outcome may be precipitated by stress. As there was no discernable benefit of ivermectin treatment to host health, we do not advocate the use of ivermectin in future, though alternative treatment protocols may be a consideration for parasites that have a demonstrated negative impact on host health (e.g. coccidia). The inclusion of TPP and PCV to further evaluate host health provided mixed results. Although TPP was lower in the presence of some parasite taxa (e.g. fleas in translocated hosts) none of our TPP values were below the reported reference value for woylies; thus it is most likely that such parasites do not negatively impact host health in woylies.

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Chapter Seven

Debilitating disease in a polyparasitised woylie (*Bettongia penicillata*): a diagnostic investigation

7.1 Debilitating disease in a polyparasitised woylie (*Bettongia penicillata*): a diagnostic investigation

The following is a published paper:

Northover, A.S., Elliot, A., Keatley, S., Lim, Z., Botero, A., Amanda, A., Godfrey, S.S., Lymbery, A.J., Wayne, A.F., Thompson, R.C.A., 2018. Debilitating disease in a polyparasitised woylie (*Bettongia penicillata*): a diagnostic investigation. International Journal for Parasitology: Parasites and Wildlife **7**: 274-279. https://doi.org/10.1016/j.ijppaw.2018.07.004.

Abstract

During monitoring of critically endangered woylie (*Bettongia penicillata*) populations within the south-west of Western Australia, an adult female woylie was euthanased after being found in extremely poor body condition with diffuse alopecia, debilitating skin lesions and severe ectoparasite infestation. *Trypanosoma copemani* G2 and *Sarcocystis* sp. were detected molecularly within tissue samples collected post-mortem. *Potorostrongylus woyliei* and *Paraustrostrongylus* sp. nematodes were present within the stomach and small intestine, respectively. Blood collected ante-mortem revealed the presence of moderate hypomagnesaemia, mild hypokalaemia, mild hyperglobulinaemia and mild hypoalbuminaemia. Diffuse megakaryocytic hypoplasia was evident within the bone marrow. We propose various hypotheses that may explain the presence of severe ectoparasite infection, skin disease and poor body condition in this woylie. Given the potential deleterious effects of parasite infection, the importance of monitoring parasites cannot be over-emphasised.

Introduction

Critically endangered woylie (brush-tailed bettong, *Bettongia penicillata*) populations have declined by more than 90% since 1999, and are now restricted to three indigenous wild populations within the south-west of Western Australia (Wayne et al., 2015). In June 2014, as part of the ongoing conservation management of this species, 182 woylies were translocated from Perup Sanctuary, a 423 ha predator-proof enclosure located 50 km east of Manjimup (34.2506 °S, 116.1425 °E), to supplement two natural populations. During monitoring within one of these populations (Walcott, situated 20 km north-west of Perup Sanctuary; 34.0592 °S, 116.3859 °E; Figure 36) six months after translocation, an adult female resident (i.e. non-translocated) woylie was found in extremely

poor body condition with diffuse alopecia and skin lesions predominantly affecting the head, hindlimbs, tail base and tail. Severe ectoparasite infestation was also apparent. Veterinary assessment deemed this animal unsuitable for release and the woylie was euthanased in the field via barbiturate injection while under inhalant anaesthesia. A post-mortem examination was carried out within seven hours of death.

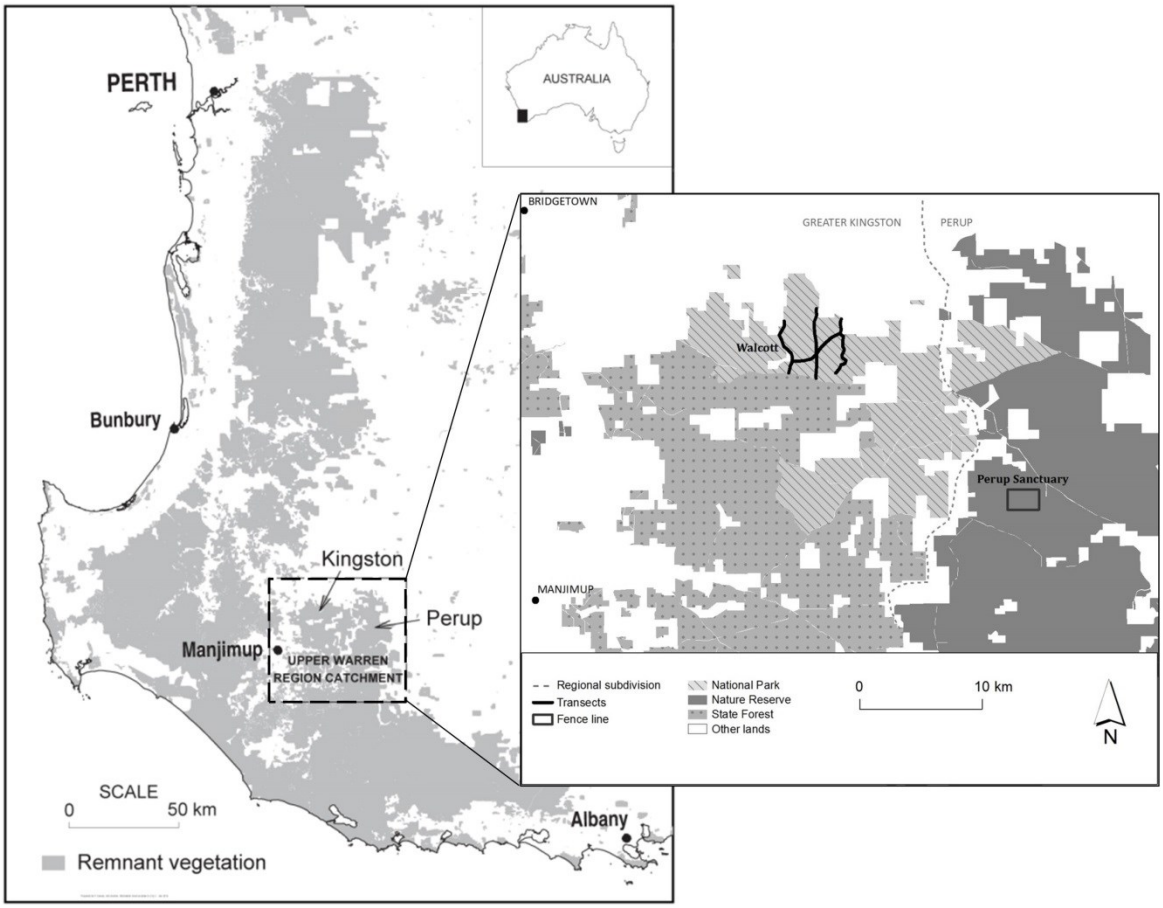

Figure 36: Map depicting our study sites within the Upper Warren region. As shown on the right, Walcott is located approximately 20 km north-west of Perup Sanctuary.

For threatened species such as the woylie, in which only small fragmented wild populations remain, parasites may have a significant impact on population dynamics and host health (Thompson et al., 2010). With regard to woylie population declines, a clear spatio-temporal pattern of decline in population size between 1999 and 2006 was identified, which suggests the potential role of an infectious disease agent (Wayne et al., 2015). Field monitoring carried out immediately prior to, and during this period, found a high prevalence of woylies with moderate to severe alopecia, skin thickening, skin excoriations and scale with a predilection for the head (periocular region and ears) and dorsal tail base/rump region (Wayne et al., 2013). Clinical signs were

strikingly similar to those described here. Despite investigation into this 'skin condition', a causative disease agent could not be identified. Since then, the focus of investigation has shifted toward the potential role of other disease agents. Trypanosomes have been of particular interest, given the demonstrated pathogenicity of *T. copemani* G2 and the association between *T. copemani* and declining woylie populations (Smith et al., 2008; Lymbery and Thompson, 2012; Botero et al., 2013; Godfrey et al., 2018). While previous investigation into the decline has focused on the effects of individual parasite species, the effect of coinfection has not been evaluated. Here we explore various hypotheses, which may explain the presence of severe ectoparasite burdens, debilitating skin disease and poor body condition in this woylie.

Materials and methods

Trapping regime

Trapping was conducted in December 2014. Woylies were captured using Sheffield cage traps (Sheffield Wire Products, Welshpool, WA), which were set along multiple transects (60 traps/night, 200 m spacing) at dusk and baited with universal bait (rolled oats, peanut butter and sardines). Newspaper was placed beneath each trap to collect faeces, which were stored chilled prior to examination. Traps were cleared within 3 hours of sunrise.

Parasitological analysis

Gastrointestinal parasites

Fresh faeces (2.6 g) were examined for eggs/oocysts using simple faecal flotation with sodium nitrate ($NaNO_3$) as described by Northover et al. (2017). The entire gastrointestinal tract was also examined for the presence of endoparasites, and specimens were morphologically identified using keys developed by Mawson (1973), Beveridge and Durette-Desset (1986), and Smales (1997; 2005).

Haemoparasites

Blood was collected ante-mortem (under anaesthesia) from the lateral caudal (tail) vein into EDTA MiniCollect tubes (Greiner Bio-One, Germany) for molecular analyses and stored at -20°C prior to processing. DNA was extracted from 200 µL aliquots of blood using the QIAamp 96 DNA blood kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. A nested polymerase chain reaction (PCR) targeting the 18S rDNA gene region was carried out using

generic trypanosome primers, as described by Maslov et al. (1996) and McInnes et al. (2009). Positive samples were subsequently screened for the presence of different *Trypanosoma* spp. using clade-specific primers designed by Botero et al. (2013) and McInnes et al. (2011). PCR reactions were performed as outlined in Cooper et al. (2018) with the exception that 2 μL of DNA was added to a 24 μL master mix.

Tissue parasites

Eleven tissue samples (spleen, liver, lung, heart, kidney, brain, oesophagus, tongue, skeletal muscle, anal glands, and bone marrow) were collected post-mortem, extensively washed with phosphate buffered saline, and stored in 100% ethanol for DNA isolation, and fixed in 10% formalin for histopathological analysis. Genomic DNA was obtained using the QIAamp tissue DNA MiniKit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA samples were screened by PCR for the presence of trypanosomes and coccidian parasites. Trypanosome PCR was performed as described above. For the detection of coccidian parasites a fragment of about 800bp from the 18SrDNA gene was amplified using the coccidia generic primers 1L and 3H as described previously (Yang et al., 2001). Sequencing was carried out to confirm parasite genotype/species using the generic coccidian primers in both directions, and using an ABI Prism TM Terminator Cycle Sequencing Kit on an Applied Bio-systems 3730 DNA Analyser (Applied Biosystems, California, USA). Sequences were aligned against reference libraries generated from GenBank®.

Ectoparasites

Prior to euthanasia, the woylie was examined in a systematic manner (under anaesthesia) and graded subjectively for ectoparasite burden (i.e. 0 = none, 1 = light, 2 = moderate, 3 = heavy) based on the number of ectoparasites visible within both ears, and the number of ectoparasites observed during standardised coat combing. A representative number of ectoparasites were collected and stored in 70% ethanol prior to identification. Ectoparasites collected during ante-mortem and post-mortem examination were identified using keys developed by Roberts (1970), von Kéler (1971), Dunnet and Mardon (1974), and Domrow (1987).

Histological analysis

Representative pieces of formalin-fixed tissue were cut and embedded in paraffin from which 3 micrometer-thick sections were cut and stained with hematoxylin and eosin (H&E) and examined microscopically, paying particular attention for the presence of parasites, or lesions that may be

associated with parasitic infection. Additional periodic acid-Schiff (PAS) histochemistry (reaction from Layton and Bancroft, 2013) and CD3, CD79 and CD20 immunohistochemistry was done on lip mucosa and head skin sections. Immunohistochemistry was undertaken using a polyclonal rabbit ant-human CD3 antibody (A0452 - Agilent Dako), a mouse monoclonal IgG1 CD79a antibody (CM067 - Biocare Medical), and a mouse monoclonal IgG2a CD20 antibody (Clone L26 - Thermo Scientific), using a horse radish peroxidase labelled polymer conjugated with secondary antibodies (Envision Dual Link System - Agilent Dako) according to the manufacturer's instructions.

Microbiological analysis

Skin from the head and lip, and intestinal tissue was submitted to Vetpath Laboratory Services for fungal culture and aerobic and anaerobic bacterial culture and sensitivity. No viral testing was performed.

Haematology/biochemistry

Blood was collected (as above) into EDTA and serum MiniCollect tubes (Greiner Bio-One, Germany) and sent to Vetpath Laboratory Services for haematological and biochemical analysis. Three thin peripheral blood smears were also submitted for microscopic examination. Physiological reference intervals for woylies obtained from Species 360 (2017) and Pacioni et al. (2013) were used to interpret the blood results. Reference intervals for magnesium were unavailable from Species 360 (2017), thus magnesium results were interpreted using a mean value from the International Species Information System (Teare, 2002).

Results

Gross examination

Veterinary examination in the field determined that this woylie was in extremely poor body condition (subjective body condition score 1.5/5 based on palpation of muscle mass/fat over the hindquarters), although body weight was within the normal adult range (1270 grams). Severe generalised musculoadipose atrophy was confirmed on internal gross examination. There was severe, diffuse alopecia and inflammation affecting the skin of the head and chin, with patchy haemorrhage (top of head, ears and mouth) and crusting (Figure 37 and 38). The lip margins were severely thickened and inflamed (Figure 38 and 39). Severe hair loss also affected the hindlimbs (medial thighs, tibia), tail base and tail (Figure 40) and mild skin thickening/flaking was evident

over the flanks and rump. The woylie was subjectively graded with a heavy burden of lice/lice eggs and a moderate burden of fleas, ticks and mites.

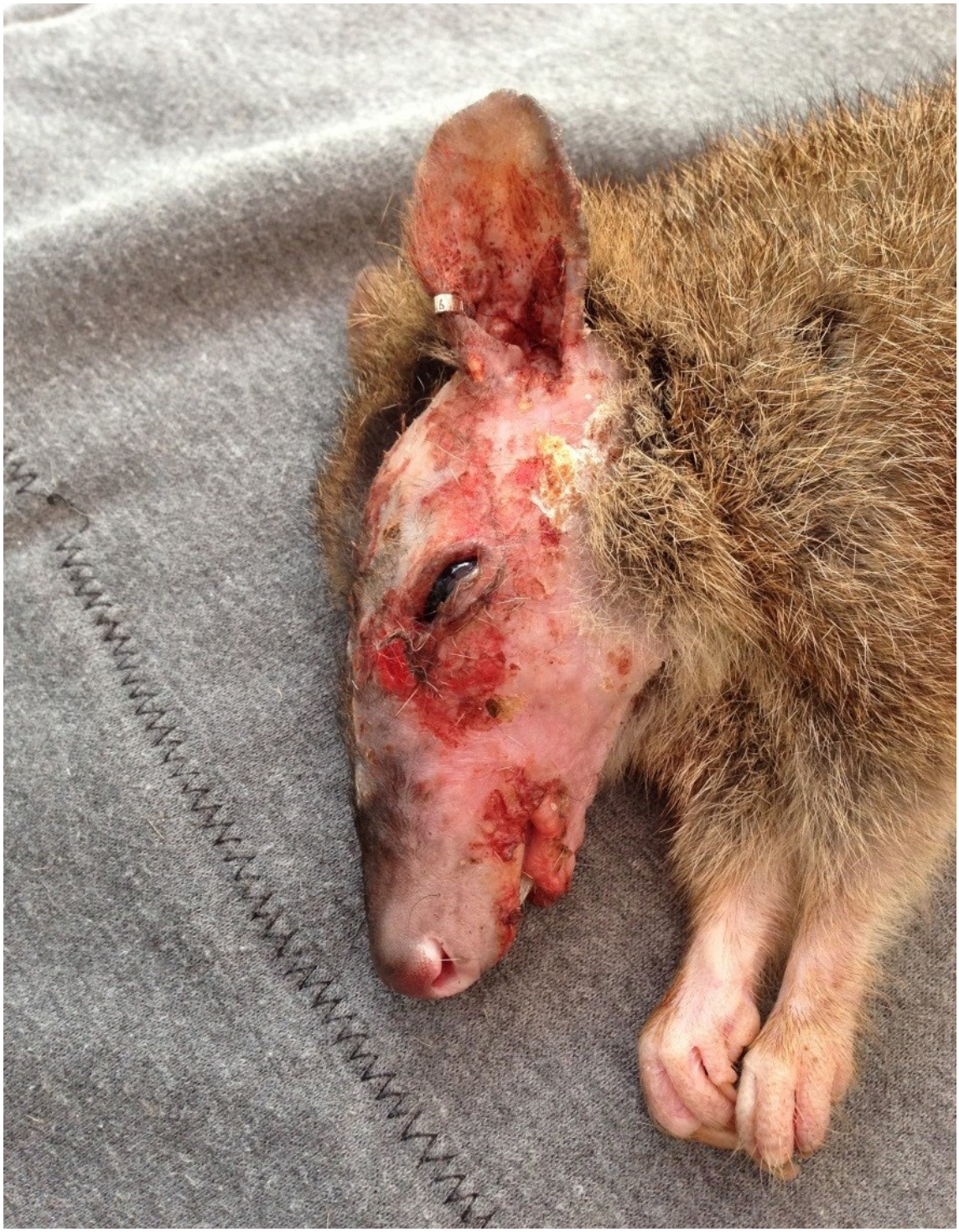

Figure 37: Lateral view of the head of the woylie, showing severe hair loss and inflammation, patchy haemorrhage (top of head, ears and mouth), and crusting.

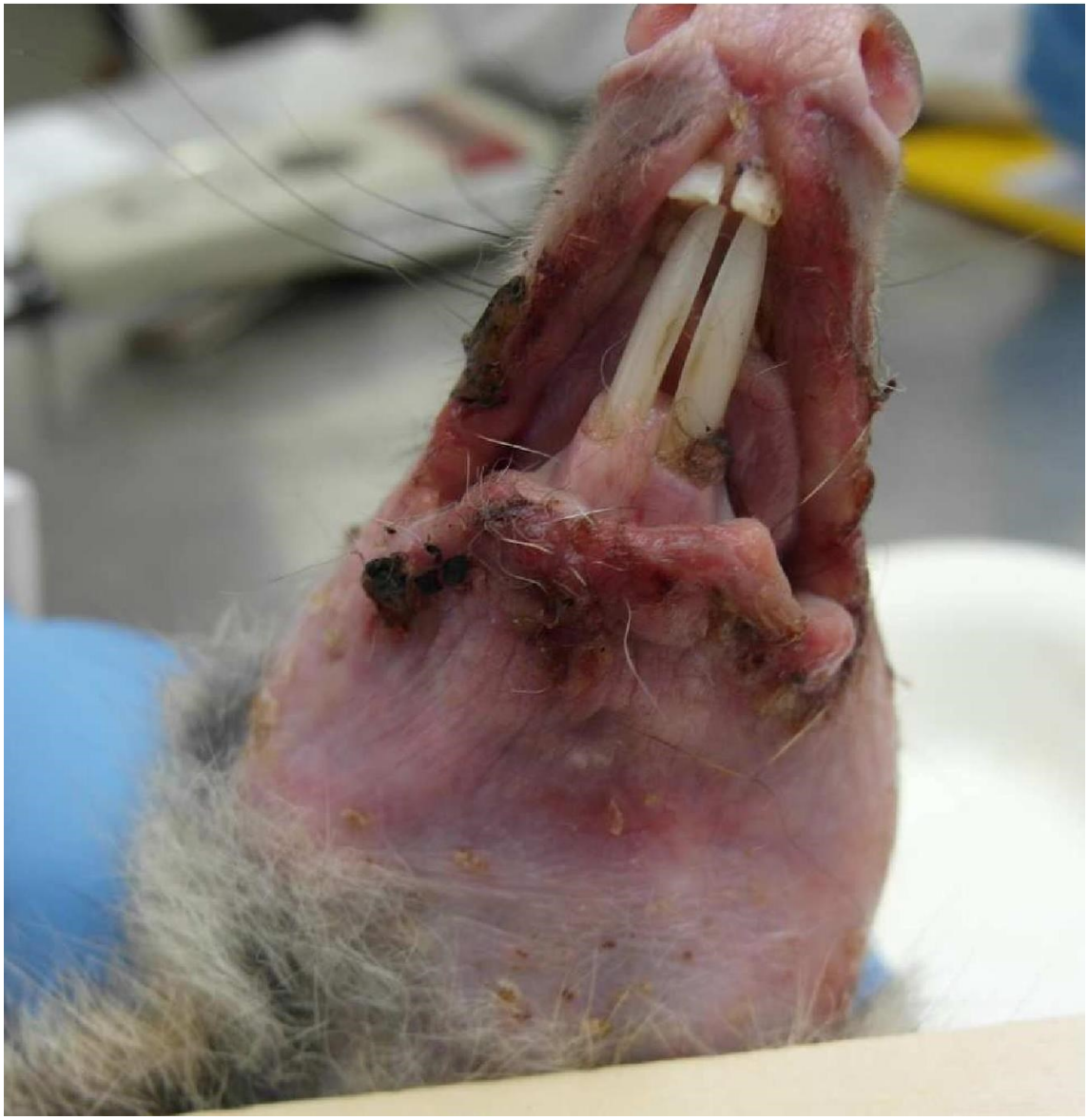

Figure 38: Ventral view of the head of the woylie, showing severe, diffuse hair loss and inflammation affecting the chin. Excoriated, thickened lip margins are also visible.

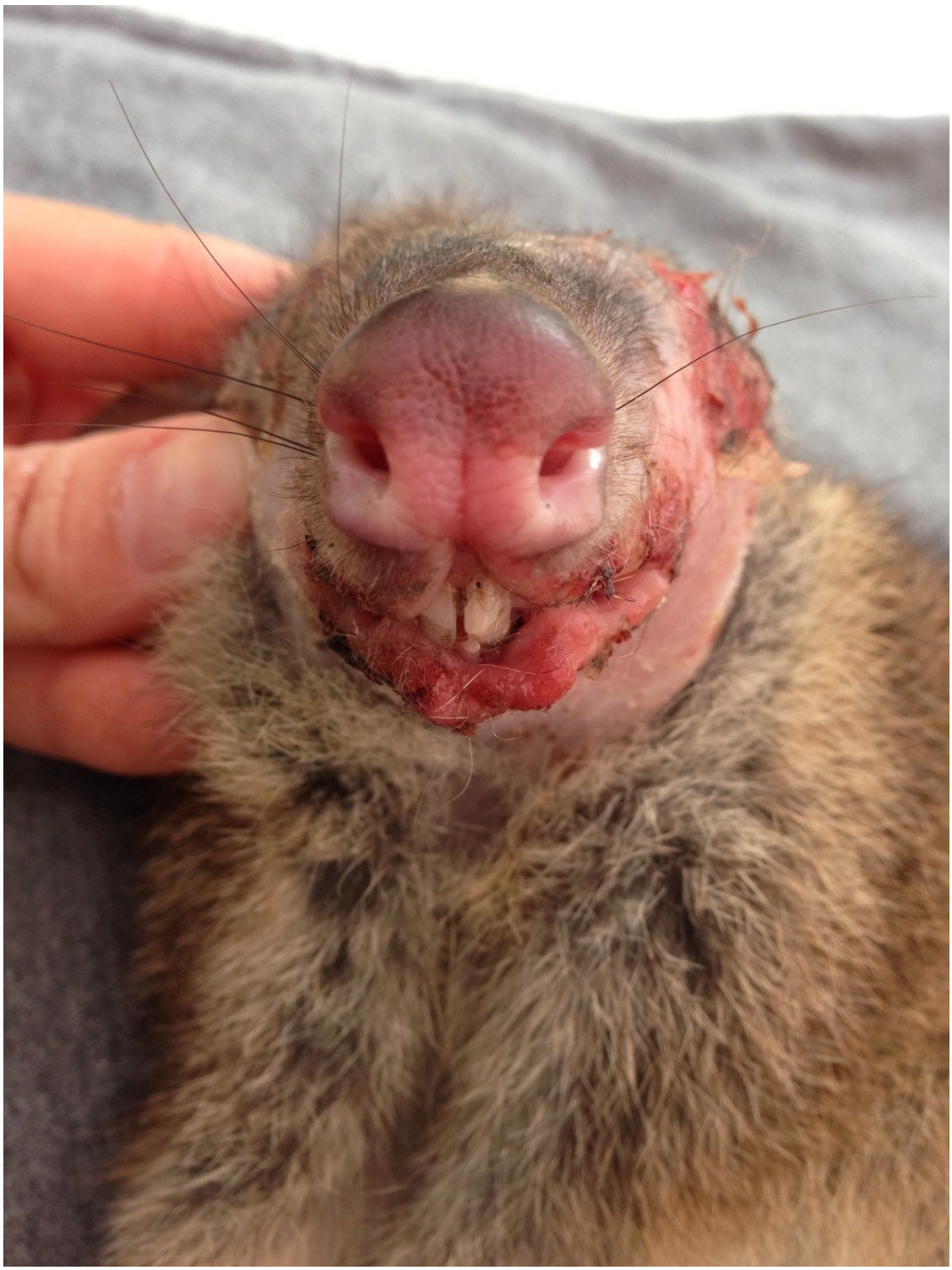

Figure 39: Frontal view of the head of the woylie, showing thickened and inflamed lip margins.

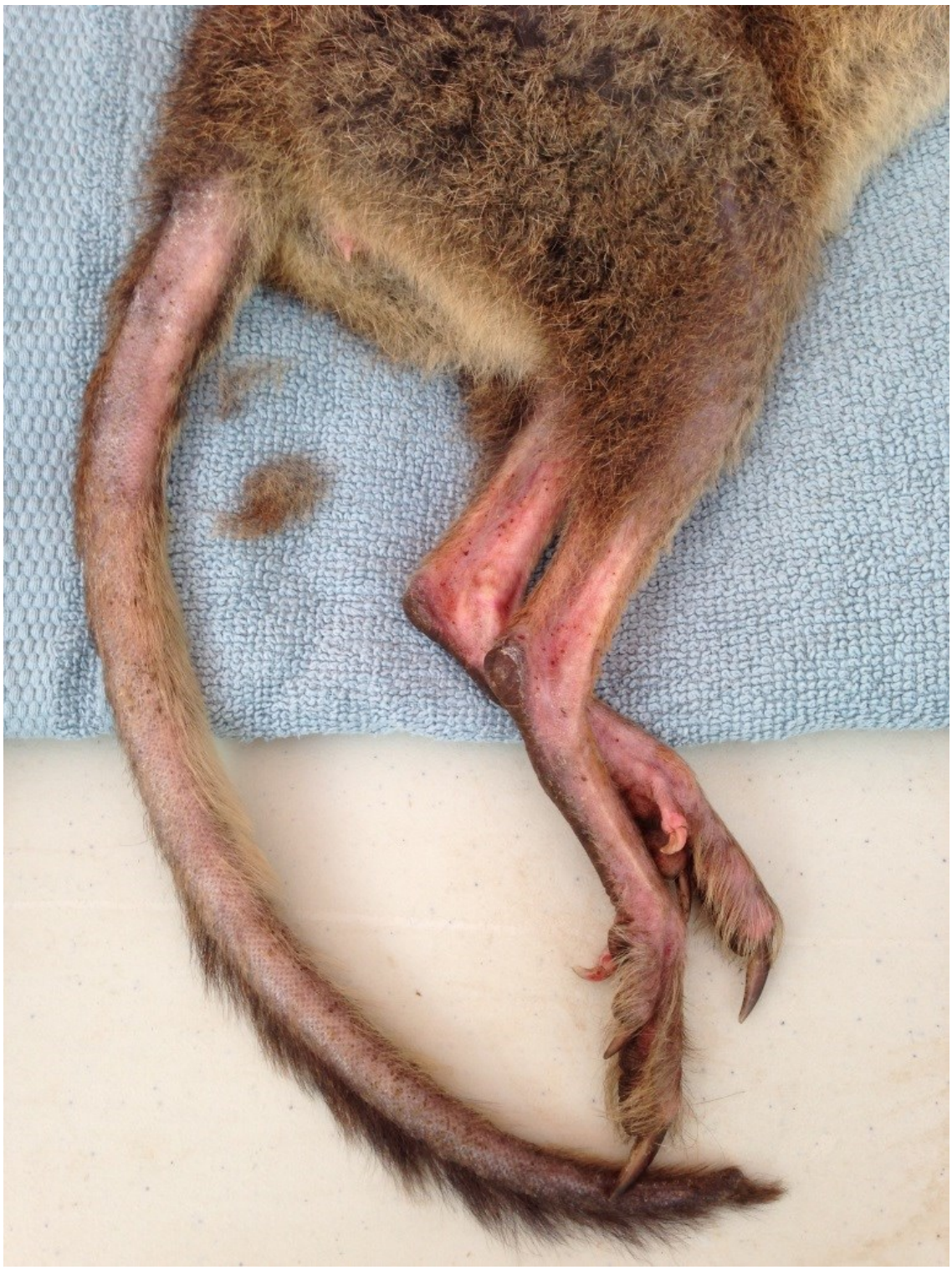

Figure 40: Lateral view of the woylie, depicting hair loss over the hindlimbs (medial thighs, tibia), tail base and tail (proximal 2 inches of tail have been clipped for blood collection) with scabbing/skin flakes evident over the flanks and rump.

Parasitology

Gastrointestinal parasites

Simple faecal flotation revealed multiple nematode larvae, 31 strongyle eggs (85 x 47.5 μm) and two *Strongyloides*-like eggs (47.5 x 32.5 μm). Within the pyloric region of the stomach, which was grossly unremarkable, 109 *Potorostrongylus woyliei* nematodes were identified. Within the duodenum, 49 *Paraustrostrongylus* sp. nematodes were present. The duodenal serosal surface had an abnormal cobblestone appearance and was oedematous. Intestinal contents were mucoid.

Haemoparasites

General PCR screening (performed twice) of blood, and peripheral blood smears were negative for trypanosomes.

Tissue parasites

PCR screening of tissue samples confirmed the presence of trypanosomes within the bone marrow, skeletal muscle, tongue, brain and liver. *Trypanosoma copemani* G2 was identified using subsequent DNA sequencing in all of these samples. PCR and DNA sequencing also revealed *Sarcocystis* sp. within the bone marrow, skeletal muscle, tongue, heart, oesophagus and spleen. *Toxoplasma gondii* was not detected.

Ectoparasites

Seven species of ectoparasite were identified; the louse *Paraheterodoxus calcaratus*, ticks (*Ixodes australiensis*, *Ixodes woyliei*, *Amblyomma* sp.), fleas (*Pygiopsylla tunneyi*, *Stephanocircus dasyuri)*; and the mite *Haemolaelaps hattanae*.

Histopathological findings

Histological changes observed within skin and lip lesions were consistent with exudative neutrophilic and eosinophilic dermatitis and cheilitis. No fungi were detected on PAS histochemistry of skin or lip lesions. Moderate numbers of intraepithelial lymphocytes were found within the lip mucosa and head skin samples and intense cytoplasmic staining was evident on CD3 immunohistochemistry. Mild eosinophilic inflammation within the soft tissues around various organs (oesophagus, skeletal muscle and eye) and within the larynx, liver, bladder and ureter was also present. Diffuse, megakaryocytic hypoplasia was detected in the bone marrow. The duodenal

tissue was moderately autolyzed, with sloughing of the superficial to mid mucosa. An unidentified larval nematode was detected within the sloughed mucosa. The stomach was histologically normal.

Microbiological findings

Bacterial culture of head and lip samples resulted in light-moderate pure growth of aerobic coagulase negative *Staphylococcus* sp. No bacterial anaerobes or fungal elements were seen. Small intestinal microbiology resulted in light growth of normal regional flora. *Salmonella* spp. were not detected, nor were bacterial anaerobes or fungal elements.

Haematological/biochemical findings

Significant biochemical changes included moderate hypomagnesaemia, mild hypokalaemia, mild hyperglobulinaemia and mild hypoalbuminaemia. The sodium/potassium (Na/K) ratio was increased and the albumin/globulin (A/G) ratio was decreased. Glutamate dehydrogenase (GLDH) and beta-hydroxybutyrate (BOHB) could not be interpreted as there are no reference values available for woylies. Using reference intervals from Pacioni et al. (2013), both haematocrit (HCT) and red blood cell (RBC) count were decreased (mild), however as both HCT and RBC count were within normal limits using Species 360 (2017) reference intervals, and haemoglobin levels were normal, these changes were deemed to be clinically insignificant. All other haematological and biochemical results were within normal limits.

Discussion

This woylie was infected with multiple parasites and had a particularly high ectoparasite burden compared with other animals encountered during this monitoring program. Due to the complicated interplay of processes by which parasites influence each other, and their host, it is difficult to determine specific parasitic influences on host health and the timeline of events that culminated in disease. Although many of the pathological changes identified in this case (poor body condition, debilitating skin disease, biochemical derangements) may be attributed to parasite infection (see below), this does not definitively establish causation.

Nematodes are usually well-tolerated by macropods, however chronic *Strongyloides* spp. infection may cause progressive anorexia, diarrhoea and weight loss (Winter, 1958; Speare et al., 1982); and mortality has been reported in captivity (Vogelnest and Portas, 2008). In this case the stomach was grossly and histopathologically unremarkable, suggesting that *Strongyloides* spp. and *Potorostrongylus woyliei* were non-pathogenic in this woylie.

In contrast, *Paraustrostrongylus* sp. nematodes were found within the small intestine and the duodenal surface had an abnormal cobblestone appearance and was oedematous. Trichostrongylid nematodes are reported to have varying degrees of pathogenicity in macropods (Vogelnest and Portas, 2008). Though little is known of the pathogenicity of *Paraustrostrongylus* sp. nematodes in woylies, closely related *Austrostrongylus* spp. are not known to be pathogenic in macropods (Spratt et al., 2008). In this case, the gross changes identified within the duodenum, and the relatively high number of *Paraustrostrongylus* sp. nematodes (extrapolating from closely related *Austrostrongylus* spp. infecting other macropods; Aussavy et al., 2011; Vendl and Beveridge, 2014), may indicate some degree of pathogenicity associated with *Paraustrostrongylus* sp. infection in this woylie; though we lack histopathological evidence to support this (duodenal tissue was autolysed).

The severe, chronic head and lip lesions associated with bacterial and parasitic infection of the skin would undoubtedly have caused discomfort while foraging and eating. Hypomagnesaemia, hypokalaemia, hypoalbuminaemia and severe generalised musculoadipose atrophy may occur with anorexia and subsequent malnutrition (Hand and Novotny, 2002; Mouw et al., 2005). Low protein has been associated with muscle wasting, impaired immunity and poor wound healing (Hand and Novonty, 2002). Gastrointestinal parasites may also promote anorexia (Symons, 1985) or induce other pathophysiological changes (e.g. malabsorption; Koski and Scott, 2001), which may promote malnutrition. As malnutrition may impair immunity and enhance susceptibility to parasite infection, a vicious cycle may ensue (Koski and Scott, 2001; Hand and Novonty, 2002).

The presence of eosinophilic inflammation is suggestive of a systemic allergic reaction, which may be secondary to endoparasite infection, but the mildness of the inflammatory infiltrate suggests that eosinophilia is unlikely to be of clinical significance. Intraepithelial lymphocytes and intense cytoplasmic staining on CD3 immunohistochemistry raise suspicion of epitheliotrophic lymphoma (Sheridan and Lefrancois, 2010; Oudejans and van der Valk, 2002). Unfortunately this could not be confirmed, as CD79 and CD20 immunohistochemistry did not work on the woylie positive control tissue (lymph node). Given the compromised state of the mucosal/skin barrier, the presence of intraepithelial lymphocytes may constitute an appropriate immune response to pathogenic microorganisms, rather than malignancy (Sheridan and Lefrancois, 2010).

While *Sarcocystis* spp. have been detected in many native mammalian intermediate hosts, including *S. bettongiae* in the closely related burrowing bettong (*Bettongia lesueur*) (O'Donaghue and Adlard, 2000), infection is thought to be generally non-pathogenic (Ladds, 2009). In other intermediate

hosts *Sarcocystis* spp. may cause debilitating disease characterised by anorexia, weight loss, anaemia, weakness, alopecia, abortion and even death (Dubey et al., 2015). In this case, we detected the molecular presence of *Sarcocystis* in various tissues, but did not identify cysts grossly or histologically; thus the pathogenicity of *Sarcocystis* in woylies remains unclear.

Likewise, the significance of the diffuse, megakaryocytic hypoplasia within the bone marrow is uncertain, particularly in the presence of a normal platelet count, and when the sample of bone marrow examined histologically was small and may not have been representative; though we cannot definitively rule out megakaryocyte destruction secondary to immune mediated or infectious disease (Feldman et al., 1988; Lachowicz et al., 2004). A decreased A/G ratio with hyperglobulinaemia is supportive of immune-mediated disease, though a decreased A/G ratio may also be associated with chronic inflammatory disease, infectious disease or neoplasia (Eckersall, 2008).

The skin lesions observed in this woylie may be attributed to ectoparasite infection. Pruritis and self-induced trauma have been documented in other macropods with ectoparasite infestations (Turni and Smales, 2001; Vogelnest and Portas, 2008). The presence of a particularly high number of normally innocuous ectoparasites in this host however, suggests the presence of immunosuppression and/or an underlying disease process. In other species, it has been proposed that trypanosomes may play an immunosuppressive role, enhancing the adverse effects of coinfecting parasites or increasing susceptibility to infection (Griffin et al., 1981; Kaufmann et al., 1992; Sileghem et al., 1994; Goossens et al., 1998; McInnes et al., 2011), although we have no evidence for this in woylies.

Immune function may also be influenced by other disease processes (e.g. neoplasia) or infectious disease agents (e.g. viral infection), which were not investigated here. Likewise, immunosuppression and enhanced vulnerability to parasite infection may occur secondary to stress (Dickens et al., 2009), which may be attributed to factors independent of (e.g. recent translocation, presence of introduced predators, season, resource availability), or associated with (e.g. malnutrition, coinfection) parasite infection; both of which may promote disease progression within a host and occur concurrently. In a parallel study (Hing et al., 2017), which examined the influence of translocation on stress physiology [measured using faecal cortisol metabolites (FCM)], FCM levels were highest in woylies from Walcott in December 2014, and there was a significant negative relationship between higher FCM levels and lower body condition. Age-related

degeneration may also compromise immune function or in the case of advanced tooth wear, may contribute to malnutrition. Tooth wear was not assessed in this case.

Conclusion

This case study highlights the complexity of parasite infection and the difficulty of elucidating mechanisms by which parasites influence host health, particularly when the chronology or the nature of host-parasite-immunological associations is unknown. Our results suggest that this woylie was unable to control normally non-pathogenic ectoparasites, which we hypothesise may be associated with immunosuppression and/or an underlying disease process. Importantly, other factors independent of disease (e.g. stress) may have induced immunosuppression and parasite-induced pathology or susceptibility to infection in this host. Given that heavy ectoparasite burdens, poor body condition and skin disease were detected during woylie population declines (Wayne et al., 2013), and an apparent association between *T. copemani* and declining woylie populations also exists, further studies are vital to explore mechanisms by which *T. copemani* G2 may influence coinfecting parasites, immune function and host health.

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Chapter Eight

Evaluating the effects of ivermectin treatment on communities of gastrointestinal parasites in translocated woylies (*Bettongia penicillata*)

8.1 Evaluating the effects of ivermectin treatment on communities of gastrointestinal parasites in translocated woylies (*Bettongia penicillata*)

The following is a published paper:

Northover, A.S., Godfrey, S.S., Lymbery, A.J., Morris, K., Wayne, A.F., Thompson, R.C.A., 2017. Evaluating the effects of ivermectin treatment on communities of gastrointestinal parasites in translocated woylies (*Bettongia penicillata*). EcoHealth 14 (Supplement 1: Health and Disease in Translocated Wild Animals): 117-127. https://doi.org/10.1007/s10393-015-1088-2. Epub 2015 Dec 30.

Abstract

Wildlife are often treated with anti-parasitic drugs prior to translocation, despite the effects of this treatment being relatively unknown. Disruption of normal host-parasite relationships is inevitable during translocation, and targeted anti-parasitic drug treatment may exacerbate this phenomenon with inadvertent impacts on both target and non-target parasite species. Here, we investigate the effects of ivermectin treatment on communities of gastrointestinal parasites in translocated woylies (*Bettongia penicillata*). Faecal samples were collected at three time points (at the time of translocation, and one and three months post-translocation) and examined for nematode eggs and coccidia oocysts. Parasite prevalence and (for nematodes) abundance were estimated in both treated and untreated hosts. In our study, a single subcutaneous injection of ivermectin significantly reduced *Strongyloides*-like egg counts one month post-translocation. Strongyle egg counts and coccidia prevalence were not reduced by ivermectin treatment, but were strongly influenced by site. Likewise, month of sampling rather than ivermectin treatment positively influenced body condition in woylies post-translocation. Our results demonstrate the efficacy of ivermectin in temporarily reducing *Strongyloides*-like nematode burdens in woylies. We also highlight the possibility that translocation-induced changes to host density may influence coinfecting parasite abundance and host body condition post-translocation.

Introduction

Fauna translocations have become an increasingly important and widely implemented conservation tool used to establish or reintroduce extirpated populations, augment existing

populations, and mitigate human-wildlife conflict (Griffith et al., 1989; Massei et al., 2010; Germano et al., 2014; Seddon et al., 2014). Our increasing reliance upon fauna translocations to augment and maintain existing populations however, has important implications for parasite transmission (using the term parasite to include both microparasites, such as viruses, bacteria and protozoa, and macroparasites, such as helminths and arthropods; Anderson and May 1992). The movement of individuals or populations from one ecosystem to another undoubtedly disrupts normal host-parasite relationships with various consequences for the translocated host and their infracommunity of parasites (Corn and Nettles, 2001; Telfer et al., 2010; Moir et al., 2012).

In addition to introducing novel parasites into naïve wild populations, and the risk of translocated hosts acquiring endemic parasites which they have not previously encountered, spill-over from wildlife to domestic and/or human hosts, and transmission between different species of wild hosts (i.e. host switching) are common disease transmission scenarios associated with translocating wildlife (Griffith et al., 1993; Thompson et al., 2010). At the population level, a sudden increase in population size post-translocation may increase contact rates between individuals thereby favouring pathogen transmission (Linklater and Swaisgood, 2008). Similarly, dispersal of hosts post-translocation may increase connectivity between susceptible hosts that may have otherwise remained spatially disconnected (Aiello et al., 2014). Importantly, amplification of parasites may occur within naive hosts subsequent to their introduction into a new environment (Kelly et al., 2009). Brush-tailed possums (*Trichosurus vulpecula*) translocated into New Zealand are a classic example, as possums became a new reservoir host for *Mycobacterium bovis* the causative agent of bovine tuberculosis following their introduction (Coleman, 1988; Daszak et al., 2001).

The effects of parasite infection in translocated hosts may be exacerbated by the stress associated with capture, clinical examination, transportation and release into a novel environment (Aiello et al., 2014). It may therefore be necessary to consider anti-parasitic drug treatment prior to translocation, particularly when dealing with critically endangered species with parasites that impose a risk to translocation success. For example, coccidian parasites are usually asymptomatic in Eurasian cranes (*Grus grus*), but may cause epidemic disease in immature birds in captive-rearing pens prior to translocation (Sainsbury and Vaughan-Higgins, 2012).

The decision to implement anti-parasitic drug treatment however, should not be taken lightly, as the ecological and evolutionary importance of parasites and the need for conserving host-parasite relationships is increasingly being recognised (Hudson et al., 2006; Gomez et al., 2012; Hatcher et

al., 2012). A growing number of studies now argue the benefit of retaining parasites during translocation, for enhancing host immunity during and post-translocation (Pizzi, 2009; McGill et al., 2010; Boyce et al., 2011), re-establishing evolutionary and ecological processes (Almberg et al., 2012) and also in conserving this element of biodiversity and the ecosystem services that it provides (Marcogliese, 2004; Gomez et al., 2012). Importantly, many parasites have established a balanced host-parasite relationship and do not induce disease in their host nor pose a threat to cohabiting species, and the very absence of these parasites may inadvertently enhance susceptibility to infection post-translocation (Viggers et al., 1993; Kock et al., 2010; Almberg et al., 2012). Furthermore, endangered species may be host to dependent host-specific parasites, and these parasites are themselves likely to be endangered (Colwell et al., 2012; Moir et al., 2012).

Unfortunately, in free-ranging wildlife, there is great difficulty in not only determining the identity, quantity and distribution of parasite communities within a host; but also elucidating how these parasites interact with their host, and each other during translocation (Sainsbury and Vaughn-Higgins, 2012; Aiello et al., 2014). Likewise, the effects of anti-parasitic drugs will vary considerably, both between- and within-species, with often unpredictable and irreversible effects in target and non-target parasite species (Pedersen and Antonovics, 2013). Alarmingly, anti-parasitic drugs are often used with no attempt to evaluate the efficacy of treatment in target or non-target parasites (Pedersen and Fenton, 2015), nor are the long-term outcomes of treatment followed up in the case of fauna translocations. For this reason, there is increasing interest in the effects of anti-parasitic drug treatment in wild hosts and their consequences on parasite community structure (Fenton, 2013; Pedersen and Antonovics, 2013; Vaclav and Blazekova, 2014; Pedersen and Fenton, 2015).

The woylie or brush-tailed bettong (*Bettongia penicillata*), is currently listed as critically endangered by the International Union for Conservation of Nature (IUCN, 2013). Having undergone a 90% decline in population size over seven years (1999-2006) the woylie is now confined to only three wild populations; the two largest of which exist within the Upper Warren region (UWR) of Western Australia (Kingston and Perup populations) (Wayne et al., 2015). While woylie numbers remain low but relatively stable within the UWR, these populations are vulnerable to local extinction due to pressure from key threats (introduced predators and disease) and stochastic events (Wayne et al., 2013). Due to the fragmented distribution of remaining woylie populations and the need to maintain genetic diversity between wild and captive populations, fauna translocations play a pertinent role in the conservation management of this species.

The focus of the current study was to evaluate the effects of ivermectin on nematode egg counts and coccidia oocyst shedding in woylies during translocation and to identify the factors that are most influential in determining parasite infection patterns of woylies post-translocation. We also wanted to determine whether ivermectin treatment conveys any benefit to the health of translocated hosts. We predicted that ivermectin would decrease nematode abundance in treated woylies, but would increase the prevalence of coccidia, as a reciprocal relationship between nematode and coccidian burdens has been previously reported in small mammals (Pederson and Antonovics, 2013). We also expected that ivermectin, by reducing the nematode burden, would improve host fitness (assessed indirectly by body condition) post-translocation.

Materials and methods

Study sites

Woylies selected for translocation were trapped from Perup Sanctuary, a 423 ha predator-proof enclosure located within the Tone-Perup Nature Reserve ~50 km east of Manjimup (34.2506 °S, 116.1425 °E). Perup Sanctuary was initially established in 2010 to support a genetically diverse insurance population of woylies during the decline. Our two destination sites, Warrup East and Walcott, are located within the Greater Kingston region of the Upper Warren approximately 15 km west and 20 km north-west of Perup Sanctuary, respectively (Figure 41). All three sites (Perup Sanctuary, Warrup East and Walcott) have a Mediterranean-style climate with very similar geography and vegetation (Wayne et al., 2015).

Trapping regime

In June 2014, 182 woylies were translocated from Perup Sanctuary to Warrup East (n = 90) and Walcott (n = 92). Woylies were captured using Sheffield cage traps (Sheffield Wire Products, Welshpool, WA), which were baited with standard universal bait (rolled oats, peanut butter and sardines). At translocation (June), woylies were captured using multiple transects (≤280 traps/night, 100 m spacing) over three consecutive nights. Post-translocation monitoring was conducted at one (July) and three (September) months post-translocation and consisted of transect trapping (≤106 traps/night, 100-200 m spacing) for three-four consecutive nights. Traps were covered with a large hessian bag to provide shelter from the weather and prevent exposure.

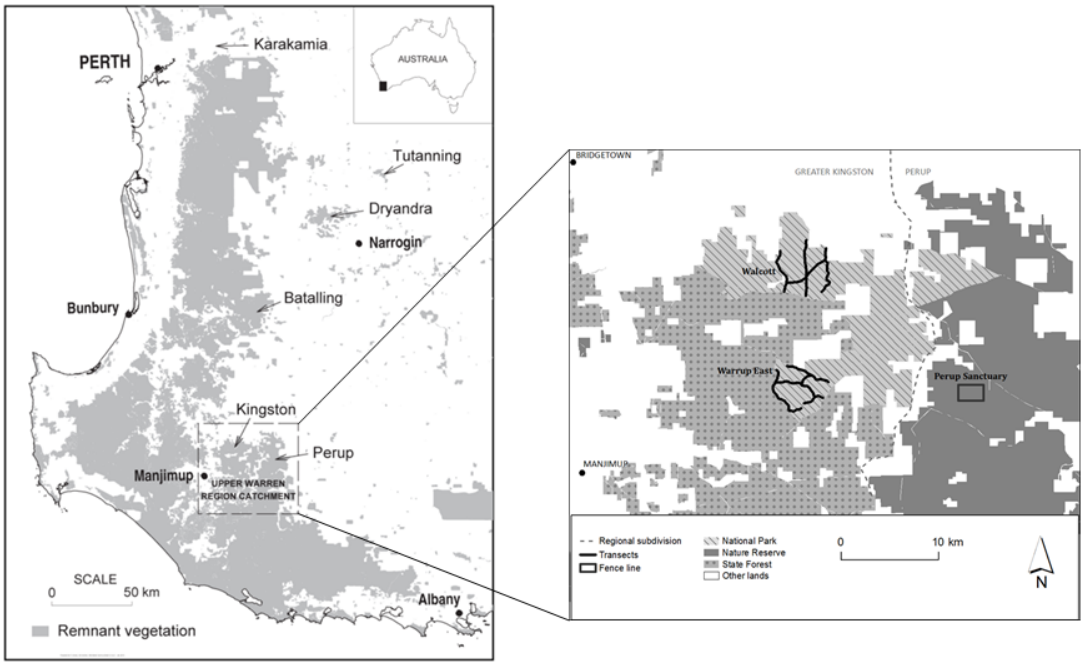

Figure 41: Map showing the Upper Warren region in the southwest of Western Australia. The box depicts Perup Sanctuary towards the East and our two destination sites, Walcott and Warrup East, within the Greater Kingston region toward the west.

Sample collection and ivermectin treatment

Woylies that were captured and deemed suitable for translocation were identified with ear-tags and their weight, age, sex, head length, pes length, and reproductive status was recorded in the field. At the point of translocation, 93 of the 182 woylies (equal ratio of male to female) were treated with a single subcutaneous injection of Ivermectin (Ivomec®) at a dose rate of 0.2 mg/kg, and approximately half of the treated individuals were translocated to each site (46 Warrup East, 47 Walcott). The remainder of the woylies were left untreated and acted as controls. During both pre- and post-translocation trapping, faecal samples were collected in the field from traps and retained for parasitological examination. For woylies that were captured more than once during a trapping session, we only included the first faecal sample collected in our analyses. Faecal samples were stored in 10 per cent buffered formalin at 4°C until processing. Storage of faecal samples was required prior to processing because of the number of hosts sampled and the remote location of the field site; 10% formalin is the best fixative for egg recovery following long-term storage (Foreyt, 1986). All except one faecal sample were processed within 6 months of collection.

Faecal analysis

An inherent difficulty in working with critically endangered species is the inability to directly quantify gastrointestinal parasite abundance via post-mortem. We therefore used egg counts as a non-invasive estimate of nematode abundance. As egg morphology alone cannot be used to distinguish gastrointestinal nematode species (Spratt et al., 2008), we identified faecal parasites as either nematode eggs or coccidia. Simple faecal flotation using sodium nitrate ($NaNO_3$) solution in deionised water (SG 1.37) was used to examine all faecal samples (modified from Zajac and Conboy, 2012). Formalin fixed faecal samples were centrifuged (2000 rpm for 2 minutes), washed with deionised water and centrifuged a second time (2000 rpm for 2 minutes). One gram of faeces was then placed into a Fecalyzer® container (EVSCO Pharmaceuticals, USA), mixed with 12.5ml of flotation solution and a 22 x 22 mm glass cover slip was placed on top. Each sample underwent flotation for fifteen minutes before being examined in a systematic manner at 10 x magnification using an Olympus BX50 microscope. For nematodes, all of the eggs observed under the cover slip and around the edges were manually counted, giving a semi-quantitative estimate of parasite abundance (e.g. O'Handley et al., 2000; Inpankaew et al., 2014). For coccidia, we scored oocysts as present or absent, but did not estimate oocyst abundance. Prevalence of infection was scored as the proportion of infected individuals, with 95% confidence intervals calculated assuming a binomial distribution.

Statistical analysis

Generalised linear mixed effects models were used to evaluate the impact of ivermectin treatment on gastrointestinal parasites of woylies during the translocation. Each parasite type was analysed separately as a dependent variable. The number of strongyle eggs was log-transformed to normality, and a Gaussian error term was used. The number of *Strongyloides*-like eggs was analysed using a negative binomial error term, and we considered the prevalence of coccidia using a model with a binomial error term. Woylie ID was included as a random effect to account for the repeated measures of individuals through time. In each model, ivermectin treatment (Y/N), month (June, July and September), site (Perup (origin), Warrup East and Walcott), and two way interactions between Ivermectin and month, and month and site were included as independent variables.

In a separate analysis, we evaluated the impact of ivermectin treatment, site and month on the body condition of woylies. We derived a measure of body condition from a mixed-model (including woylie ID to account for repeated measures on individuals) regression between body weight (g) and head length, separately for each sex (males and females). Head length was the only

morphometric variable linearly related to body weight for both males ($r = 0.301$, $X^2 = 5.36$, $df = 1$, $P = 0.021$) and females ($r = 0.354$, $X^2 = 8.29$, $df = 1$, $P = 0.004$). The residuals from these regressions were used as an index of body condition, and included as a dependent variable in the models. We ran the model separately for males and females, including the same set of independent variables and interaction terms as for the parasite analyses, with a Gaussian error term. For females, we included pouch young size (mm) as an additional variable to account for the influence of pouch young on calculations of body condition.

Results

In total, we analysed 139 faecal samples (Table 10). Two visually distinct nematode eggs were recognised (Figure 42). The first, which we refer to as strongyle eggs, were typically unembryonated eggs measuring between 80-100 µm in length. The second, which we refer to as *Strongyloides*-like eggs, were embryonated eggs measuring roughly 50 µm in length. We also identified unsporulated coccidia measuring approximately 30 µm in length (Figure 42).

Table 10: Summary of faecal samples that were analysed from each site (treated *versus* untreated). A total of 27 woylies were captured at both time points.

	Walcott		Warrup East		Total
	Treated	Untreated	Treated	Untreated	
June	10	12	20	14	56
July	4	10	13	8	35
September	8	11	18	11	48
	22	33	51	33	**139**

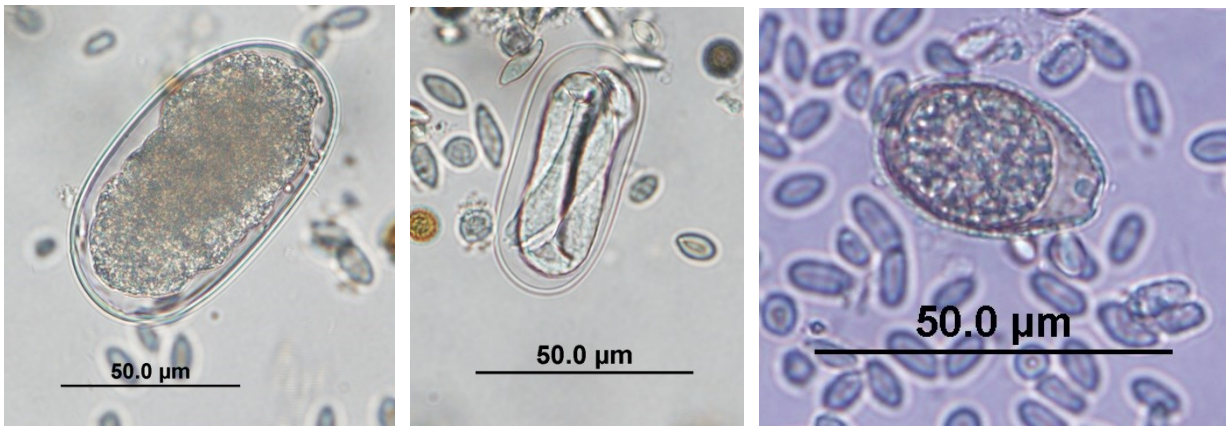

Figure 42 (left to right)**:** Typical strongyle egg, *Strongyloides*-like egg and unsporulated coccidian oocyst detected in woylie faeces.

Treatment with ivermectin significantly reduced *Strongyloides*-like egg counts in woylies post-translocation (Table 11). *Strongyloides*-like egg counts were also influenced by month, with egg counts significantly lower in treated hosts one month post-translocation. An interaction between month and ivermectin was also identified where egg counts increased in treated hosts between one and three months post-translocation. By three months post-translocation there was little difference between the egg counts of treated and untreated hosts (Figure 43).

Table 11: Results from Generalised Linear Mixed Model analysis of factors influencing nematode egg counts and coccidia prevalence post-translocation.

	Strongyles			*Strongyloides*			Coccidia		
	X^2	*df*	*P*	X^2	*df*	*P*	X^2	*df*	*P*
Month	5.93	2	**0.051**	10.08	2	**0.006**	0.81	2	0.666
Ivermectin	0.17	1	0.677	4.50	1	**0.034**	0.59	1	0.442
Site	66.21	1	**<0.001**	0.46	1	0.497	0.11	1	0.741
Month x Ivermectin	2.77	2	0.250	20.58	2	**<0.001**	0.69	2	0.709
Month x Site	0.45	1	0.503	0.78	1	0.378	9.77	1	**0.002**

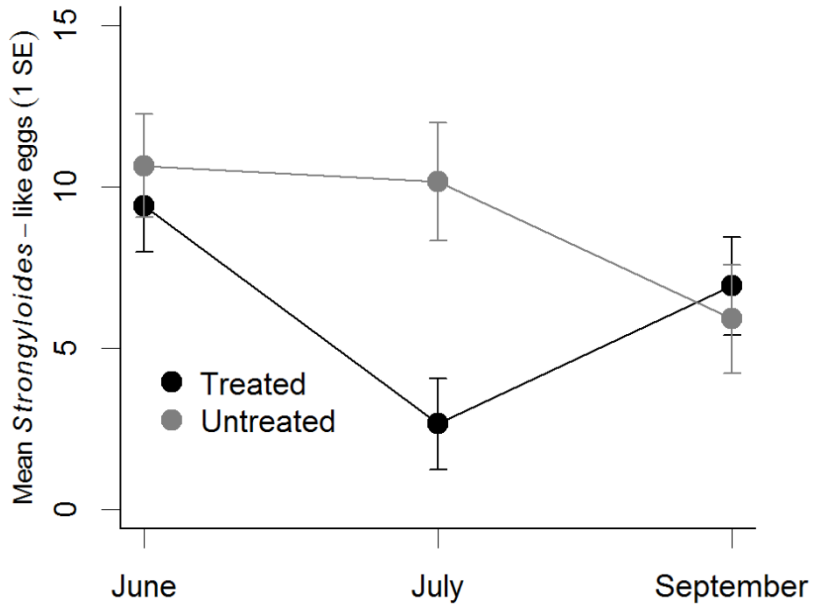

Figure 43: Effect of ivermectin treatment on *Strongyloides*-like egg counts in woylies.

Ivermectin did not significantly reduce overall strongyle egg counts in translocated woylies, and there was no interaction between month and ivermectin. However there was a marked effect of site (Table 11) with egg counts higher within Walcott compared to Warrup East in July and September (Figure 44). Similarly, coccidia prevalence was not influenced by ivermectin treatment,

month of sampling or translocation site, but a significant interaction between month and site was detected (Table 11). Within Walcott we observed an increase in prevalence one month post-translocation followed by a subsequent decline between one and three months post-translocation. Woylies that were translocated into Warrup East in comparison, had a slight decrease in prevalence one month post-translocation followed by a small increase between one and three months post-translocation (Figure 45).

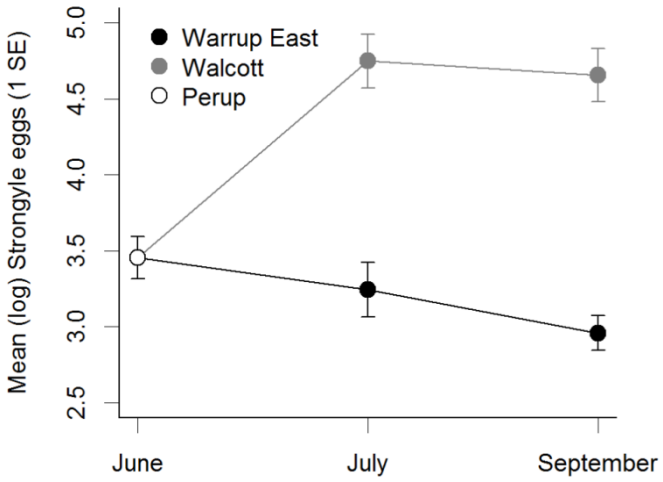

Figure 44: Effect of site on strongyle egg counts in woylies post-translocation.

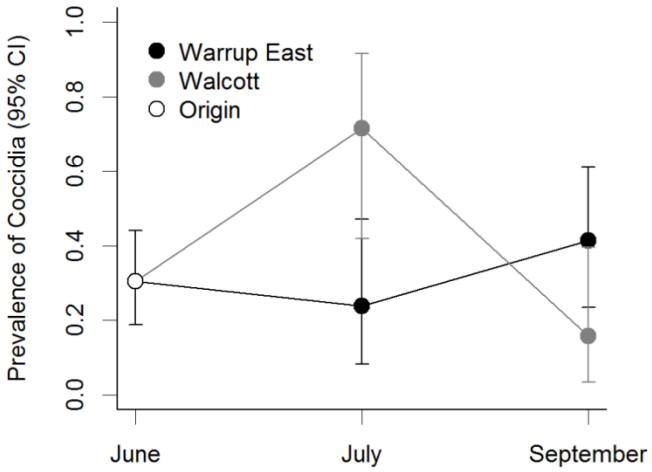

Figure 45: Coccidia oocyst prevalence in woylies pre- (June) and post-translocation (July and September).

Month, but not ivermectin treatment or site, significantly influenced body condition in woylies post-translocation (Table 12). We observed an overall increase in body condition post-translocation, with body condition increasing in July and again in September (Figure 46).

Table 12: Results from Generalised Linear Mixed Model analysis of factors influencing body condition in woylies post-translocation.

	Males			Females		
	X^2	*df*	*P*	X^2	*df*	*P*
PY size	-	-	-	**6.574**	**1**	**0.010**
Ivermectin	0.508	1	0.476	0.001	1	0.990
Month	**26.260**	**2**	**<0.001**	**25.843**	**2**	**<0.001**
Site	1.502	1	0.220	0.575	1	0.448
Month x Site	1.247	1	0.264	0.580	1	0.446
Ivermectin x Month	1.718	2	0.423	2.338	2	0.310

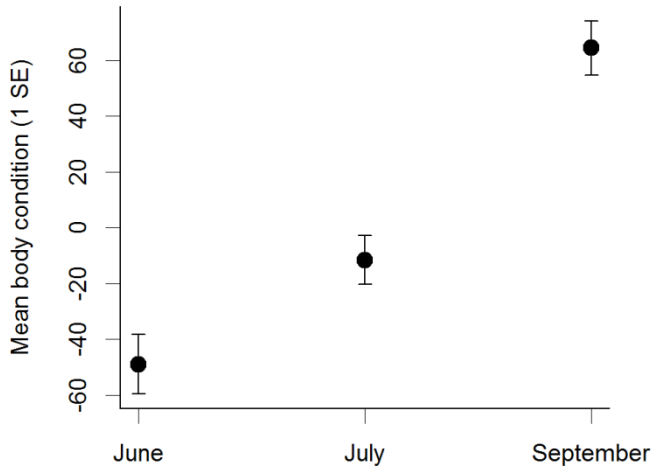

Figure 46: Effect of month on body condition in woylies post-translocation.

Discussion

The administration of anti-parasitic drugs to wildlife prior to translocation is inconsistently carried out, and the efficacy of such treatment is rarely followed up to determine its effectiveness and the potential impacts on both target and non-target parasites, and benefits to the host. Here we demonstrate that ivermectin administered to woylies at the point of translocation was only effective in temporarily reducing *Strongyloides*-like egg counts. There were no detectable effects of treatment on other target or non-target gastrointestinal parasites in this study. The fact that we only observed an effect of treatment in *Strongyloides*-like nematodes may reflect interspecies or inter-individual variation in susceptibility to ivermectin. Cripps et al. (2013) assessed the anthelmintic efficacy of ivermectin at recommended dose rates in free-ranging eastern grey kangaroos (*Macropus giganteus*) and found poor efficacy of this drug in reducing egg counts in strongylid nematodes. *Strongyloides* spp. nematodes also have a systemic migratory phase, which may enhance susceptibility to ivermectin when in the tissues compared to strongyle nematodes in the gut.

The *Strongyloides*-like eggs that we describe here are likely to be similar to those described in other macropods, and at least one undescribed *Strongyloides* sp. has been identified in woylies (Elliot pers. comm., 2015). Given that infection with species of *Strongyloides* has been associated with morbidity and mortality in captive macropods (Speare et al., 1982) and that "all macropod species should be considered susceptible to infection with this parasite" (Vogelnest and Portas, 2008), elimination of *Strongyloides* sp. may confer some benefits to the host, particularly during the immediate post-translocation period in which stress-induced physiological changes may negatively impact host health (Aiello et al., 2014). Despite a significant effect of ivermectin treatment in reducing *Strongyloides*-like egg counts in in this study, we found no benefit of treatment to host health, as measured by body condition.

In a review of anti-parasitic drug treatment experiments in wildlife, Pedersen and Fenton (2015) found that in many cases there was no benefit of treatment to host health, and treatment efficacy varied between and within host-parasite systems. Anthelmintic treatment in juvenile eastern grey kangaroos for example, had no effect on body condition (Cripps et al., 2014). We speculate that the overall increase in body condition that we observed over time following translocation is secondary to enhanced resource availability and reduced competition within our destination sites. As woylies were translocated from a site with greater density (Wayne pers. comm., 2015) body condition would be expected to improve in the absence of resource limitation and competition.

As ivermectin is well known for its broad-spectrum activity against gastrointestinal nematodes in domestic and companion animals (Campbell and Benz, 1984) we also expected treatment to reduce strongyle egg counts. However, we found no effect of treatment on strongyle abundance. There are a number of possible explanations for this unexpected result. Firstly, using faecal flotation to screen woylies for the presence of gastrointestinal parasites has its limitations. Stringer et al. (2014) support the use of faecal egg counts as a reliable indicator of parasite abundance for strongyle-type nematodes, however egg counts may underestimate parasitism when there is low parasite burden, reduced fecundity, immature or arrested parasites, intermittent egg shedding or mixed infections (Bordes and Morand, 2011; Emery, 2014). Likewise, seasonal variation can influence faecal egg production in macropods (Cripps et al., 2015).

Another consideration that also highlights one of the major limitations of using anti-parasitic treatment in wildlife, is whether we selected the appropriate drug, administered it at the correct dose and/or via the correct route. We administered ivermectin subcutaneously, the route which

offers the greatest bioavailability, longest duration of activity and improved efficacy (Canga et al., 2007). We also know that each individual received the exact dose, as compared to oral administration in which there is a risk that not all of the dose will be swallowed or topical administration in which not all of the drug may be absorbed.

Administering ivermectin via injection however, is not fool-proof. Due to the propensity of ivermectin to accumulate within fat tissue, faster absorption may be observed in animals with poorer body condition, which may reduce its presence in the bloodstream thereby decreasing long-term efficacy (Canga et al., 2007). Cripps et al. (2013) suggest that macropod species in general have fewer fat reserves compared to domestic livestock and may therefore require higher doses. Eastern grey kangaroos that were administered twice the recommended dose of moxidectin had a greater reduction in faecal egg counts than kangaroos that were administered standard doses, providing some evidence to support this theory (Cripps et al., 2013). Unfortunately there are no clinical trials that demonstrate the efficacy of ivermectin in reducing endoparasite loads in woylies. Previous studies report the use of ivermectin in closely related species such as the Eastern bettong (*Bettongia gaimardi*) (Portas et al., 2014), although follow-up data evaluating its effectiveness in reducing gastrointestinal parasites is not yet available.

Ivermectin was administered at the dose reported by Portas et al. (2014), and toward the lower end of the reference range (0.2-0.4 mg/kg) recommended for the treatment of helminthiasis in macropods (Vogelnest and Portas, 2008). Vogelnest and Portas (2008) specify that repeat doses may be required, but this is not practical in the case of translocating woylies, and may have contributed to the results in our study.

Finally, we found no effect of treatment on coccidia prevalence. While ivermectin treatment would not be expected to have any direct effect on coccidia, Pederson and Antonovics (2013) found that experimental reduction of intestinal nematodes with ivermectin resulted in a reciprocal increase in coccidia prevalence in the white-footed mice (*Peromyscus leucopus*). Václav and Blažeková (2014), however, found no effect on oocyst shedding in marmot (Marmot marmot) treated with a combination of ivermectin and praziquantel. Fenton (2013) theoretically describes how de-worming an individual with a low to moderate helminth parasite burden can be detrimental to a host that is co-infected with microparasites (e.g. protozoa). This occurs because the presence of helminths may either assist the host in eradicating co-infecting pathogens, or diminish the pathogen's negative impact on the host. The interaction between co-infecting helminths and

microparasites, however, can be complex and context dependent, depending not only immune interactions, but also on resource competition among parasite species (Graham, 2008; Fenton, 2013). As we found no effect of treatment on strongyle nematodes, a reduction in *Strongyloides*-like nematodes alone may have been insufficient to reverse any antagonistic force imposed by nematodes within the gut; or *Strongyloides*-like nematodes may have no influence on coccidia prevalence.

Ivermectin did not influence coccidia prevalence post-translocation, but there was a significant interaction between month and site, with a spike in prevalence detected at Walcott one month post-translocation. In addition, the percentage of woylies classified as having a severe coccidia burden increased from 3.6% to 20% (n = 7) during this timeframe, and 71.4% (n = 5) of these cases were reported in woylies at Walcott. Stress associated with translocation may explain the post-translocation spike in coccidia prevalence at Walcott, which had a higher density of woylies than Warrup East. In marsupials, translocation has been identified as a significant stressor capable of inducing physiological changes within a host (Hing, 2014) and coccidia are capable of inducing clinical disease in a novel host in a new environment (Kock et al., 2010).

We also detected a significant effect of site on strongyle egg counts, which increased almost three-fold within Walcott post-translocation. During pre-translocation sampling, we captured 66 resident woylies within Walcott compared to 33 resident woylies within Warrup East. With twice the woylie density of Warrup East, we postulate that density dependent transmission may be driving parasite abundance at Walcott. Stringer and Linklater (2015) found that host density powerfully predicts the abundance of strongyle nematodes in Black rhinoceros (*Diceros bicornis*) at the population level. As the density of woylies within Perup Sanctuary is higher than either Walcott or Warrup East, by increasing the overall number of woylies within a moderately dense site such as Walcott, woylie density may have been high enough to maintain a high prevalence of infection within Walcott post-translocation.

It is evident that fauna translocations create a unique scenario for parasite transmission and the unpredictable effects of anti-parasitic treatment may add another realm of uncertainty. Some studies acknowledge that there should be some benefit of treatment for the host that outweighs the cost of eliminating this parasite from the ecosystem before anti-parasitic treatment is administered (Stringer and Linklater, 2014). However anti-parasitic treatments are still often applied in an ad hoc manner and without a justifiable reason for doing so. With few studies that

document the effects of anti-parasitic drug treatment in wild populations, or lack of follow up in many cases, it is difficult to determine what best practice should be.

Conclusion

This study provides empirical evidence that a single subcutaneous dose of ivermectin (0.2 mg/kg) temporarily reduces *Strongyloides*-like egg counts in woylies post-translocation. We observed no effect of treatment in other target or non-target gastrointestinal parasites, and we found no benefit of treatment to host health. Instead, translocation-induced perturbations to population density were influential in driving parasite abundance and shaping host health. With no apparent benefit of treatment to host health, we question the use of ivermectin in woylies during translocation. Further studies are needed to investigate the effects of alternative anti-parasitic drugs, particularly those that target different parasite groups (e.g. protozoa) to determine whether anti-parasitic treatment is warranted during translocation. Likewise, studies that explore the impacts of different parasite groups on host health may help influence drug selection and improve translocation outcomes.

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Chapter Nine

General Discussion

9.1 General discussion

This thesis aimed to investigate the impact of parasite infection and ivermectin treatment on parasite community structure and host health in a critically endangered marsupial host, the woylie (*Bettongia penicillata*), following translocation. This longitudinal, field-based study involved monitoring over 600 individual woylies for up to 12 months during the course of two fauna translocations to supplement three different sites; a large-scale undertaking, which has provided some of the first comprehensive insights into how parasite community structure changes following translocation. This is the first study to examine the broader parasite community (i.e. blood-borne endo- and ectoparasites) and monitor the response of parasites following translocation in both translocated and resident animals. Insights gained from this study are relevant to the management of threatened species and their parasite taxa, with a number of key considerations for the future planning and implementation of fauna translocations. The main overarching observations from this study are discussed below, with particular reference to how these observations are transferable to the broader field.

Key results from this study are summarised as follows. In Chapter 1, I provided a general overview of the way in which fauna translocations and antiparasitic drug treatment may influence parasite community structure, host health and translocation success; emphasising the knowledge gaps in this field, concerns regarding the ad-hoc use of antiparasitic drugs, and the inadequacy of current parasite monitoring during fauna translocations. I then introduced the study species, and defined the study aims, objectives and hypotheses. In Chapter 2, I described the overall study design, outlining the study sites, trapping regime, sample collection/processing methodology, and the 'ivermectin treatment trial'.

In Chapter 3, I examined how the broader parasite community (i.e. gastrointestinal, blood-borne and ectoparasite taxa) changed following translocation and in response to ivermectin treatment. Following translocation, the parasite communities of translocated and resident animals converged to become more similar over time, with failure of some parasite taxa to persist and new host-parasite associations emerging. Ivermectin treatment did not significantly reduce the prevalence or abundance of any target parasites, though a significant interaction between ivermectin treatment and site was identified for trypanosomes. Changes to the parasite community were most pronounced immediately after translocation and varied significantly across sites. In Chapter 4, I formally described a novel species of *Eimeria* (*E. woyliei*) which was identified from woylies during

this study. This chapter also genetically characterises three other *Eimeria* spp. (*E. gaimardi*, *E. potoroi*, and *E. mundayi*) from other potoroid marsupials; no potoroid *Eimeria* spp. had been genetically characterised prior to this study and *E. woyliei* is the sixth species of *Eimeria* to be described from Potoroidae.

Chapter 5 investigated the effects of parasite infection and ivermectin treatment on a finer scale, by monitoring the response of haemoparasites [(*Trypanosoma* spp. and piroplasms (*Babesia* and *Theileria* spp.)] following translocation. Similar trends to those identified in Chapter 3 were reinforced, while *Trypanosoma* spp. richness and the prevalence of haemoparasite coinfection increased following translocation, suggesting that the outcome of fauna supplementations may differ from reintroductions where parasite loss typically occurs. Indirect effects of ivermectin treatment were identified for *Trypanosoma vegrandis* and *Trypanosoma* spp. polyparasitism, however these effects were small, and varied between sites, making it difficult to deduce the biological significance of these changes.

Chapter 6 evaluated the impact of parasite infection and ivermectin treatment on host health [as measured using body condition (BCI), packed cell volume (PCV) and total plasma protein (TPP)]. Changes to BCI after translocation were strongly associated with site and TST. The presence of coccidia during the first three months following translocation and higher *Strongyloides*-like egg counts were associated with lower body condition in translocated woylies, while strongyle infection and higher parasite infracommunity richness were associated with lower body condition in resident woylies. In translocated woylies, ivermectin treatment did not significantly reduce parasite prevalence or mean FEC in target parasites, and there was no discernible benefit of treatment to host health.

Chapter 7 investigated disease aetiology in a resident woylie, which was found in extremely poor body condition with diffuse alopecia, debilitating skin lesions and severe ectoparasite infestation six months after translocation. Clinical signs were similar to those observed in woylies during the decline and *T. copemani* G2 was observed in the tissues of this host, emphasising the need to further explore mechanisms by which potentially pathogenic *Trypanosoma* spp. may impact coinfecting parasites, immune function and host health.

Lastly, Chapter 8 focuses on the effects of ivermectin treatment on gastrointestinal parasites and host health in a subset of woylies. Results from this preliminary study (data collected during the

first translocation only) empirically demonstrated a temporary reduction in *Strongyloides*-like egg counts in response to ivermectin treatment. This effect was only transitory however, and with no apparent benefit of treatment to host health, the use of ivermectin was questioned.

Overall, this study enhances our knowledge of parasites infecting the woylie, and substantially increases our understanding of host-parasite dynamics during fauna translocations. Notably, three main observations were identified, all of which have relevance to conservation translocations and improving translocation outcomes, regardless of species. Each of these observations are expanded upon (including study limitations) in subsequent sections of this chapter.

1. The response of parasites (and host health) following translocation differed significantly between sites. Changes to the parasite community of translocated hosts were strongly impacted by site-associated factors including the type and prevalence/abundance of parasite fauna infecting resident woylies. The dominant site-specific response of parasites reinforces the value of parasite monitoring to enhance our fundamental understanding of perturbations in host-parasite systems during translocation, in particular the site-level drivers of parasite dynamics.

2. The parasite communities of translocated and resident hosts generally converged to become more similar over time. Loss of parasite taxa and new host-parasite associations are likely to occur following translocation, thus it is imperative that translocation protocols incorporate long-term parasite monitoring and consider the intrinsic biodiversity value of parasites (Colwell et al., 2012; Thompson et al., 2018).

3. With the exception of a temporary reduction in *Strongyloides*-like egg counts in a subset of woylies, the administration of ivermectin did not significantly reduce the prevalence or abundance of target parasites during this study, and there was no discernible benefit of treatment to host health. In the absence of a justifiable reason to use antiparasitic drugs (i.e. evidence that treatment reduces the prevalence of target parasites and improves host health), parasite conservation and preservation of the fundamental host-parasite relationship should be a key consideration in the future planning and implementation of fauna translocations.

9.2 Site specificity of parasite dynamics

Parasite prevalence and abundance were highly site-specific, such that site had the strongest effect on parasite dynamics following translocation. Site was also an important factor governing host health. The dominant site-specific response of parasites following translocation reinforces the importance of parasite monitoring and well-designed scientific studies to gain a greater understanding of host-parasite ecology, in particular the site-level drivers of parasite dynamics. Based on the observations from this study, host density was postulated to be a potential driver of parasite dynamics, however various other ecological factors (e.g. contrasting environmental conditions, contact with cohabiting species or vector prevalence) may also affect parasite dynamics and were not evaluated here. Likewise, as I did not monitor the source populations from which translocated hosts may have acquired infection, it is difficult to separate 'new' infections (i.e. those obtained within the release site) from those that subsequently became detectable following translocation (i.e. recrudescence of pre-existing, previously undetectable infection). While increased detection of pre-existing infections is likely to be true for certain parasite taxa (e.g. gastrointestinal parasites such as coccidia), this is less likely to be the case for ectoparasites or *Trypanosoma* spp. (with the exception of *T. copemani* G2). Differences in the response of specific parasites (e.g. *T. copemani* within Dryandra; Chapter 5) suggest intrinsic parasitic traits (e.g. the proposed ability of specific *Trypanosoma* sp. genotypes to invade cells) may also be an important driver of parasite dynamics post-release; although not an effect of site per se, conducting this study across different sites enabled me to identify this phenomenon.

Given the significant impact of site, investigating the parasite taxa infecting conspecifics within a planned release site prior to translocation will provide valuable insight into the type of parasite taxa a translocated animal is likely to encounter following release. During this study, I monitored resident woylies for up to two months prior to translocation. This enabled me to establish baseline data of the parasite taxa endemic within each destination site, however this relatively short timeframe only provided a snapshot of the parasite community at that particular time of year; both time and financial constraints were limiting factors influencing this decision. Thus, seasonal fluctuations in parasite prevalence/abundance were not specifically examined, reducing the ability to interpret changes in parasite dynamics following translocation. For example, an increase in parasite prevalence at a particular time may represent normal temporal trends in parasite prevalence or abundance, rather than a response to translocation. The inclusion of a control site that monitors woylies in the absence of translocation would enhance the ability to interpret such

changes. Likewise, monitoring the source populations from which translocated hosts were obtained would enable the separation of 'new' from 'pre-existing' infections.

Longer-term monitoring (i.e. over several years) would have been ideal for identifying 'normal' parasite trends and evaluating the outcome of specific parasite taxa following release (e.g. strongyle nematodes within Dryandra; Chapter 3). While post-translocation monitoring over a three year time frame was initially planned, a second translocation into a spatially independent study site (Dryandra) was incorporated into the study design to gain a greater understanding of the factors impacting parasite dynamics during translocation. Although the time period of post-translocation monitoring was reduced, monitoring was undertaken at regular intervals for up to 12 months; the minimum acceptable standard for post-release monitoring (to estimate population size) proposed by Sutherland et al. (2010). Importantly, the inclusion of a spatially independent study site (Dryandra) emphasised the significance of site as a factor impacting parasite community structure following translocation.

Despite the acknowledgement that post-release monitoring should form an essential component of translocation protocols (IUCN/SSC, 2013) there are no standardised recommendations for parasite monitoring. In the field of wildlife health, this is largely due to scarcity of data. Knowledge regarding the type of parasites, their prevalence, lifecycles and potential pathogenicity within wild populations is limited, as is our understanding of host-parasite ecology. Significant biological variability between different host species, their parasites and site-specific ecosystem processes that affect host-parasite interactions also limits a one-size-fits-all approach. While guidelines for wildlife disease risk analysis promote health surveillance (e.g. OIE/IUCN, 2014; Hartley and Sainsbury, 2017), the timeframe of parasite screening is often limited (e.g. a one off sampling event prior to translocation) and there is considerable reliance on published scientific literature for identifying potential disease hazards. As the literature on parasites infecting wild populations of threatened species if often limited, I propose that active parasite screening (e.g. Vaughan-Higgins et al., 2016) should always be employed during translocation.

With increasing recognition of the intrinsic biodiversity value of parasites (Colwell et al., 2012; Thompson et al., 2018), the potential loss of parasite fauna should also be a serious consideration when planning fauna translocations; particularly with regard to host-specific parasites that are likely to be endangered themselves (Colwell et al., 2012). Given the significant impact of site, it would have been valuable to examine the effects of translocation on individual parasite species,

instead of higher level taxa. For host specific parasites such as *Ixodes woyliei* for example, the risk of extinction following translocation is likely to be higher than generalist ectoparasites with multiple host species (e.g. *Amblyomma* sp. ticks; Waudby et al., 2007).

Lastly, a number of parasite taxa identified in this study appeared to be site specific. For parasites with low prevalence (e.g. lungworm larvae and *Potoroxyuris* sp. nematodes), it was difficult to interpret whether their absence from certain sites was real or an effect of sampling bias. For example, *Potoroxyuris* sp. nematodes were predominantly detected in March, however Walcott and Warrup East were not sampled at this time. For comparative purposes, careful consideration of the timing (i.e. frequency, duration and synchronicity) of parasite sampling is important. Despite a number of time points being consistent during this study, not all were comparable. During the first translocation, for example, woylies were not monitored in August, a significant time point within Dryandra; thus important trends may have been missed. While every effort was made to ensure trapping times/regimes were similar between sites, the timing of fieldwork was ultimately determined by DBCA (the collaborative partner in this study) and the availability of staff. Trap numbers also had to be modified according to site to ensure that ethics requirements were met and all traps were cleared within three hours of sunrise each day (i.e. fewer traps were set in sites with higher animal density such as Walcott).

9.3 Changes to the parasite community over time

Translocation-induced changes to the parasite community are seemingly likely and inevitable. This study, for example, showed that the parasite communities of translocated and resident woylies generally converged to become more similar over time. Loss of parasite taxa and new host-parasite associations were also observed following translocation. Of particular concern is whether these changes have the potential to significantly impact host health and translocation outcomes. Parasite monitoring not only enhances our ability to anticipate such changes, but it also enables us to identify and respond to biologically significant changes that do occur, thereby improving the management of threatened fauna and their parasite taxa during future translocations.

An important observation from this study was that major changes to the parasite community (including loss of parasite taxa) occurred relatively quickly (i.e. within the first few months after translocation). As highlighted previously, there are no universal guidelines for parasite monitoring during translocations. Results from this study indicate that the immediate post-translocation period is an important time to conduct follow-up monitoring. The detection of novel host-parasite associations at 9-12 months following translocation also demonstrates the importance of long-term parasite monitoring, which ultimately should be the goal. Long-term monitoring is important for drawing sound conclusions regarding the outcomes of parasite infection following translocation. Within Dryandra, for example, fleas were not detected in translocated hosts after August, suggesting that this parasite taxon may have failed to persist. However, given that fleas were detected in a Dryandra resident woylie 12 months after translocation, it is possible that translocated hosts might have acquired flea-infection after monitoring ceased.

Adequate sample size is also essential for the detection of parasites (and determining infection prevalence) within a population (Nusser et al., 2008). Likewise, comparable sample sizes across the time series, between groups (e.g. resident versus translocated woylies), and between sites at specific time points is necessary for determining the effect of translocation on parasite dynamics. During this study, confounding factors such as year and variation in sample size could not be separated from translocation-associated effects given the unbalanced nature of the data (i.e. woylies were released into unfenced wild sites and had variable recapture rates post-release). When sampling small populations of threatened species in the wild, this is often unavoidable, though it is important to acknowledge that differences in sample size may explain the changes in parasite dynamics I observed following translocation. For example, as woylie recaptures decreased over

time, differences in sample size at the two time points I selected to examine changes in community composition pre- and post-translocation (Chapters 3 and 5) may have reduced the power of the analyses (i.e. smaller sample size post-release) and therefore influenced the conclusions made; though the very large difference in the strength of the associations at each time point suggests that this is unlikely.

Based on the observations from this study, I propose that translocation protocols incorporate pre- and post-release assessment of the parasite fauna of translocated hosts (and their source populations) and resident conspecifics within a planned release site for a period of at least 12 months. Other studies demonstrate the benefit of long-term health monitoring of translocated and closely related species for the purpose of identifying disease hazards if and when they arise (i.e. an adaptive and ongoing management approach) and for determining the success of translocation. In reintroduced pool frogs (*Pelophylax lessonae*), for example, it took six years for the population to be considered stable following reintroduction (Sainsbury et al., 2017). Ideally, parasite monitoring should include cohabiting species that also impact parasite dynamics within a population; this is particularly important when compiling baseline data of parasite fauna present within different sites, as cohabiting species may be reservoirs of disease. Based on disease risk analyses in other species (e.g. Hartley and Sainsbury, 2017), closely related sympatric species appear to be the most appropriate targets. If long-term parasite monitoring is not practical, then there is still considerable value in monitoring within the immediate post-translocation period. For projects with limited resources, much could be learned from focusing efforts within this time frame.

During this study, intermittent detection of haemoparasites over time was common. False negative results may occur, for example, when parasitaemia is below the detectable limit, or parasites have invaded the tissues and left the peripheral circulation (e.g. *T. copemani*; Thompson et al., 2013). Determining detection limits using diagnostic screening methods is fundamental to the field of parasitology, however culturing parasites to verify these limits, is often not achievable. Dunlop et al. (2014) used *T. copemani* to determine the sensitivity of the *Trypanosoma* genus-specific primers (663 parasites/ml with 95% confidence) and *T. copemani* primers (960 parasites/ml with 95% confidence) used in this study. The sensitivity of *T. vegrandis* and *T. noyesi* primers used in this study was not evaluated as these trypanosomes could not be cultured at the time of screening. Nonetheless, Dunlop et al. (2014) proposed the detection limit for wildlife trypanosomiasis to be in the scale of thousands of trypanosomes per ml. Piroplasms (*Theileria* and *Babesia* spp.) were

detected in this study using the PCR protocol of Jefferies et al. (2007) with a parasitaemia detection limit of 2.7 x 10^{-7} % using a sample of a known concentration.

Primer design may also result in intermittent detection of infection depending on the targeted region of interest. As such, novel genotypes and species may go unnoticed and the true prevalence of polyparasitism is likely to be under-represented during this study. This is particularly true for *T. gilletti*, as species-specific primers for *T. vegrandis* are unable to discriminate between these two species, thus a positive result as *T. vegrandis* could unknowingly indicate the presence of *T. gilletti*. Without DNA sequencing I was unable to detect the presence of *T. gilletti*, thus the true prevalence of *T. gilletti* in woylies is unknown. In all cases where *T. gilletti* was identified during this study, coinfection with *T. vegrandis* was apparent. Additionally, replication slippage during PCR may result in primer misalignment resulting in false negatives. All samples were screened at least twice to reduce the likelihood of false negatives occurring. Targeted amplicon next generation sequencing (NGS), which can be superior for detecting coinfection and more accurately estimating parasite prevalence (Cooper et al., 2018), should be a consideration for future research. Additionally, High Resolution Melt quantitative PCR (HRM-qPCR) would be a useful tool for not only quantifying parasite load, but also the detection of mixed infections through the generation of species specific melt curves (e.g. Ghorashi et al., 2015).

9.4 Efficacy of ivermectin treatment

With the exception of a temporary reduction in *Strongyloides*-like egg counts in a subset of woylies, ivermectin treatment did not significantly alter the prevalence or abundance of target parasites during this study, or provide any discernible benefit to host health. Dead lice were observed on treated hosts following translocation, and although lice prevalence was lower in treated compared to untreated woylies at every sampling point during which ivermectin should be effective (≥ 3 months after administration), this effect was not statistically significant and only temporary; treated hosts became reinfected with lice at the same, or greater, prevalence than prior to translocation. A similar phenomenon has been observed with lice in ivermectin treated free-ranging Australian sea lions (*Neophoca cinerea*) (Marcus et al., 2015), where reinfection from conspecifics occurred as ivermectin became ineffective.

The lack of an observed effect in most target parasites (and a more pronounced effect in *Strongyloides* nematodes and lice) does not necessarily indicate that ivermectin is ineffective. As discussed in Chapters 3 and 8, no clinical trials have been undertaken to demonstrate the efficacy of ivermectin in macropods, thus the dose, route of administration or dosage regime may have been suboptimal. Likewise, different parasite species are likely to vary in their susceptibility to ivermectin treatment. Examining the effects of ivermectin on individual species, as opposed to higher level taxa, may have revealed species-specific responses to antiparasitic drug treatment that were missed. The dose administered to woylies was at the lower end of the reference range recommended for macropods (Vogelnest and Portas, 2008), but was selected based on its apparent safe use in the closely related eastern bettong (*Bettongia gaimardi*; Portas et al., 2014); follow-up monitoring of translocated bettongs in that study (Portas et al., 2016) did not investigate the effects of ivermectin treatment on host health. Given that there is uncertainty regarding the dose of ivermectin administered to woylies, it may be worthwhile testing a higher dose of ivermectin, an alternative drug or using a different dosage regime (e.g. repeat dosing) in future; the latter option is often logistically unfeasible however during wild-to-wild translocations.

While parasite reduction, rather than parasite eradication is advocated and justifiable during the translocation of other species (e.g. McGill et al., 2010), treatment confers a known benefit to host health in such cases. One of the aims of the ivermectin treatment trial was to investigate the value of retaining parasites by empirically evaluating how the removal of parasites affects host health following translocation. During this study, I observed no benefit of ivermectin treatment to host

health, despite a correlation between certain parasite taxa (i.e. strongyloid nematodes and coccidia) and lower body condition, and treatment having some form of effect on one of these parasites (*Strongyloides*-like nematodes). Thus, there is no evidence to support the use of ivermectin (administered at 0.2 mg/kg subcutaneously) in future; in fact, perturbations to the prevalence of non-target trypanosomes (Chapters 3 and 5) pose a potential concern to woylie health.

To further evaluate the impact of parasites on host health, experimental manipulation using alternative antiparasitic drugs could be undertaken for parasites of potential concern, as alternative treatment protocols are likely to deliver different outcomes for parasites and host health. For coccidia, the use of a coccidiostatic drug (e.g. toltrazuril; Vogelnest and Portas, 2008) could be considered, although oral dosing would be more difficult, impose greater stress to the animal (as their head would have to be exposed) and is inherently less reliable (Chapter 7); repeat dosing is also required, which in most instances is unfeasible. Manipulating *Trypanosoma* spp. is not an option given the notorious risk of toxicity and species-specific efficacy of trypanocides in other animals (Giordani et al., 2016). Experimental manipulation of trypanosome vectors could be considered, though the vector of trypanosomes in Australian marsupials is currently unknown.

Ultimately it would also be ideal to examine survival differences between treated and untreated hosts, although this was not addressed during this study. Given that all woylies were released into unfenced, wild sites and I had no means of monitoring their movement following translocation (i.e. they were not collared), I did not obtain mark-recapture data from all individuals, which poses uncertainty as to whether individuals which were not re-trapped dispersed from the release site or were deceased (e.g. secondary to disease or predation, or potentially a side-effect of treatment). During this study, a number of Walcott resident woylies were captured in an adjacent site (Winnejup), demonstrating that woylies naturally travel large distances (in this instance up to 5 km). Dispersal following translocation is a well-known phenomenon (Le Gouar, Mihoub and Sarrazin, 2012), and may be more pronounced with increased competition or stress (Aiello et al., 2014). Coincidentally, Walcott had the highest estimated woylie density and the lowest recapture rate (63%) of any site. Nonetheless, a number of translocated animals established within the release sites, many of which were recaptured and sampled repeatedly following translocation, enabling us to obtain mark-recapture data and assess host health and reproductive success; this data could potentially be used to evaluate survival differences, adding to the overall picture of the impacts of ivermectin treatment during translocation. As maintaining genetic diversity within the last remaining indigenous woylie populations is one of the principal objectives of woylie

translocations, the detection of multiple generations of offspring in translocated woylies was an indication of translocation success. Evidence of population growth was also apparent following translocation (e.g. six months after the first translocation woylie numbers had doubled within the release sites).

In the absence of data that directly quantifies parasite abundance (and potential disease pathology), and the knowledge that parasite conservation during translocation may enhance host immunity (Pizzi, 2009; McGill et al., 2010; Boyce et al., 2011) and improve translocation outcomes (Rideout et al., 2016), it is difficult to advocate the use of antiparasitic drugs such as ivermectin based on the results of this study; particularly in the absence of any apparent benefit to host health. Further studies are required to evaluate the effects of gastrointestinal nematodes, coccidia and *Trypanosoma* spp. on woylie health. Given the difficulty, potential risks and large degree of uncertainty associated with manipulating the parasite community in woylies, I propose that future management practices should focus on preserving the parasite community and its structure, and enhance parasite monitoring to better understand what drives parasite dynamics during translocation. This is particularly important given that antiparasitic drugs may exacerbate parasite loss that typically occurs during translocation (Moir et al., 2012) and disrupt the parasite community within a host with unknown consequences for host health.

9.5 Future directions

This observational field-based study provides rare insights into host-parasite dynamics following translocation and highlights the value of well-designed scientific studies that examine parasite dynamics across a broad enough spatial and temporal scope to be adequately interpreted. Results from this study reinforce the need for ongoing parasite surveillance of native wildlife in conjunction with long-term field-based studies to elucidate the impacts of translocation on host-parasite relationships. Scientific research that examines parasite dynamics following experimental manipulation is vital for assessing the value of antiparasitic drugs in translocations. If antiparasitic drugs are to be considered during translocation, studies that explore the impacts of different parasite taxa on host health are vital for (a) identifying the need for antiparasitic treatment, and (b) selecting appropriate antiparasitic drugs. Ultimately, the use antiparasitic drugs must be justified. In the absence of a clear purpose/benefit for antiparasitic drug use, preservation of the host-parasite relationship should be a key consideration in the design and implementation of fauna translocation programs.

Given the complexity of host-parasite interactions and the difficulty of elucidating mechanisms by which parasites influence each other and host health, the value of high-frequency, long-term monitoring (i.e. mark-recapture studies) of translocated and resident animals (including cohabiting species) should not be underestimated. For woylies, more research is needed to better understand the life-cycle, transmission and potential clinical impact of parasites, in particular trypanosomes, and investigating potential trypanosome vectors. For completeness, and to determine the evolutionary relationship between all described potoroid *Eimeria* species, it would be useful to characterise the other two known *Eimeria* species (*E. aepyprymni* from the rufous bettong and *E. burdi* from the burrowing bettong). Given the association between *T. copemani*, the woylie decline, and the unusual behaviour of this parasite following translocation, further studies are vital to explore mechanisms by which *T. copemani* genotypes may affect coinfecting parasites, immune function and host health. Studies that utilise comprehensive molecular techniques such as NGS and HRM-qPCR will be more proficient at monitoring coinfection and detecting changes to the parasite community during translocation. Likewise, studies that investigate the link between stress and parasite infection will add to our understanding of what might be driving parasite dynamics and enhance our knowledge of host-parasite-immunological associations. Understanding the nature and drivers of health conditions (parasites or otherwise), such as those identified in Chapter 7, have great importance for the conservation management of the woylie.

Studies such as this, which aim to evaluate the effects of parasite infection (and changes to the parasite community) on host health, would be greatly enhanced by evaluating host fecundity and survivorship, to provide an empirically informed framework for the management of threatened fauna and their parasite taxa during future translocations. More research is needed to determine the biological significance of parasites and altering host-parasite associations on individuals (e.g. reproductive fitness, survivorship), host populations (e.g. population health, growth rates) and ultimately translocation success. Wildlife disease is poorly understood and while identified as a key threat to some species at risk of extinction, its importance is likely to be globally underestimated, as are the benefits of conserving parasites and host-parasite associations. As such, it is important to monitor and broadly disseminate information regarding the impacts of parasite infection and antiparasitic drug treatment during fauna translocations to progressively improve our understanding in this area and develop more refined translocation protocols for hosts and their parasites.

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Chapter Ten

Appendices

Table A1: The number of translocated and resident woylies sampled within **Dryandra**. For each sampling point (month), the prevalence of infection with 95% CI is recorded for each parasite taxa. For strongyle and *Strongyloides*-like eggs, mean faecal egg counts are also shown.

			2015					2016	
			JUN	JUL	AUG	SEP	DEC	MAR	JUN
Strongyle eggs	Translocated	**(No. sampled)**	**(56)** 96.4%	**(28)** 100%	**(25)** 92%	**(22)** 86.4%	**(17)** 70.6%	**(13)** 76.9%	**(7)** 71.4%
		Prevalence	89.0 - 99.3%	93.4 - 100%	76.7 - 98.3%	67.9 - 96.0%	47.0 - 87.8%	50.3 - 93.0%	35.2 - 93.5%
		95% CI	**59.4**	**45.5**	**33.2**	**18.2**	**9.0**	**3.1**	**2.7**
	Resident	**Mean egg count**	**(19)** 0.0%	**(40)** 0.0%	**(35)** 0.0%	**(33)** 0.0%	**(26)** 3.8%	**(19)** 10.5%	**(23)** 0.0%
			0.0 - 9.5%	0.0 - 4.7%	0.0 - 5.3%	0.0 - 5.6%	0.4 - 16.6%	2.3 - 29.7%	0.0 - 7.9%
			0.0	**0.0**	**0.0**	**0.0**	**0.2**	**1.2**	**0.0**
Strongyloides-like eggs	Translocated	**(No. sampled)**	**(56)** 55.4%	**(28)** 78.6%	**(25)** 56%	**(22)** 68.2%	**(17)** 23.5%	**(13)** 30.8%	**(7)** 57.1%
		Prevalence	42.3 - 67.8%	61.1 - 90.5%	36.8- 73.9%	47.4 - 84.5%	8.5 - 46.7%	11.4 - 57.7%	23.5 - 86.1%
		95% CI	**1.8**	**3.5**	**2.4**	**2.0**	**0.4**	**0.5**	**1.3**
	Resident	**Mean egg count**	**(19)** 10.5%	**(40)** 7.5%	**(35)** 0.0%	**(33)** 15.2%	**(26)** 7.7%	**(19)** 15.8%	**(23)** 39.1%
			2.3 - 29.7%	2.2 - 18.7%	0.0 - 5.3%	6.0 - 30.1%	1.6 - 22.5%	4.7 - 36.4%	21.4 - 59.4%
			0.2	**0.1**	**0.0**	**0.2**	**0.1**	**0.2**	**0.8**
Coccidian oocysts	Translocated	**No. sampled**	**(56)** 25%	**(28)** 0.0%	**(25)** 12%	**(22)** 4.5%	**(17)** 0.0%	**(13)** 0.0%	**(7)** 0.0%
		Prevalence	15.1 - 37.4%	0.0 - 6.6%	3.5 - 28.7%	0.5 - 19.3%	0.0 - 10.5%	0.0 - 13.5%	0.0 - 23.2%
	Resident	95% CI	**(19)** 0.0%	**(40)** 2.5%	**(35)** 8.6%	**(33)** 3.0%	**(26)** 0.0%	**(19)** 0.0%	**(23)** 0.0%
			0.0 - 9.5%	0.3 - 11.1%	2.5 - 21.1%	0.3 - 13.3%	0.0 - 7.1%	0.0 - 9.5%	0.0 - 7.9%
Lungworm larvae	Translocated	**No. sampled**	**(56)** 0.0%	**(28)** 0.0%	**(25)** 0.0%	**(22)** 0.0%	**(17)** 0.0%	**(13)** 0.0%	**(7)** 0.0%
		Prevalence	0.0 - 3.4%	0.0 - 6.6%	0.0 - 7.3.%	0.0 - 8.3%	0.0 - 10.5%	0.0 - 13.5%	0.0 - 23.2%
	Resident	95% CI	**(19)** 0.0%	**(40)** 0.0%	**(35)** 0.0%	**(33)** 0.0%	**(26)** 0.0%	**(19)** 0.0%	**(23)** 0.0%
			0.0 - 9.5%	0.0 - 4.7%	0.0 - 5.3%	0.0 - 5.6%	0.0 - 7.1%	0.0 - 9.5%	0.0 - 7.9%
Cestode eggs	Translocated	**No. sampled**	**(56)** 0.0%	**(28)** 0.0%	**(25)** 0.0%	**(22)** 0.0%	**(17)** 0.0%	**(13)** 0.0%	**(7)** 28.6%
		Prevalence	0.0 - 3.4%	0.0 - 6.6%	0.0 - 7.3.%	0.0 - 8.3%	0.0 - 10.5%	0.0 - 13.5%	6.5 - 64.8%
	Resident	95% CI	**(19)** 10.5%	**(40)** 17.5%	**(35)** 8.6%	**(33)** 12.1%	**(26)** 0.0%	**(19)** 0.0%	**(23)** 13.0%
			2.3 - 29.7%	8.2 - 31.3%	2.5 - 21.1%	4.2 - 26.3%	0.0 - 7.1%	0.0 - 9.5%	3.8 - 30.9%
Potoroxyuris sp. eggs	Translocated	**No. sampled**	**(56)** 0.0%	**(28)** 0.0%	**(25)** 0.0%	**(22)** 0.0%	**(17)** 0.0%	**(13)** 0.0%	**(7)** 0.0%
		Prevalence	0.0 - 3.4%	0.0 - 6.6%	0.0 - 7.3.%	0.0 - 8.3%	0.0 - 10.5%	0.0 - 13.5%	0.0 - 23.2%
	Resident	95% CI	**(19)** 0.0%	**(40)** 0.0%	**(35)** 0.0%	**(33)** 0.0%	**(26)** 0.0%	**(19)** 0.0%	**(23)** 0.0%
			0.0 - 9.5%	0.0 - 4.7%	0.0 - 5.3%	0.0 - 5.6%	0.0 - 7.1%	0.0 - 9.5%	0.0 - 7.9%
Ticks	Translocated	**No. sampled**	**(69)** 91.3%	**(30)** 86.7%	**(27)** 55.6%	**(22)** 63.6%	**(17)** 64.7%	**(13)** 76.9%	**(8)** 87.5%
		Prevalence	83.0 - 96.3%	71.3 - 95.3%	37.1 - 72.9%	42.9 - 81.1%	41.1 - 83.7%	50.3 - 93.0%	54.6 - 98.6%
	Resident	95% CI	**(21)** 95.2%	**(42)** 57.1%	**(36)** 58.3%	**(34)** 61.8%	**(27)** 37.0%	**(26)** 84.6%	**(35)** 88.6%
			79.8 - 99.5%	42.1 - 71.2%	42.1 - 73.3%	45.0 - 76.6%	20.9 - 55.8%	67.5 - 94.6%	75.1 - 96.0%
Lice	Translocated	**No. sampled**	**(69)** 85.5%	**(30)** 93.3%	**(27)** 96.3%	**(22)** 100%	**(17)** 100%	**(13)** 84.6%	**(8)** 100%
		Prevalence	75.8 - 92.3%	80.3 - 98.6%	84.0 - 99.6%	91.7 - 100%	89.5- 100%	59.1 - 96.7%	79.2 - 100%
	Resident	95% CI	**(21)** 95.2%	**(41)** 85.4%	**(36)** 94.4%	**(34)** 97.1%	**(27)** 92.6%	**(26)** 88.5%	**(35)** 85.7%
			79.8 - 99.5%	72.3 - 93.7%	83.4 - 98.8%	87.1 - 99.7%	78.3 - 98.4%	72.3 - 96.6%	71.5 - 94.3%
Mites	Translocated	**No. sampled**	**(69)** 42.0%	**(30)** 13.3%	**(27)** 37.0%	**(22)** 13.6%	**(17)** 29.4%	**(13)** 7.7%	**(8)** 25.0%
		Prevalence	30.9 - 53.8%	4.7 - 28.7%	20.9 - 55.8%	4.0 - 32.1%	12.2 - 53.0%	0.8 - 30.7%	5.6 - 59.2%
	Resident	95% CI	**(21)** 4.8%	**(41)** 2.4%	**(36)** 27.8%	**(34)** 17.6%	**(27)** 25.9%	**(26)** 0.0%	**(35)** 28.6%
			0.5 - 20.2%	0.3 - 10.8%	15.3 - 43.7%	7.7 - 32.8%	12.4 - 44.3%	0.0 - 7.1%	15.7 - 44.8%
Fleas	Translocated	**No. sampled**	**(69)** 53.6%	**(30)** 0.0%	**(27)** 3.7%	**(22)** 0.0%	**(17)** 0.0%	**(13)** 0.0%	**(8)** 0.0%
		Prevalence	41.9 - 65.0%	0.0 - 6.2%	0.4 - 16.0%	0.0 - 8.3%	0.0 - 10.5%	0.0 - 13.5%	0.0 - 20.8%
	Resident	95% CI	**(21)** 9.5%	**(41)** 2.4%	**(36)** 0.0%	**(34)** 0.0%	**(27)** 0.0%	**(26)** 0.0%	**(35)** 2.9%
			2.0 - 27.2%	0.3 - 10.8%	0.0 - 5.2%	0.0 - 5.5%	0.0 - 6.8%	0.0 - 7.1%	0.3 - 12.6%
Trypanosomes	Translocated	**No. sampled**	**(69)** 53.6%	**(30)** 66.7%	**(26)** 80.8%	**(22)** 68.2%	**(17)** 76.5%	**(12)** 66.7%	**(8)** 62.5%
		Prevalence	41.9 - 65.0%	48.9 - 81.4%	62.9 - 92.3%	47.4 - 84.5%	53.3 - 91.5%	38.8 - 87.5%	29.5 - 88.1%
	Resident	95% CI	**(22)** 36.4%	**(40)** 32.5%	**(36)** 41.7%	**(34)** 2.9%	**(26)** 57.7%	**(25)** 32.0%	**(35)** 11.4%
			18.9 - 57.1%	19.6 - 47.8%	26.7 - 57.9%	0.3 - 12.9%	37.1 - 72.9%	16.4 - 51.5%	5.7 - 28.5%

Table A2: The number of translocated and resident woylies sampled within **Walcott**. For each sampling point (month), the prevalence of infection with 95% CI is recorded for each parasite taxa. For strongyle and *Strongyloides*-like eggs, mean faecal egg counts are also shown.

			2014						2015	
			APR	MAY	JUN	JUL	SEP	DEC	APR	MAY
Strongyle eggs	Translocated	**(No. sampled)** Prevalence 95% CI **Mean egg count**	Not sampled	Not sampled	**(29)** 100% 93.6 - 100% **63.0**	**(17)** 100% 89.5 - 100% **130.2**	**(19)** 100% 90.5 - 100% **124.3**	**(13)** 100% 86.5 - 100% **65.7**	**(9)** 100% 81.3 - 100% **53.9**	**(1)** 100% 22.9 - 100% **96.0**
	Resident		**(21)** 100% 91.4 - 100% **52.9**	**(45)** 100% 95.8 - 100% **18.1**	Not sampled	**(12)** 100% 85.5 - 100% **69.8**	**(23)** 100% 92.1 - 100% **71.8**	**(45)** 100% 95.8 - 100% **79.1**	**(21)** 100% 91.4 - 100% **73.8**	**(8)** 100% 79.2 - 100% **93.0**
Strongyloides-like eggs	Translocated	**(No. sampled)** Prevalence 95% CI **Mean egg count**	Not sampled	Not sampled	**(29)** 79.3% 62.2 - 90.9% **6.1**	**(17)** 76.5% 53.3 - 91.5% **3.2**	**(19)** 52.6% 31.2 - 73.4% **5.5**	**(13)** 46.2% 22.1 - 71.7% **1.8**	**(9)** 55.6% 25.4 - 82.7% **1.2**	**(1)** 100% 22.9 - 100% **5.0**
	Resident		**(21)** 61.9% 40.7 - 80.1% **4.3**	**(45)** 48.9% 34.7- 63.2% **1.1**	Not sampled	**(12)** 91.7% 67.2 - 99.1% **3.3**	**(23)** 82.6% 63.8 - 93.8% **3.3**	**(45)** 55.6% 41.1 - 69.4% **3.2**	**(21)** 85.7% 66.6 - 95.8% **2.1**	**(8)** 37.5% 11.9 - 70.5% **3.3**
Coccidian oocysts	Translocated	**No. sampled** Prevalence 95% CI	Not sampled	Not sampled	**(29)** 27.6% 14.0 - 45.4%	**(17)** 64.7% 41.1 - 83.7%	**(19)** 21.1% 7.6 - 42.6%	**(13)** 7.7% 0.8 - 30.7%	**(9)** 11.1% 1.2 - 41.4%	**(1)** 0.0% 0.0 - 77.1%
	Resident		**(21)** 19.0% 6.8 - 39.2%	**(45)** 26.7% 15.5 - 40.7%	Not sampled	**(12)** 25.0% 7.6 - 52.9%	**(23)** 13.0% 3.8 - 30.9%	**(45)** 11.1% 4.4 - 22.7%	**(21)** 19.0% 6.8 - 39.2%	**(8)** 12.5% 1.4 - 45.4%
Lungworm larvae	Translocated	**No. sampled** Prevalence 95% CI	Not sampled	Not sampled	**(29)** 13.8% 4.8 - 29.5%	**(17)** 0.0% 0.0 - 10.5%	**(19)** 5.3% 0.6 - 22.1%	**(13)** 7.7% 0.8 - 30.7%	**(9)** 11.1% 1.2 - 41.4%	**(1)** 0.0% 0.0 - 77.1%
	Resident		**(21)** 0.0% 0.0 - 8.6%	**(45)** 0.0% 0.0 - 4.2%	Not sampled	**(12)** 0.0% 0.0 - 14.5%	**(23)** 0.0% 0.0 - 7.9%	**(45)** 0.0% 0.0 - 4.2%	**(21)** 0.0% 0.0 - 8.6%	**(8)** 0.0% 0.0 - 20.8%
Cestode eggs	Translocated	**No. sampled** Prevalence 95% CI	Not sampled	Not sampled	**(29)** 0.0% 0.0 - 6.4%	**(17)** 0.0% 0.0 - 10.5%	**(19)** 0.0% 0.0 - 9.5%	**(13)** 0.0% 0.0 - 13.5%	**(9)** 0.0% 0.0 - 18.7%	**(1)** 0.0% 0.0 - 77.1%
	Resident		**(21)** 0.0% 0.0 - 8.6%	**(45)** 0.0% 0.0 - 4.2%	Not sampled	**(12)** 0.0% 0.0 - 14.5%	**(23)** 0.0% 0.0 - 7.9%	**(45)** 0.0% 0.0 - 4.2%	**(21)** 0.0% 0.0 - 8.6%	**(8)** 0.0% 0.0 - 20.8%
Potoroxyuris sp. eggs	Translocated	**No. sampled** Prevalence 95% CI	Not sampled	Not sampled	**(29)** 6.9% 1.5 - 20.3%	**(17)** 0.0% 0.0 - 10.5%	**(19)** 0.0% 0.0 - 9.5%	**(13)** 0.0% 0.0 - 13.5%	**(9)** 0.0% 0.0 - 18.7%	**(1)** 0.0% 0.0 - 77.1%
	Resident		**(21)** 0.0% 0.0 - 8.6%	**(45)** 0.0% 0.0 - 4.2%	Not sampled	**(12)** 0.0% 0.0 - 14.5%	**(23)** 0.0% 0.0 - 7.9%	**(45)** 0.0% 0.0 - 4.2%	**(21)** 0.0% 0.0 - 8.6%	**(8)** 0.0% 0.0 - 20.8%
Ticks	Translocated	**No. sampled** Prevalence 95% CI	Not sampled	Not sampled	**(92)** 70.7% 60.8 - 79.2%	**(17)** 100% 89.5 - 100%	**(21)** 100% 91.4 - 100%	**(13)** 100% 86.5 -100%	**(10)** 100% 82.9 - 100%	**(1)** 100% 22.9 - 100%
	Resident		**(20) 100**.0% 91.0 - 100%	**(48)** 100% 96.1 - 100%	Not sampled	**(21)** 100% 91.4 - 100%	**(16)** 93.8 74.3 - 99.3%	**(125)** 97.6% 93.7 - 99.3%	**(102)** 91.2% 84.5 - 95.5%	**(18)** 100% 90.0 - 100%
Lice	Translocated	**No. sampled** Prevalence 95% CI	Not sampled	Not sampled	**(92)** 87.0% 79.0 - 92.7%	**(16)** 100% 88.9 - 100%	**(20)** 85.0% 65.1 - 95.6%	**(13)** 92.3% 69.3 - 99.2%	**(10)** 100% 82.9 - 100%	**(1)** 100% 22.9 - 100%
	Resident		**(19)** 84.2% 63.6 - 95.3%	**(47)** 91.5% 81.0 - 97.1%	Not sampled	**(17)** 100% 89.5 - 100%	**(14)** 100% 87.4 - 100%	**(123)** 91.9% 86.1 - 95.7%	**(102)** 100% 98.1 - 100%	**(18)** 100% 90.0 - 100%
Mites	Translocated	**No. sampled** Prevalence 95% CI	Not sampled	Not sampled	**(91)** 68.1% 58.1 - 77.0%	**(16)** 75.0% 50.9 - 90.9%	**(20)** 30.0% 13.6 - 51.7%	**(13)** 84.6% 59.1 - 96.7%	**(10)** 60.0% 30.4 - 84.7%	**(1)** 0.0% 0.0 - 77.1%
	Resident		**(20)** 75.0% 53.6 - 89.8%	**(45)** 71.1% 56.9 - 82.7%	Not sampled	**(17)** 82.4% 60.0 - 94.8%	**(14)** 78.6% 53.1 - 93.6%	**(123)** 82.9% 75.6 - 88.8%	**(102)** 52.0% 42.3 - 61.5%	**(18)** 77.8% 55.4 - 92.0%
Fleas	Translocated	**No. sampled** Prevalence 95% CI	Not sampled	Not sampled	**(91) 40.7**% 31.0 - 50.9%	**(17)** 88.2% 67.3 - 97.5%	**(21)** 95.2% 79.8 - 99.5%	**(12)** 58.3% 31.2 - 82.0%	**(10)** 70.0% 39.4 - 90.7%	**(1)** 100% 22.9 - 100%
	Resident		**(19)** 68.4% 46.1 - 85.6%	**(46)** 80.4% 67.3 - 89.9%	Not sampled	**(19)** 89.5% 70.3 - 97.7%	**(15)** 80.0% 55.6 - 94.0%	**(122)** 63.9% 55.2 - 72.0%	**(102)** 62.7% 53.1 - 71.7%	**(18)** 61.1% 38.3 - 80.6%
Trypanosomes	Translocated	**No. sampled** Prevalence 95% CI	Not sampled	Not sampled	**(92)** 26.1% 18.0 - 35.7%	**(17)** 58.8% 35.6 - 79.3%	**(20)** 95.0% 78.9 - 99.5%	**(12)** 91.7% 67.2 - 99.1%	**(10)** 60.0% 30.4 - 84.7%	**(1)** 100% 22.9 - 100%
	Resident		**(18)** 72.2% 49.4 - 88.5%	**(45)** 66.7% 52.2 - 79.1%	Not sampled	**(12)** 58.3% 31.2 - 82.0%	**(14)** 50.0% 25.9 - 74.1%	**(119)** 89.1% 82.5 - 93.7%	**(92)** 72.8% 63.1 - 81.1%	**(18)** 83.3% 61.9 - 95.1%

Table A3: The number of translocated and resident woylies sampled within **Warrup East**. For each sampling point (month), the prevalence of infection with 95% CI is recorded for each parasite taxa. For strongyle and *Strongyloides*-like eggs, mean faecal egg counts are also shown.

			2014						2015	
			APR	MAY	JUN	JUL	SEP	DEC	APR	MAY
Strongyle eggs	Translocated	**(No. sampled)** Prevalence 95% CI **Mean egg count**	Not sampled	Not sampled	**(36)** 97.2% 87.7 - 99.7% **45.8**	**(20)** 100% 91.0 - 100% **35.2**	**(29)** 100% 93.6 - 100% **22.6**	**(25)** 100% 92.7 - 100% **11.4**	**(17)** 94.1% 75.6 - 99.4% **27.4**	**(3)** 100% 55.6 - 100% **18.3**
	Resident		**(6)** 83.3% 44.2 - 98.1% **24.7**	**(24)** 79.2% 60.2 - 91.6% **12.3**	Not sampled	**(12)** 100% 85.5 - 100% **40.0**	**(15)** 100% 88.2 - 100% **46.3**	**(42)** 90.5% 78.9 - 96.7% **15.6**	**(16)** 100% 88.9 - 100% **35.9**	**(1)** 100% 22.9 - 100% **30.0**
Strongyloides - like eggs	Translocated	**(No. sampled)** Prevalence 95% CI **Mean egg count**	Not sampled	Not sampled	**(36)** 86.1% 72.2 - 94.5% **12.6**	**(20)** 35.0% 17.2 - 56.8% **1.2**	**(29)** 55.2% 37.3 - 72.1% **3.3**	**(25)** 20.0% 8.1 - 38.4% **0.2**	**(17)** 47.1% 25.4 - 69.7% **1.7**	**(3)** 66.7% 17.7 - 96.1% **0.7**
	Resident		**(6)** 0.0% 0.0 - 26.4% **0.0**	**(24)** 12.5% 3.6 - 29.7% **0.1**	Not sampled	**(12)** 25.0% 7.6 - 52.9% **0.3**	**(15)** 53.3% 29.4 - 76.1% **0.9**	**(42)** 14.3% 6.2 - 27.1% **0.2**	**(16)** 56.3% 32.6 - 77.8% **1.0**	**(1)** 0.0% 0.0 - 77.1% **0.0**
Coccidian oocysts	Translocated	**No. sampled** Prevalence 95% CI	Not sampled	Not sampled	**(36)** 25% 13.2 - 40.7%	**(20)** 25.0% 10.2 - 46.4%	**(29)** 41.4% 25.0 - 59.4%	**(25)** 4.0% 0.4 - 17.2%	**(17)** 0.0% 0.0 - 10.5%	**(3)** 0.0% 0.0 - 44.4%
	Resident		**(6)** 0.0% 0.0 - 26.4%	**(24)** 20.8% 8.4 - 39.8%	Not sampled	**(12)** 8.3% 0.9 - 32.8%	**(15)** 40.0% 18.8 - 64.7%	**(42)** 11.9% 4.7 - 24.1%	**(16)** 12.5% 2.7 - 34.4%	**(1)** 0.0% 0.0 - 77.1%
Lungworm larvae	Translocated	**No. sampled** Prevalence 95% CI	Not sampled	Not sampled	**(36)** 8.3% 2.4 - 20.6%	**(20)** 0.0% 0.0 - 9.0%	**(29)** 6.9% 1.5 - 20.3%	**(25)** 0.0% 0.0 - 7.3%	**(17)** 5.9% 0.6 - 24.4%	**(3)** 0.0% 0.0 - 44.4%
	Resident		**(6)** 0.0% 0.0 - 26.4%	**(24)** 0.0% 0.0 - 7.6%	Not sampled	**(12)** 0.0% 0.0 - 14.5%	**(15)** 0.0% 0.0 - 11.8%	**(42)** 0.0% 0.0 - 4.4%	**(16)** 0.0% 0.0 - 11.1%	**(1)** 0.0% 0.0 - 77.1%
Cestode eggs	Translocated	**No. sampled** Prevalence 95% CI	Not sampled	Not sampled	**(36)** 0.0% 0.0 - 5.2%	**(20)** 0.0% 0.0 - 9.0%	**(29)** 0.0% 0.0 - 6.4%	**(25)** 0.0% 0.0 - 7.3%	**(17)** 0.0% 0.0 - 10.5%	**(3)** 0.0% 0.0 - 44.4%
	Resident		**(6)** 0.0% 0.0 - 26.4%	**(24)** 0.0% 0.0 - 7.6%	Not sampled	**(12)** 0.0% 0.0 - 14.5%	**(15)** 0.0% 0.0 - 11.8%	**(42)** 0.0% 0.0 - 4.4%	**(16)** 0.0% 0.0 - 11.1%	**(1)** 0.0% 0.0 - 77.1%
Potoroxyuris sp. eggs	Translocated	**No. sampled** Prevalence 95% CI	Not sampled	Not sampled	**(36)** 0.0% 0.0 - 5.2%	**(20)** 0.0% 0.0 - 9.0%	**(29)** 0.0% 0.0 - 6.4%	**(25)** 0.0% 0.0 - 7.3%	**(17)** 5.9% 0.6 - 24.4%	**(3)** 0.0% 0.0 - 44.4%
	Resident		**(6)** 0.0% 0.0 - 26.4%	**(24)** 0.0% 0.0 - 7.6%	Not sampled	**(12)** 0.0% 0.0 - 14.5%	**(15)** 0.0% 0.0 - 11.8%	**(42)** 0.0% 0.0 - 4.4%	**(16)** 0.0% 0.0 - 11.1%	**(1)** 0.0% 0.0 - 77.1%
Ticks	Translocated	**No. sampled** Prevalence 95% CI	Not sampled	Not sampled	**(90)** 84.4% 75.9 - 90.8%	**(21)** 90.5% 72.8 - 98.0%	**(30)** 70.0% 52.3 - 84.0%	**(26)** 96.2% 83.4 - 99.6%	**(22)** 40.9% 22.5 - 61.5%	**(3)** 100% 55.6 - 100%
	Resident		**(7)** 57.1% 23.5 - 86.1%	**(29)** 86.2% 70.5 - 95.2%	Not sampled	Not sampled	**(12)** 75.0% 47.1 - 92.4%	**(53)** 88.7% 78.1 - 95.1%	**(59)** 42.4% 30.4 - 55.1%	**(9)** 100% 81.3 - 100%
Lice	Translocated	**No. sampled** Prevalence 95% CI	Not sampled	Not sampled	**(89)** 91.1% 83.8 - 95.7%	**(21)** 85.7% 66.6 - 95.8%	**(29)** 75.9% 58.4 - 88.5%	**(26)** 96.2% 83.4 - 99.6%	**(22)** 100% 91.7 - 100%	**(3)** 100% 55.6 - 100%
	Resident		**(7)** 57.1% 23.5 - 86.1%	**(29)** 96.6% 85.0 - 99.6%	Not sampled	Not sampled	**(11)** 90.9% 64.7 - 99.0%	**(51)** 96.1% 88.0 - 99.2%	**(59)** 91.5% 82.4 - 96.7%	**(9)** 100% 81.3 - 100%
Mites	Translocated	**No. sampled** Prevalence 95% CI	Not sampled	Not sampled	**(88)** 58.0% 47.5 - 67.9%	**(21)** 42.9% 23.7 - 63.8%	**(29)** 72.4% 54.6 - 86.0%	**(26)** 76.9% 58.5 - 89.7%	**(22)** 81.8% 62.4 - 93.5%	**(3)** 33.3% 3.9 - 82.3%
	Resident		**(7)** 0.0% 0.0 - 23.2%	**(29)** 34.5% 19.3 - 52.6%	Not sampled	Not sampled	**(12)** 91.7% 67.2 - 99.1%	(51) 76.5% 63.6 - 86.4%	**(59)** 76.3% 64.3 - 85.7%	**(9)** 55.6% 25.4 - 82.7%
Fleas	Translocated	**No. sampled** Prevalence 95% CI	Not sampled	Not sampled	**(89)** 46.1% 36.0 - 56.4%	**(21)** 66.7% 45.4 - 83.7%	**(30)** 50.0% 32.8 - 67.2%	**(26)** 26.9% 12.9 - 45.7%	**(22)** 31.8% 15.5 - 52.6%	**(3)** 66.7% 17.7 - 96.1%
	Resident		**(7)** 14.3% 1.6 - 50.1%	**(29)** 65.5% 47.4 - 80.7%	Not sampled	Not sampled	**(11)** 72.7% 43.5 - 91.7%	**(51)** 29.4% 18.3 - 42.8%	**(59)** 55.9% 43.2 - 68.1%	**(9)** 100% 81.3 - 100%
Trypanosomes	Translocated	**No. sampled** Prevalence 95% CI	Not sampled	Not sampled	**(89)** 31.5% 22.5 - 41.6%	**(20)** 60.0% 38.4 - 78.9%	**(31)** 22.6% 10.7 - 39.3%	**(26)** 50.0% 31.6 - 68.4%	**(22)** 54.5% 34.3 - 73.7%	**(4)** 75.0% 28.4 - 97.2%
	Resident		**(6)** 16.7% 1.9 - 55.8%	**(27)** 59.3% 40.6 - 76.1%	Not sampled	Not sampled	**(11)** 54.5% 27.0 - 80.0%	**(51)** 78.4% 65.8 - 88.0%	**(51)** 62.7% 49.1 - 75.0%	**(1)** 100% 22.9 - 100%

Table A4: Generic and species-specific primers used for the detection of haemoparasites in woylies.

	External primer (5'-3')	Annealing temperature	Internal primer (5'-3')	Annealing temperature	Band size
Nested PCR					
Generic Trypanosome	SLF GCTTGTTTCAAGGACTTAGC	55°C	S825F ACCGTTTCGGCTTTTGTTGG	56°C	959bp
	S762R GACTTTTGCTTCCTCTAATG		SLIR ACATTGTAGTGCGCGTGTC		
Generic Piroplasm	BTF1 GGCTCATTACAACAGTTATAG	58°C	BTF2 CCGTGCTAATTGTAGGGCTAATAC	62°C	800bp
	BTR1 CCCAAAGACTTTGATTTCTCTC		BTR2 GGACTACGACGGTATCTGATCG		
Species-specific PCR					
Trypanosoma copemani	S825F ACCGTTTCGGCTTTTGTTGG	56°C	WOF GTGTTGCTTTTTTGGTCTTCACG	56°C	457bp
	SLIR ACATTGTAGTGCGCGTGTC		WOR CACAAAGGAGGAAAAAAGGGC		
Trypanosoma vegrandis	TVEF GGGGTCCTTTTATTTTATTTG	58°C	TVIF GACCAAAAACGTGCACGTG	58°C	350bp
	TVER TAATTTATTGGCCAGACAAA		TVIR AAATCGTCTCCGCTTTAAC		
Trypanosoma noyesi	H25EF GCCGACAGTGCATTTTGT	58°C	H25IF TTTGAGGCGCAATGGTTTAG	62°C	400bp
	H25ER GAGCGAGATGAACTCGACC		H25IR CGAGTTGAGGGAAGGTGGC		

Table A5: Molecular methods for piroplasm PCR and Sanger sequencing/phylogenetic analyses of haemoparasites.

PCR protocol - piroplasms (modified from Jefferies et al., 2007)

A 24 μL master mix was made up of 0.8 μM of each primer (forward and reverse; see Table A4), 2 mM $MgCl_2$, 200 μM dNTPs and 0.2 U of Taq Pol, with the subsequent addition of 2 μL of DNA template. The cycling conditions consisted of a pre-PCR step of 94°C for 3 min, 58°C for 1 min and 72°C for 2 min, followed by 35 cycles of 94°C for 30 sec, 58°C for 20 sec, and 72°C for 30 sec, with a final extension temperature of 72°C for 7 min.

Sanger sequencing protocol

Purified amplicons were sequenced in both directions using an ABI Prism™ Terminator Cycle Sequencing Kit on an Applied Bio-systems 3730 DNA Analyser (Applied Bio-systems, California, USA). To control for GC rich regions in the samples, the denaturation stage of the sequencing PCR was extended from 2 min to 10 min, and a 3:1 ratio of BigDye Terminator v3.1 Ready Reaction mix and dGTP BigDye Terminator v3.0 Ready Reaction mix was used (as opposed to just BigDye Terminator v3.1) in order to generate high quality chromatograms. Sequence identity for each sample was confirmed by using MUSCLE (Edgar et al., 2004) to align each sequence against reference libraries downloaded from GenBank using Geneious v 8.1.

Phylogenetic analyses

The construction of each tree was dependent on the substitution model selected by jModeltest (Posada, 2008). All trees were generated used a GTR + G + I substitution model. For *Trypanosoma* sp. ANU2 and *Bodo* sp. ANO4, bootstrap support for 1000 replicates was performed for maximum likelihood analysis using MEGA v.7 (Tamura et al., 2013). The Bayesian method (10,000,000 generations, burn- in 3,000 and sampling frequency of 1,000) was selected to construct a phylogenetic tree for *Theileria apogeana* genotype ANO2. The tree was run using Mr Bayes v. 3.1.2 (Ronquist and Huelsenbeck, 2003).

Table A6: Number of woylies sampled within **Dryandra**, with prevalence of infection (and Jeffrey's 95% CI).

			2015					2016	
			JUN	JUL	AUG	SEP	DEC	MAR	JUN
Trypanosoma copemani	Translocated	**No. sampled** Prevalence 95% CI	**(69)** 34.8% 24.4 - 46.5%	**(30)** 43.3% 26.9 - 61.0%	**(26)** 57.7% 38.7 - 75.0%	**(22)** 63.6% 42.9 - 81.1%	**(17)** 47.1% 25.4 - 70.0%	**(12)** 25.0% 7.6 - 52.9%	**(8)** 62.5% 29.5 - 88.1%
	Resident		**(22)** 13.6% 4.0 - 32.1%	**(40)** 7.5% 2.2 - 18.7%	**(36)** 8.3% 2.4 - 20.6%	**(34)** 0.0% 0.0 - 5.5%	**(26)** 3.8% 0.4 - 16.6%	**(24)** 0.0% 0.0 - 7.6%	**(35)** 8.6% 2.5 - 21.1%
Trypanosoma vegrandis	Translocated	**No. sampled** Prevalence 95% CI	**(69)** 18.8% 11.0 - 29.2%	**(30)** 30.0% 16.0 - 47.7%	**(26)** 30.8% 15.8 - 49.8%	**(22)** 9.1% 1.9 - 26.1%	**(17)** 47.1% 25.4 - 69.7%	**(12)** 50.0% 24.3 - 75.7%	**(8)** 25.0% 5.6 - 59.2%
	Resident		**(22)** 27.3% 12.3 - 47.8%	**(40)** 27.5% 15.6 - 42.5%	**(36)** 27.8% 15.3 - 43.7%	**(34)** 2.9% 0.3 - 12.9%	**(26)** 53.8% 35.1 - 71.8%	**(25)** 24% 10.7 - 42.9%	**(35)** 8.6% 2.5 - 21.1%
Trypanosoma noyesi	Translocated	**No. sampled** Prevalence 95% CI	**(69)** 7.2% 2.8 - 15.2%	**(30)** 3.3% 0.4 - 14.5%	**(26)** 7.7% 1.6 - 22.5%	**(22)** 0.0% 0.0 - 8.3%	**(17)** 5.9% 0.6 - 24.4%	**(12)** 16.7% 3.6 - 43.6%	**(8)** 0.0% 0.0 - 20.8%
	Resident		**(22)** 4.5% 0.5 - 19.3%	**(40)** 2.5% 0.3 - 11.1%	**(36)** 11.1% 3.9 - 24.3%	**(34)** 0.0% 0.0 - 5.5%	**(26)** 0.0% 0.0 - 7.1%	**(25)** 8.0% 1.7 - 23.3%	**(35)** 2.9% 0.3 - 12.6%
Trypanosoma sp. ANU2	Translocated	**No. sampled** Prevalence 95% CI	**(69)** 1.4% 0.2 - 6.6%	**(30)** 0.0% 0.0 - 6.2%	**(26)** 0.0% 0.0 - 7.1%	**(22)** 0.0% 0.0 - 8.3%	**(17)** 0.0% 0.0 - 10.5%	**(12)** 0.0% 0.0 - 14.5%	**(8)** 0.0% 0.0 - 20.8%
	Resident		**(22)** 0.0% 0.0 - 8.3%	**(40)** 0.0% 0.0 - 4.7%	**(36)** 0.0% 0.0 - 5.2%	**(34)** 0.0% 0.0 - 5.5%	**(26)** 0.0% 0.0 - 7.1%	**(25)** 0.0% 0.0 - 7.3%	**(35)** 0.0% 0.0 - 5.3%
Theileria penicillata	Translocated	**No. sampled** Prevalence 95% CI	**(35)** 85.7% 71.5 - 94.3%	**(29)** 86.2% 70.5 - 95.2%	**(26)** 92.3% 77.5 - 98.4%	**(22)** 81.8% 62.4 - 93.5%	Not sampled	Not sampled	Not sampled
	Resident		Not sampled	Not sampled	Not sampled	Not sampled	Not sampled	Not sampled	Not sampled
Theileria apogenana genotype ANO2	Translocated	**No. sampled** Prevalence 95% CI	**(35)** 0.0% 0.0 - 5.3%	**(29)** 3.4% 0.4 - 15.0%	**(26)** 0.0% 0.0 - 7.1%	**(22)** 0.0% 0.0 - 8.3%	Not sampled	Not sampled	Not sampled
	Resident		Not sampled	Not sampled	Not sampled	Not sampled	Not sampled	Not sampled	Not sampled
Babesia sp.	Translocated	**No. sampled** Prevalence 95% CI	**(35)** 11.4% 4.0 - 24.9%	**(29)** 10.3% 3.0 - 25.1%	**(26)** 4.0% 0.4 - 16.6%	**(22)** 13.6% 4.0 - 32.1%	Not sampled	Not sampled	Not sampled
	Resident		Not sampled	Not sampled	Not sampled	Not sampled	Not sampled	Not sampled	Not sampled

Table A7: Number of woylies sampled within **Walcott**, with prevalence of infection (and Jeffrey's 95% CI).

			2014						2015	
			APR	MAY	JUN	JUL	SEP	DEC	APR	MAY
Trypanosoma copemani	Translocated	**No. sampled** Prevalence 95% CI	Not sampled	Not sampled	**(92)** 14.1% 8.2 - 22.3%	**(17)** 35.3% 16.3 - 58.9%	**(20)** 25.0% 10.2 - 46.4%	**(12)** 25.0% 7.6 - 52.9%	**(10)** 10.0% 1.1 - 38.1%	**(1)** 100% 22.9 - 100%
	Resident		**(18)** 5.6% 0.6 - 23.2%	**(45)** 13.3% 5.8 - 25.4%	Not sampled	**(12)** 8.3% 0.9 - 32.8%	**(14)** 7.1% 0.8 - 28.8%	**(119)** 44.5% 35.8 - 53.5%	**(92)** 19.6% 12.5 - 28.5%	**(18)** 22.2% 8.0 - 44.6%
Trypanosoma vegrandis	Translocated	**No. sampled** Prevalence 95% CI	Not sampled	Not sampled	**(92)** 1.1% 0.1 - 5.0%	**(17)** 35.3% 16.3 - 58.9%	**(20)** 90.0% 71.6 - 97.9%	**(12)** 75.0% 47.1 - 92.4%	**(10)** 60.0% 30.4 - 84.7%	**(1)** 100% 22.9 - 100%
	Resident		**(18)** 55.6% 33.2 - 76.3%	**(45)** 44.4% 30.6 - 58.9%	Not sampled	**(12)** 50.0% 24.3 - 75.7%	**(14)** 42.9% 20.3 - 68.1%	**(119)** 71.4% 62.9 - 79.0%	**(92)** 44.6% 34.7 - 54.8%	**(18)** 38.9% 19.4 - 61.7%
Trypanosoma noyesi	Translocated	**No. sampled** Prevalence 95% CI	Not sampled	Not sampled	**(92)** 15.2% 9.0 - 23.6%	**(17)** 23.5% 8.5 - 46.7%	**(20)** 10.0% 2.1 - 28.4%	**(12)** 0.0% 0.0 - 14.5%	**(10)** 0.0% 0.0 - 17.1%	**(1)** 0.0% 0.0 - 77.1%
	Resident		**(18)** 5.6% 0.6 - 23.2%	**(45)** 15.6% 7.2 - 28.1%	Not sampled	**(12)** 25.0% 7.6 - 52.9%	**(14)** 0.0% 0.0 - 12.6%	**(119)** 0.8% 0.1 - 3.9%	**(92)** 2.2% 0.5 - 6.8%	**(18)** 11.1% 2.4 - 31.1%
Trypanosoma sp. ANU2	Translocated	**No. sampled** Prevalence 95% CI	Not sampled	Not sampled	**(92)** 5.4% 2.1 - 11.5%	**(17)** 5.9% 0.6 - 24.4%	**(20)** 0.0% 0.0 - 9.0%	**(12)** 0.0% 0.0 - 14.5%	**(10)** 0.0% 0.0 - 17.1%	**(1)** 0.0% 0.0 - 77.1%
	Resident		**(18)** 5.6% 0.6 - 23.2%	**(45)** 6.7% 1.9 - 16.7%	Not sampled	**(12)** 16.7% 3.6 - 43.6%	**(14)** 7.1% 0.8 - 28.8%	**(119)** 0.8% 0.1 - 3.9%	**(92)** 2.2% 0.5 - 6.8%	**(18)** 0.0% 0.0 - 10.0%
Theileria penicillata	Translocated	**No. sampled** Prevalence 95% CI	Not sampled	Not sampled	**(28)** 39.3% 23.0 - 57.7%	**(17)** 94.1% 75.6 - 99.4%	**(20)** 100% 91.0 - 100%	Not sampled	Not sampled	Not sampled
	Resident		Not sampled	Not sampled	Not sampled	Not sampled	Not sampled	Not sampled	Not sampled	Not sampled
Theileria apogenana genotype ANO2	Translocated	**No. sampled** Prevalence 95% CI	Not sampled	Not sampled	**(28)** 39.3% 23.0 - 57.7%	**(17)** 5.9% 0.6 - 24.4%	**(20)** 0.0% 0.0 - 9.0%	Not sampled	Not sampled	Not sampled
	Resident		Not sampled	Not sampled	Not sampled	Not sampled	Not sampled	Not sampled	Not sampled	Not sampled
Babesia sp.	Translocated	**No. sampled** Prevalence 95% CI	Not sampled	Not sampled	**(28)** 3.6% 0.4 - 15.5%	**(17)** 0.0% 0.0 - 10.5%	**(20)** 0.0% 0.0 - 9.0%	Not sampled	Not sampled	Not sampled
	Resident		Not sampled	Not sampled	Not sampled	Not sampled	Not sampled	Not sampled	Not sampled	Not sampled

Table A8: Number of woylies sampled within **Warrup East**, with prevalence of infection (and Jeffrey's 95% CI).

			2014						2015	
			APR	MAY	JUN	JUL	SEP	DEC	APR	MAY
Trypanosoma copemani	Translocated	**No. sampled** Prevalence 95% CI	Not sampled	Not sampled	**(89)** 13.5% 7.6 - 21.7%	**(20)** 30.0% 13.6 - 51.7%	**(31)** 16.1% 6.4 - 31.8%	**(26)** 30.8% 15.8 - 49.8%	**(22)** 31.8% 15.5 - 52.6%	**(4)** 75.0% 28.4 - 97.2%
	Resident		**(6)** 0.0% 0.0 - 26.4%	**(27)** 11.1% 3.2 - 26.8%	Not sampled	Not sampled	**(11)** 36.4% 13.7 - 65.2%	**(51)** 27.5% 16.7 - 40.7%	**(51)** 17.6% 9.1 - 29.7%	**(1)** 0.0% 0.0 - 77.1%
Trypanosoma vegrandis	Translocated	**No. sampled** Prevalence 95% CI	Not sampled	Not sampled	**(89)** 2.2% 0.5 - 7.0%	**(20)** 10.0% 2.1 - 28.4%	**(31)** 9.7% 2.8 - 23.6%	**(26)** 30.8% 15.8 - 49.8%	**(22)** 31.8% 15.5 - 52.6%	**(4)** 0.0% 0.0 - 36.2%
	Resident		**(6)** 0.0% 0.0 - 26.4%	**(27)** 44.4% 27.1 - 62.9%	Not sampled	Not sampled	**(11)** 27.3% 8.3 - 56.5%	**(51)** 47.1% 33.8 - 60.6%	**(51)** 5.9% 1.7 - 14.9%	**(1)** 100% 22.9 - 100%
Trypanosoma noyesi	Translocated	**No. sampled** Prevalence 95% CI	Not sampled	Not sampled	**(89)** 14.6% 8.4 - 23.0%	**(20)** 15.0% 4.4 - 34.9%	**(31)** 6.5% 1.4 - 19.1%	**(26)** 11.5% 3.4 - 27.7%	**(22)** 4.5% 0.5 - 19.3%	**(4)** 0.0% 0.0 - 36.2%
	Resident		**(6)** 0.0% 0.0 - 26.4%	**(27)** 7.4% 1.6 - 21.7%	Not sampled	Not sampled	**(11)** 18.2% 4.0 - 46.7%	**(51)** 2.0% 0.2 - 8.8%	**(51)** 7.8% 2.7 - 17.6%	**(1)** 0.0% 0.0 - 77.1%
Trypanosoma sp. ANU2	Translocated	**No. sampled** Prevalence 95% CI	Not sampled	Not sampled	**(89)** 3.4% 1.0 - 8.7%	**(20)** 15.0% 4.4 - 34.9%	**(31)** 0.0% 0.0 - 6.0%	**(26)** 0.0% 0.0 - 7.1%	**(22)** 4.5% 0.5 - 19.3%	**(4)** 0.0% 0.0 - 36.2%
	Resident		**(6)** 16.7% 1.9 - 55.8%	**(27)** 3.7% 0.4 - 16.0%	Not sampled	Not sampled	**(11)** 0.0% 0.0 - 15.7%	**(51)** 2.0% 0.2 - 8.8%	**(51)** 0.0% 0.0 - 3.7%	**(1)** 0.0% 0.0 - 77.1%
Theileria penicillata	Translocated	**No. sampled** Prevalence 95% CI	Not sampled	Not sampled	**(37)** 45.9% 30.7 - 61.8%	**(20)** 50.0% 29.3 - 70.7%	**(30)** 76.7% 59.6 - 88.9%	Not sampled	Not sampled	Not sampled
	Resident		Not sampled	Not sampled	Not sampled	Not sampled	Not sampled	Not sampled	Not sampled	Not sampled
Theileria apogenana genotype ANO2	Translocated	**No. sampled** Prevalence 95% CI	Not sampled	Not sampled	**(37)** 37.8% 23.6 - 53.9%	**(20)** 50.0% 29.3 - 70.7%	**(30)** 20.0% 8.8 - 36.7%	Not sampled	Not sampled	Not sampled
	Resident		Not sampled	Not sampled	Not sampled	Not sampled	Not sampled	Not sampled	Not sampled	Not sampled
Babesia sp.	Translocated	**No. sampled** Prevalence 95% CI	Not sampled	Not sampled	**(37)** 0.0% 0.0 - 5.0%	**(20)** 0.0% 0.0 - 9.0%	**(30)** 0.0% 0.0 - 6.2%	Not sampled	Not sampled	Not sampled
	Resident		Not sampled	Not sampled	Not sampled	Not sampled	Not sampled	Not sampled	Not sampled	Not sampled

Table A9: Genetic similarity between haemoparasites isolated during this study and other known species.

Haemoparasite species	GenBank ID	Similarity	Source/Host species	Reference
Trypanosoma sp. ANU2	MF459652			
Trypanosoma copemani G1	KC753530	91.2%	Woylie, *Bettongia Penicillata*	Botero et al., 2013
Trypanosoma copemani G2	KC753531	91.1%	Woylie, *Bettongia Penicillata*	Botero et al., 2013
Trypanosoma copemani Charlton	GU966588	91.1%	Koala, *Phascolarctos cinereus*	McInnes et al., 2011
Theileria apogeana genotype ANO2	MK182522			
Theileria apogeana	MG758116	96.7%	Tick, *Ixodes tasmani*	Greay et al., 2018
Theileria brachyuri PSC12	DQ437685	94.1%	Quokka, *Setonix brachyurus*	Clark and Spencer, 2007
Theileria worthingtonorum n. sp. ITF5	MG758121	93.5%	Tick, *Ixodes tasmani*	Greay et al., 2018
Theileria fuliginosus	DQ437686	89.4%	Western grey kangaroo, *Macropus fuliginosus*	Clark and Spencer, 2007
Babesia sp. 28	JQ682873			
Babesia sp. ALT strain 2	JQ437266	95.2%	Eastern grey kangaroo, *Macropus giganteus*	Dawood et al., 2013
Babesia sp. voucher AB2015-P38	KX361183	94.5%	Northern brown bandicoot, *Isoodon macrourus*	Barbosa et al., 2017
Babesia occultans	EU376017	92.7%	Sable antelope, *Hippotragus niger*	Oosthuizen et al., 2008
Bodo sp. ANO4	MK182523			
Bodo curvifilus	AY425015	93.4%	Red deer (*Cervus elaphus*) faeces	Von der Heyden et al., 2004
Bodo sorokini strain ATCC	AY425018	86.1%	Russia (A.p. Mylnikov)	Von der Heyden et al., 2004

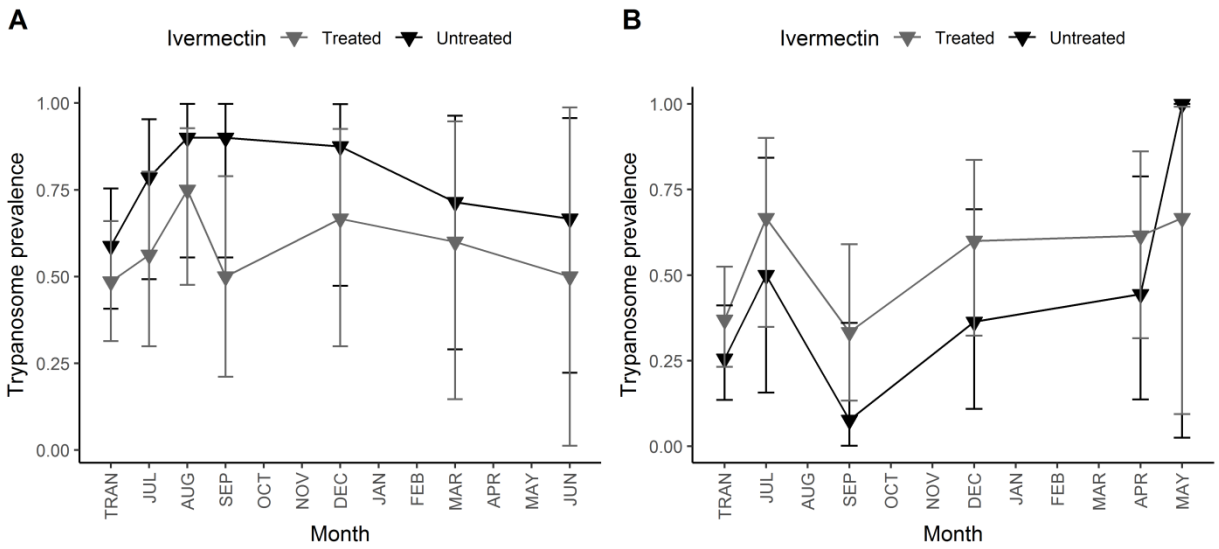

Figure A1: Trypanosome prevalence (with 95% CI) in treated *versus* untreated translocated woylies within (A) Dryandra and (B) Warrup East. TRAN: time of translocation.

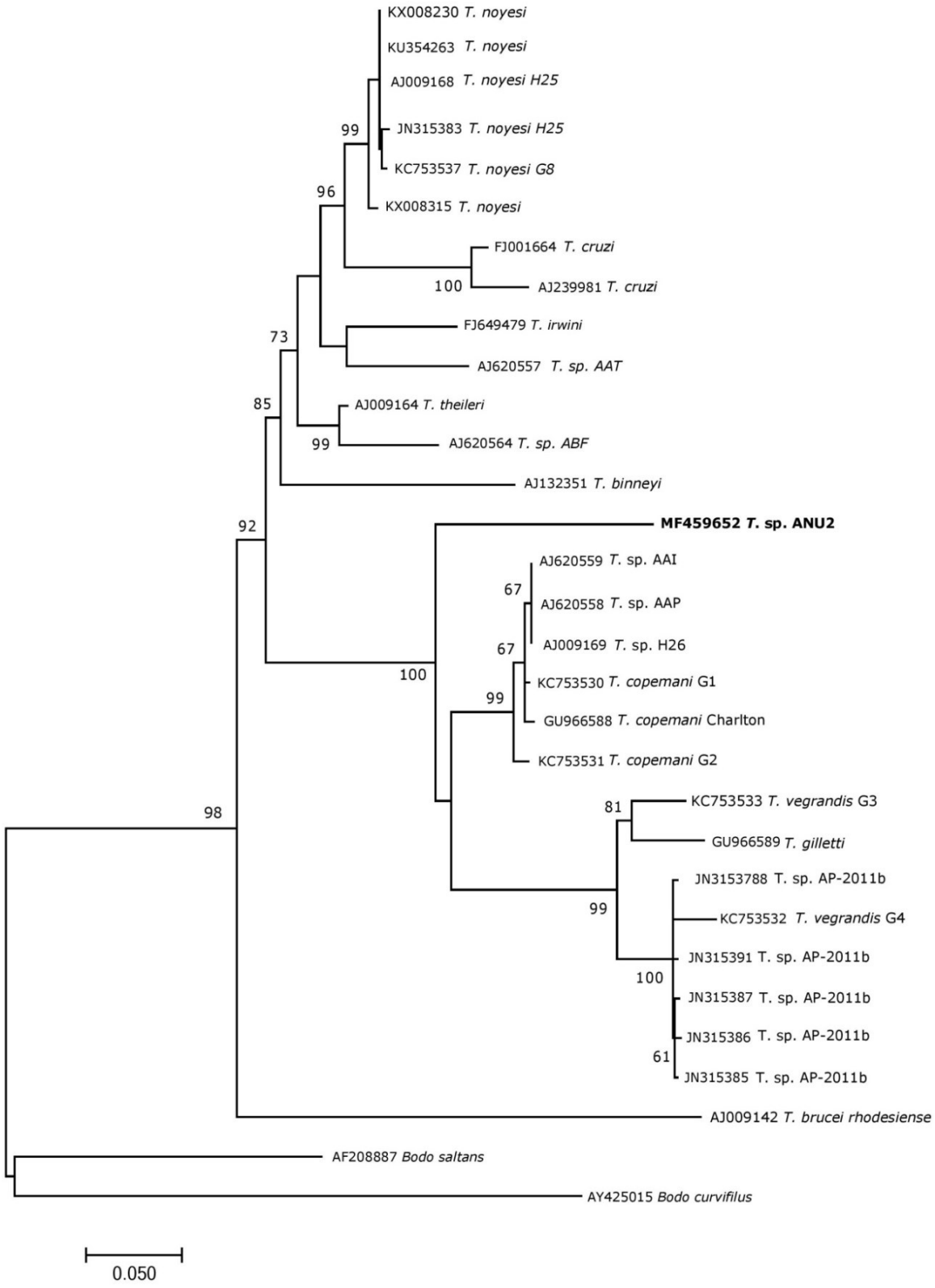

Figure A2: Phylogenetic relationship of *Trypanosoma* sp. ANU2 MF459625 compared to other trypanosomes, inferred using maximum likelihood analysis. We have included 30 sequences from GenBank, including three outgroups (*Bodo saltans*, *Bodo curvifilus* and *Trypanosoma brucei rhodesiense*), to validate the 1371 bp *18S* rDNA alignment.

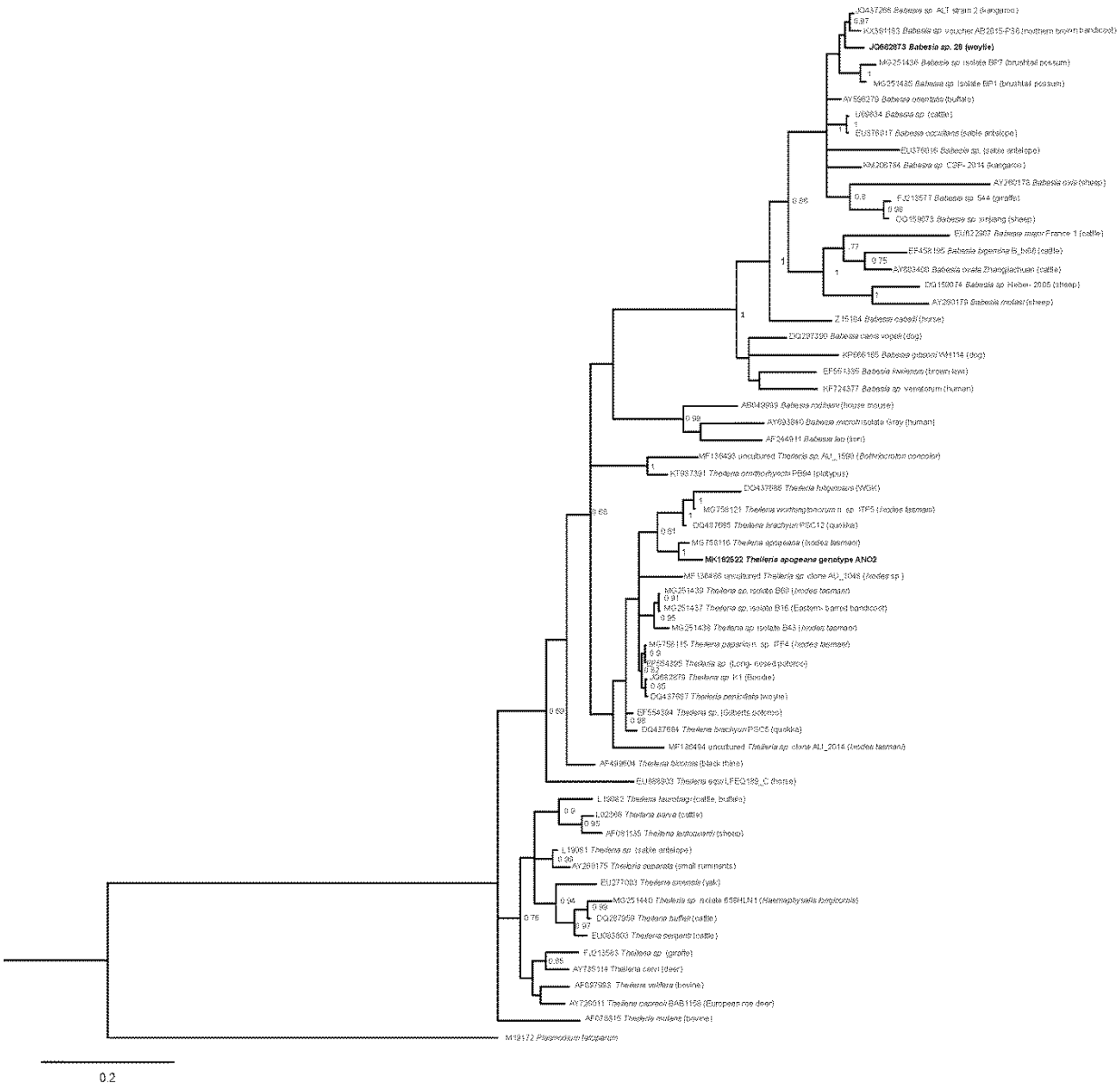

Figure A3: Phylogenetic relationship of *Theileria apogeana* genotype ANO2 MK182522 (and *Babesia* sp. 28 JQ682873) compared to other piroplasms, inferred using the Bayesian method. Fifty nine sequences from GenBank, plus one outgroup sequence (*Plasmodium falciparum* M19171), have been included to validate the 693 bp *18S* rDNA alignment.

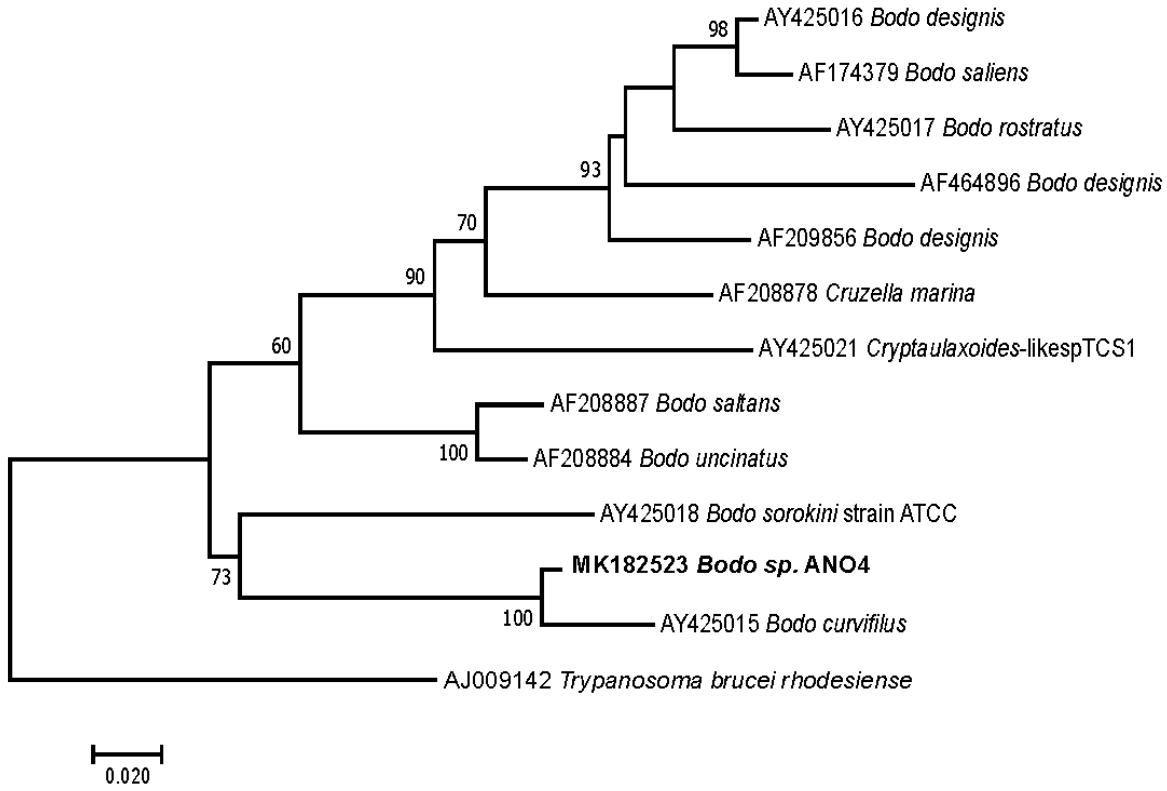

Figure A4: Phylogenetic relationship of *Bodo* sp. ANO4 MK182523 compared to other bodonida species, inferred using the maximum likelihood analysis. Eleven sequences from GenBank, plus one outgroup sequence (AJ009142), have been included to validate the 725 bp *18S* rDNA alignment.

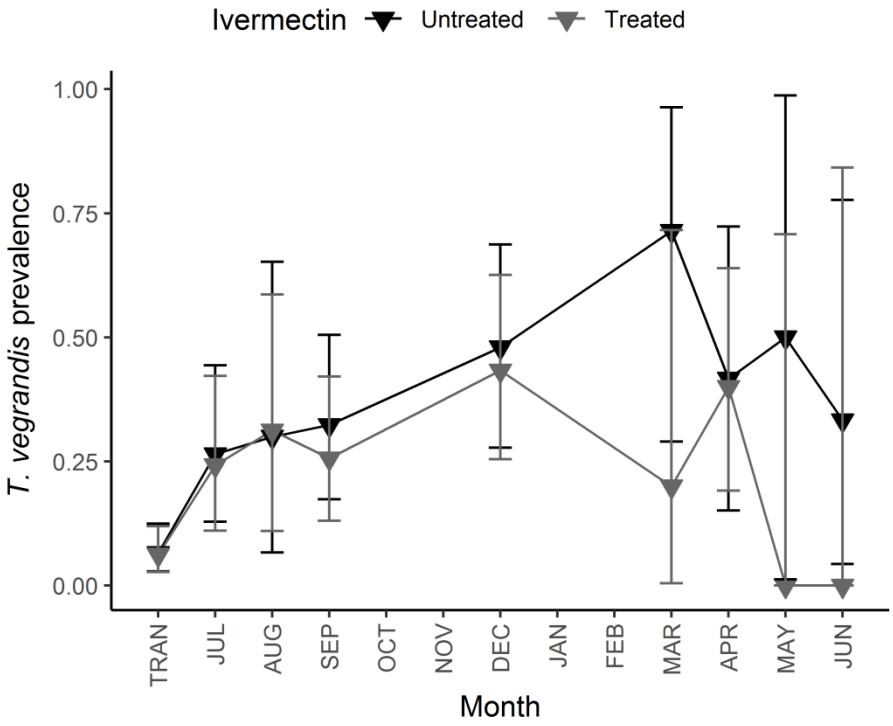

Figure A5: *Trypanosoma vegrandis* prevalence (all sites combined) over time (with 95% CI) in treated *versus* untreated translocated woylies (TRAN: time of translocation).

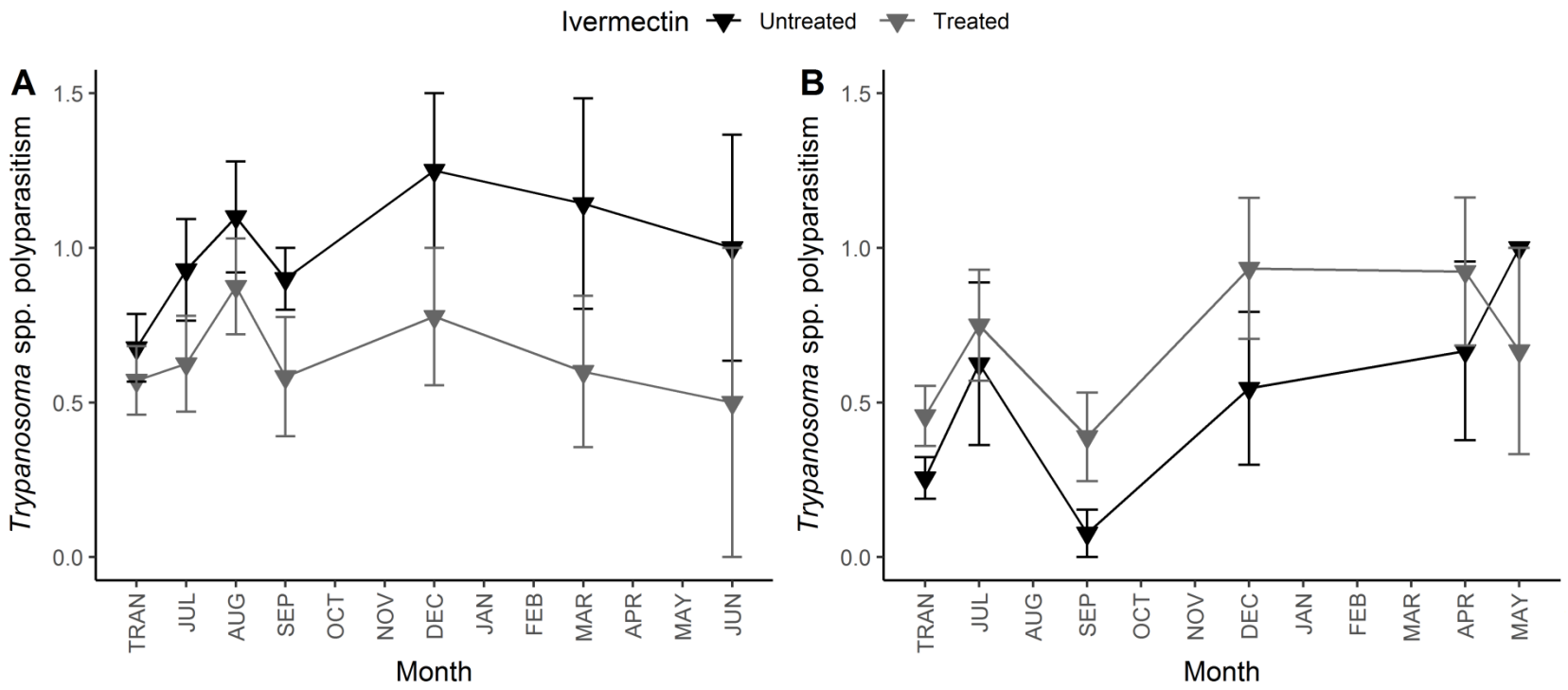

Figure A6: *Trypanosoma* spp. polyparasitism over time (with standard error bars representing 1 SE) in treated *versus* untreated translocated woylies in (A) Dryandra and (B) Warrup East (TRAN: time of translocation).

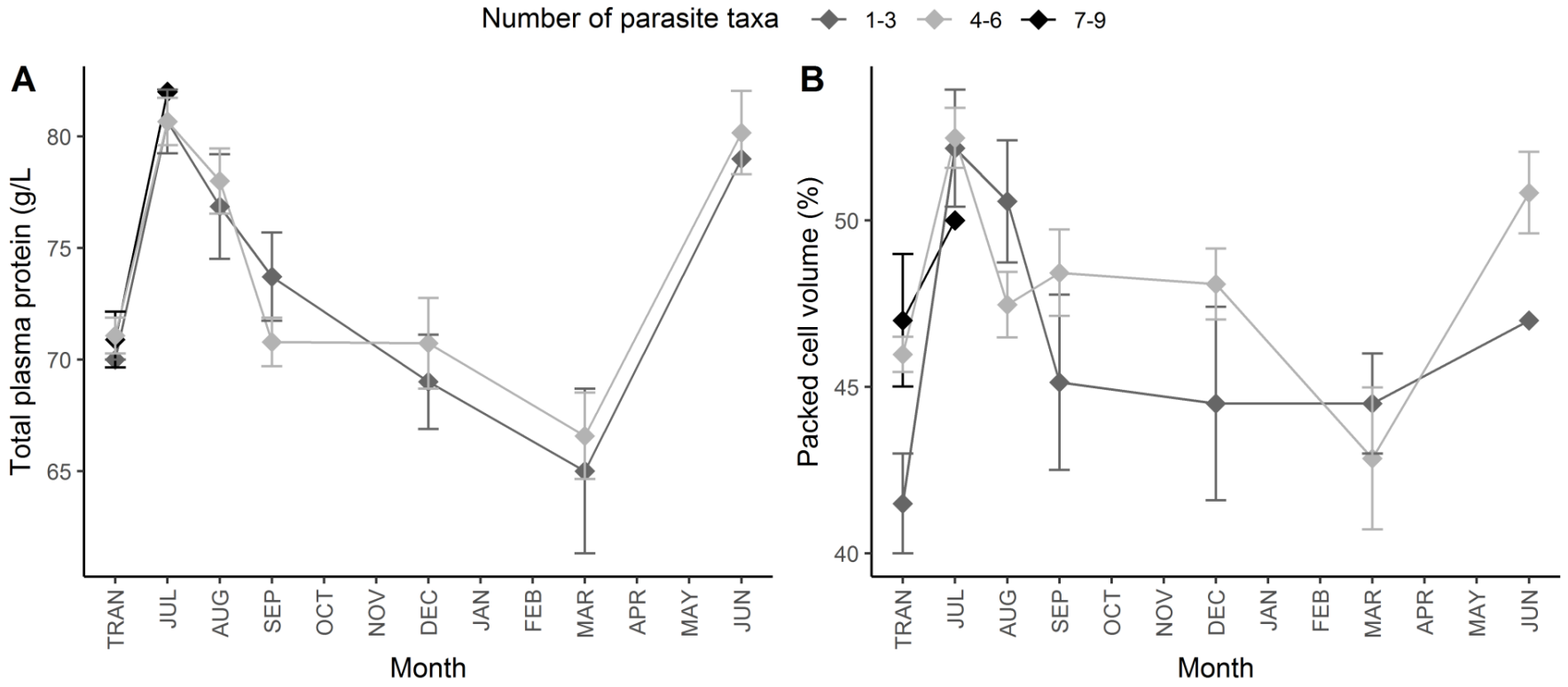

Figure A7: Total plasma protein (A) and packed cell volume (B) over time in relation to the number of parasite taxa infecting woylies translocated into Dryandra. After July we did not detect more than six parasite taxa infecting a host. TRAN: time of translocation; Error bars represent one standard error.

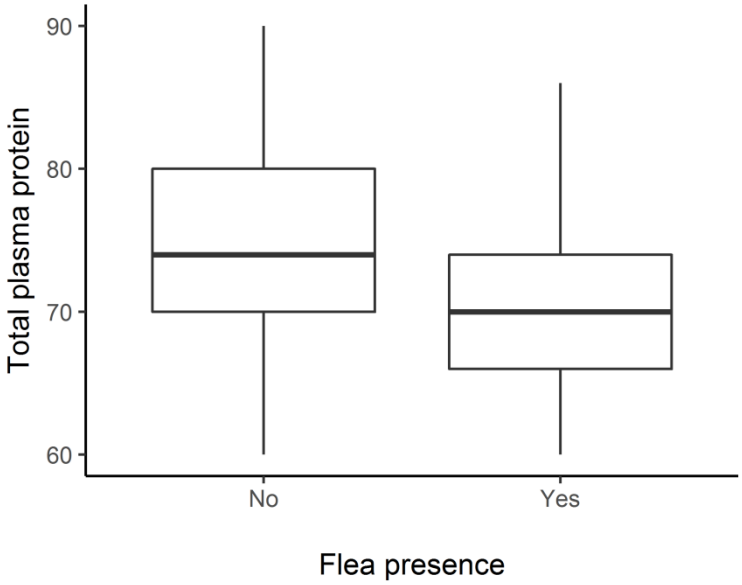

Figure A8: Boxplots depicting total plasma protein (g/L) values in woylies translocated into Dryandra in the presence (n = 29) and absence (n = 135) of fleas. Each box is delimited by the first (lower) and third (upper) quartile with the median represented by the thick horizontal line; whiskers represent the 1.5 interquartile range; solid black dots represent outliers.

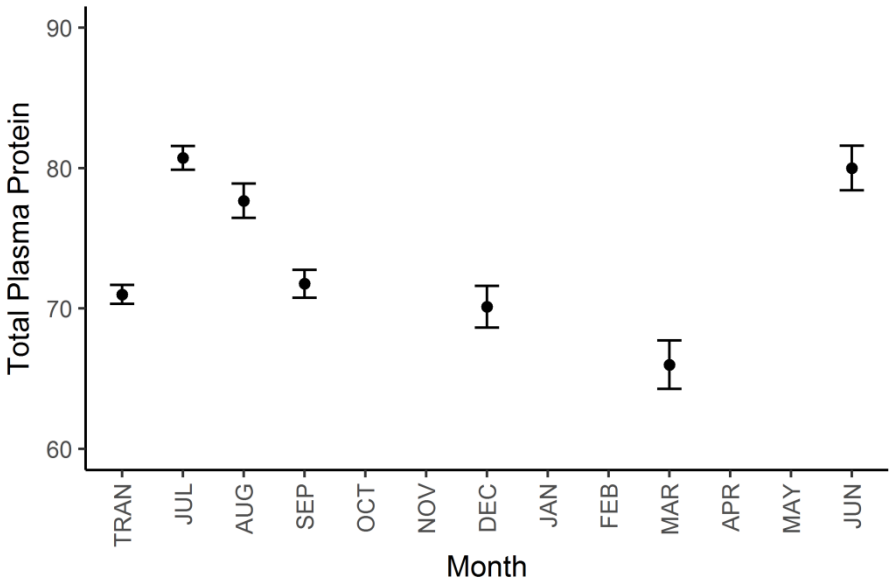

Figure A9: Mean total plasma protein values (g/L) over time in woylies translocated into Dryandra. TRAN: time of translocation. Error bars represent one standard error.

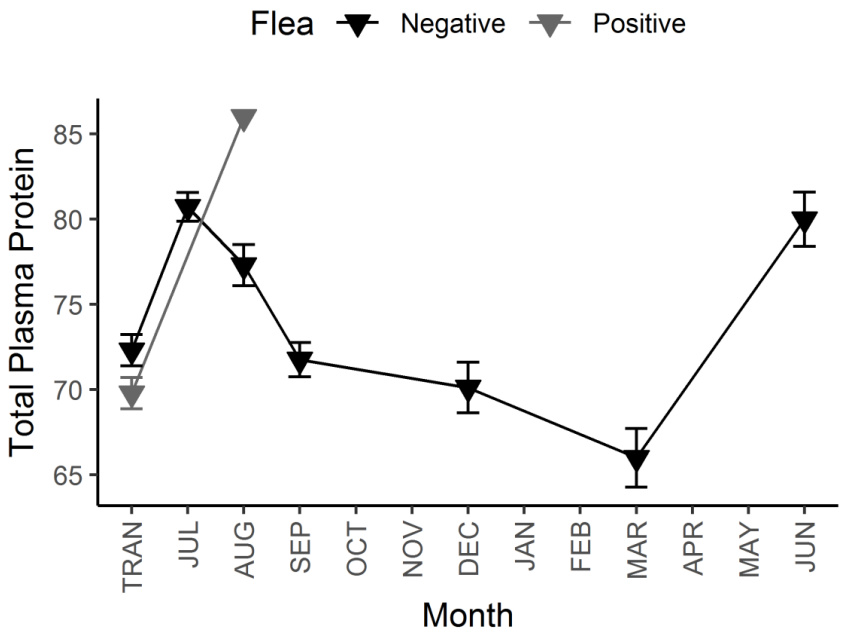

Figure A10: Mean total plasma protein values (g/L) in (Dryandra) translocated woylies in the presence and absence of fleas. No fleas were detected in translocated hosts after August. TRAN: time of translocation; Error bars represent one standard error.

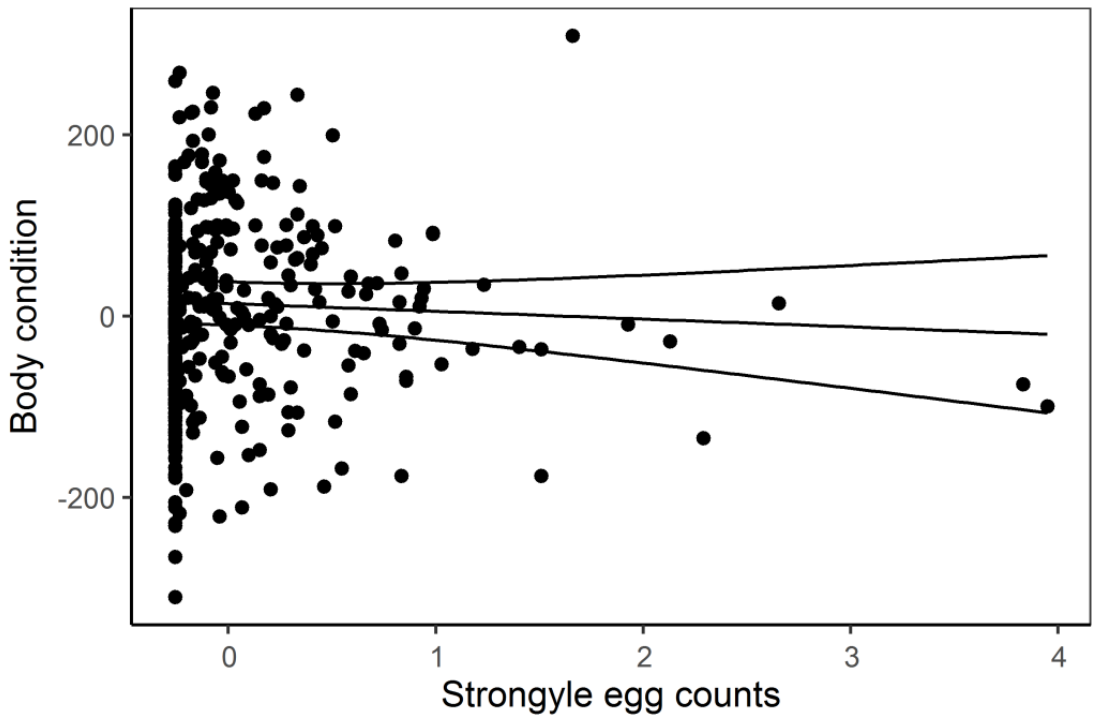

Figure A11: The relationship between body condition and strongyle egg counts in resident woylies (all three sites combined, sampled over two years). Data points above zero reflect above average body condition, while those below zero reflect below average body condition.

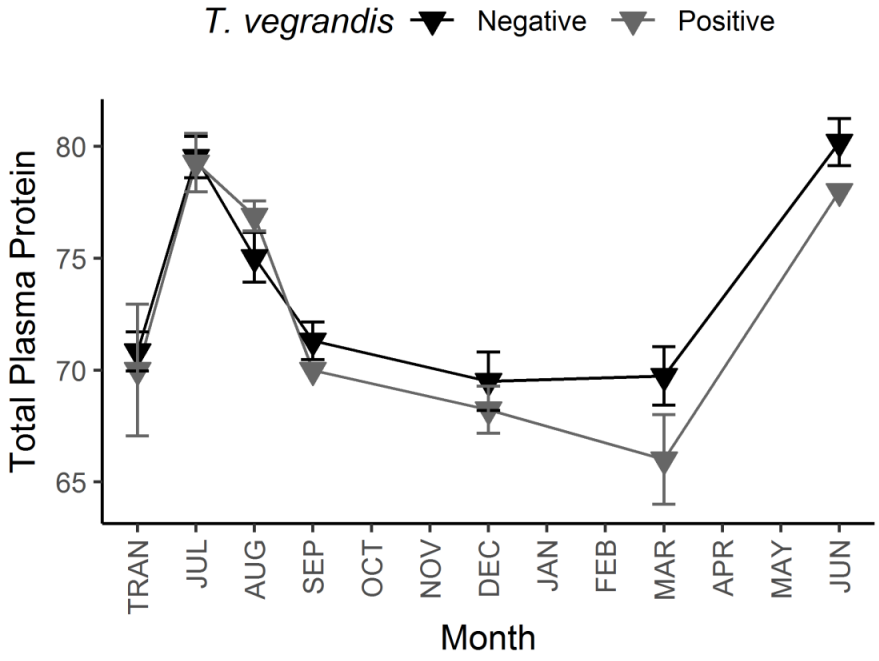

Figure A12: Mean total plasma protein values (g/L) over time in *T. vegrandis*-positive *versus T. vegrandis*-negative Dryandra resident woylies. TRAN: time of translocation; Error bars represent one standard error.