

SARCOPTIC MANGE AND ITS ICONIC HOSTS IN SOUTHEASTERN AUSTRALIA

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STATEMENT OF ORIGINALITY

This statement certifies that to the best of my knowledge, this thesis content is entirely of my own work. The research presented in this thesis was undertaken while I was enrolled as a Doctor of Philosophy candidate with the School of Life and Environmental Sciences at the University of Sydney. This thesis has not been submitted to the University of Sydney or any other institution.

I certify that the intellectual content presented in this thesis is entirely the product of my own work and to the best of knowledge, I have appropriately acknowledged all sources of information, collaboration and assistance received in preparing this thesis. Generative AI was not used at any stage in the development of this thesis.

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THESIS ABSTRACT

Introduced pathogens are a major source of infectious disease in wildlife. The *Sarcoptes scabiei* mite, causing sarcoptic mange, is a notable example of an invasive pathogen which has expanded its geographic and host species distribution through the movement of humans, domestic animals and wildlife. Sarcoptic mange causes a range of impacts across host species, including severe disease, population declines and even extirpation events. Effective strategies to mitigate disease impacts are in demand for many affected species, including Australia's iconic koalas and bare-nosed wombats.

The aim of this thesis was to advance our understanding of the impact of sarcoptic mange, as an introduced pathogen, on two Australian iconic animals, koalas and bare-nosed wombats. Firstly, to advance understanding of sarcoptic mange as an emerging disease in koalas, I undertook research to investigate epidemiology of the disease in northern Victoria (Chapter 2). In my study area, regular cases come into care, suggesting mange may have become enzootic. By investigating koala mange admission records, my research suggested that sarcoptic mange causes severe disease in koalas, and that male koalas may play an important role in seasonal disease dynamics.

Secondly, I tested the safety and pharmacokinetics of a novel chemotherapeutic agent in koalas to provide an evidence base for treatment options (Chapter 3). Fluralaner (Bravecto® MSD Animal Health), has been demonstrated as a long-lasting and efficacious chemotherapeutic option against sarcoptic mange in mammalian wildlife, and may also be beneficial for impacted koalas. I evaluated the pharmacokinetics and clinical safety of 85mg/kg of transdermal fluralaner in healthy, captive koalas. Overall, this research suggests fluralaner is a safe and long-lasting chemotherapeutic agent that may be efficacious against *S. scabiei* in koalas.

I then used applied research to contribute to our understanding of broad-scale landscape epidemiology of sarcoptic mange in bare-nosed wombats. In Chapter 4, I investigated the influence of bushfire on sarcoptic mange in bare-nosed wombats. Large, high intensity wildfires can have dramatic impacts on wildlife, but how fires shape wildlife disease is virtually unknown. I sought to investigate the impact of the Black Summer bushfires 2019/2020 in NSW on the dynamics of wombat-mange.

Using two independent lines of evidence, in a Before After Control Impact (BACI) study design, I tested the effect of the occurrence of fire and the effect of fire severity on the occurrence of sarcoptic mange in wombats. Results suggest that mange temporarily increases as a function of the extent of local area impacted by severe fire conditions, but does not persist beyond the year of the fire.

Lastly, I incorporated fire into broad-scale landscape epidemiological investigation in eastern Victoria and contrast my findings with other landscape epidemiology studies of mange in wombats (Chapter 5). I investigated the landscape epidemiology of wombat-mange using 188,982 remote camera trap images of bare-nosed wombats and remote sensed environmental data. I found that wombats, and wombats with mange, were widespread. Mange occurrence in wombats was most strongly predicted by year, was higher in areas with more topographic roughness, lower annual mean precipitation and with increasing distance to freshwater sources. By comparing my results with landscape epidemiology studies from Tasmania and New South Wales, I found a consistent negative association between mean annual precipitation and mange. The relationships between mange and both mean annual temperature and temperature annual range switched from positive in Tasmania and Victoria, to negative in New South Wales.

This thesis has: i) advanced our understanding of the individual and population level impacts of mange in koalas, including the clinical presentation, seasonality, transmission dynamics, prevalence of mange and interannual variation in sarcoptic mange patterns in koalas, ii) identified a novel treatment that is safe and which may be of use in treating and managing sarcoptic mange in koalas, iii) fire can have short-term effects on wombat mange, but this effect is mediated by the extent and severity of the local landscape burned, and iv) advanced our understanding of the landscape epidemiology of wombat-mange on landscape scale and across latitudes. This thesis has direct implications for future disease management and findings here also contribute to our broader understanding of an invasive visual pathogen on iconic host species, with global significance.

NOTES ON FORMATTING

This thesis is a compilation of multiple individual manuscripts, some published, and others formatted for publication. For the published and unpublished chapters, the formatting is reflective of the relevant journals requirements and therefore there may be differences between chapters, and particularly the reference lists for each section. Furthermore, since each chapter has been developed independently as standalone manuscripts there is inevitably repetition, particularly around the introductions for each chapter.

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CHAPTER 1.

GENERAL INTRODUCTION



Image credit: Ellyssia Young

CHAPTER 1: GENERAL INTRODUCTION

1.1 IMPACT OF INTRODUCED PATHOGENS ON WILDLIFE

Many important diseases impacting wildlife are caused by introduced pathogens, and the significance of introduced pathogens has increased worldwide (Tompkins et al., 2015). Introduced pathogens of wildlife are often due to spillover, when species are moved into new geographic regions by humans and domestic animals, and through landscape modifications which alter wildlife movement and contact with species in which they do not normally interact (Daszak et al., 2000). Introduced pathogens have had substantive consequences on biodiversity, animal conservation, and animal health and welfare, and are a key threatening process for many wildlife species globally (Daszak et al., 2000, White et al., 2018, Gottdenker et al., 2014)

Variable impacts of invasive pathogens on wildlife host species

The impacts of invasive pathogens vary greatly, among host species affected by the same pathogen, among pathogens themselves, and at different spatial scales (individual, population, ecosystem). The causes of variation are also complex, often fundamentally shaped by physiological and environmental mechanisms (Gallana et al., 2013). Due to this, understanding the species-specific differences in the impacts of introduced pathogens has become a critical area of wildlife research and species management (Lips, 2016, Lewin et al., 2023). For example, host specific behavioural, physiological, and habitat attributes, have been shown to influence invasion dynamics of pathogens, host susceptibility, disease prevalence and morbidity (Biggins and Kosoy, 2001, Martin et al., 2019a, Lewin et al., 2023). As a result, the epidemiology of invasive pathogens can range from low-level endemic disease presence to population declines and localised extinction (Martin et al., 2018, Carver et al., 2023). Temporal patterns of disease may also oscillate between low levels and sporadic outbreaks, even well after introduction of the pathogen, with persistent consequences on host populations. Some of the most significant pathogens impacting wildlife worldwide include: chytridiomycosis, which affects over 700 amphibian species (Lips, 2016); bat white-nose syndrome which is currently affecting over 60 species of hibernating bat (Hoyt et al., 2021); and sarcoptic mange which affects over 150 species of mammal (Escobar et al., 2022).

1.2 SARCOPTIC MANGE: AN IMPORTANT DISEASE OF WILDLIFE GLOBALLY

Sarcoptic mange is an emerged and emerging pathogen impacting mammalian wildlife worldwide (Astorga et al., 2018). The parasite has been documented in over 150 species of mammal across 12 orders and 39 families and is present on every continent except Antarctica (Escobar et al., 2022). Mange is caused by the epidermal burrowing parasitic mite *Sarcoptes scabiei* (Pence and Ueckermann, 2002). Despite being among the most host-generalist of mammalian parasitic diseases, the parasite continues to be documented in new geographic regions and host species, facilitated by direct and indirect transmission modes (Escobar et al., 2022, Browne et al., 2022).

1.3 MORPHOLOGY AND LIFECYCLE OF *SARCOPTES SCABIEI*

The morphology and lifecycle of the *S. scabiei* mite is well understood (Friedman, 1947, Bochkov, 2010, Klompen, 1992, Arlian and Morgan, 2017, Currier et al., 2011). In brief, both male and female sarcoptic mites have a roughly oval body shape, with slight dorso-ventral flattening and short legs (idiosoma) with small variation between sexes (Bochkov, 2010; Figure 1). As an obligate ectoparasite, the mange mite's whole lifecycle occurs on the host, with potential for short-term survival off the host as an environmental reservoir (Arlian and Morgan, 2017). The *S. scabiei* lifecycle has five stages of development: egg, larva, protonymph, tritonymph and adult, all of which occur within the stratum corneum (outermost layer of the epidermis) (Figure 1.1) (Yabsley et al., 2025). Reports from *in vivo* animal models, suggest the lifecycle from egg to adult takes about 14 days with variations between some hosts and environmental conditions (Wendel and Rompalo, 2002, Walton et al., 2004, Arlian and Morgan, 2017). Because *S. scabiei* can also survive for up to 19 days off the host (under low temperature and high humidity conditions) (Arlian and Morgan, 2017), the parasite can influence transmission dynamics through direct transmission (between individual animals) and indirect transmission (between individual animal and an environmental reservoir) mechanisms (Browne et al., 2022). Flexibility in parasite transmission dynamics contributes to spatial and temporal heterogeneity based on bioclimatic and environmental attributes (Martin et al., 2019a, Burgess et al., 2023, Ringwaldt et al., 2023).

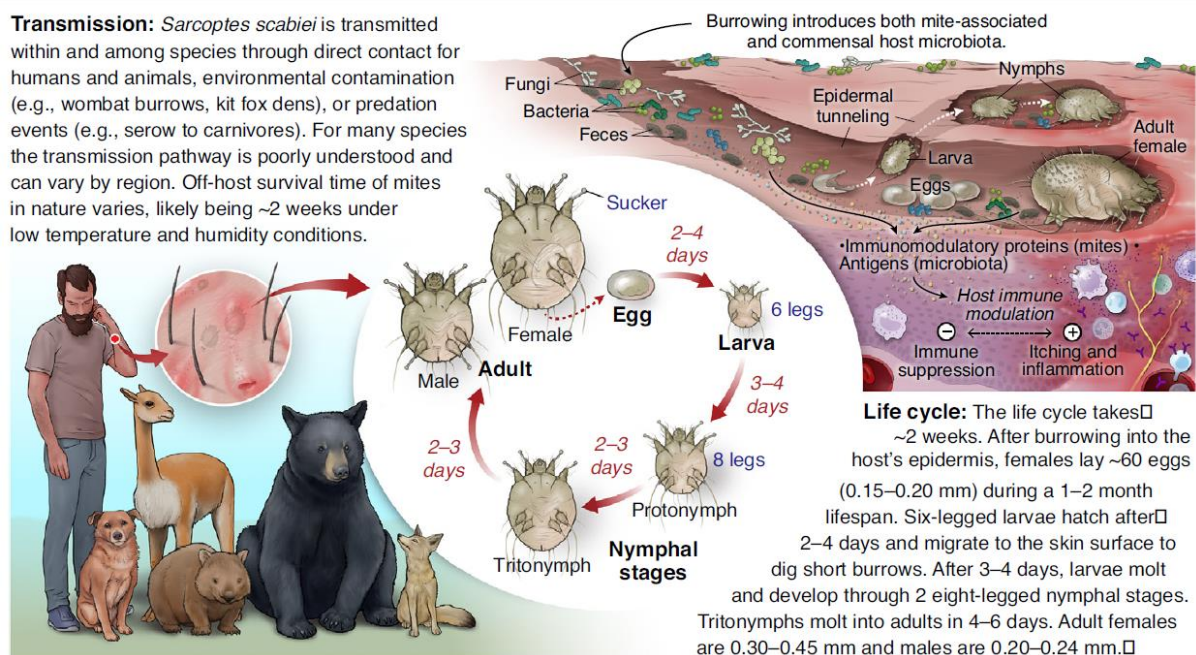


Figure 1.1. *Sarcoptes scabiei* mite lifecycle. Source: Yabsley et al. (2025).

1.4 IMPACT OF SARCOPTIC MANGE ON AUSTRALIAN WILDLIFE

It is generally accepted that sarcoptic mange was introduced during European colonisation of Australia, via humans (otherwise known as scabies in humans) and their domestic animals (dogs and possibly also agricultural and invasive animals such as red foxes, *Vulpes vulpes*) (Fraser et al., 2016). Available evidence suggests that there were likely multiple introduction events, and subsequent spillovers (where pathogens are transferred from endemic to novel hosts) to Australian wildlife (Fraser et al., 2017). Sarcoptic mange is an important parasitic disease in humans and domestic dogs in northern regions of Australia and is predominantly a wildlife disease issue in southern Australia (Currie and Carapetis, 2000, Munoz et al., 1992, Walton et al., 2004). Currently there are at least 11 wild Australian mammal species that have been documented to be affected by sarcoptic mange: bare-nosed wombat (*Vombatus ursinus*) (Martin et al., 1998), southern hairy nosed wombat (*Lasiorhinus latifrons*) (Ruykys et al., 2009), koala (*Phascolarctos cinereus*) (Young et al., 2024, Speight et al., 2017), agile wallaby (*Macropus agilis*) (McLelland and Youl, 2005), swamp wallaby (*Wallabia bicolor*) (Holz et al., 2011), dingo (*Canis lupus dingo*) (Hoyte, 1961), quenda (*Isoodon fusciventer*) (Botten et al., 2022), ringtail possum (Fraser et al., 2016), Tasmanian devil (*Sarcophilus harrisii*) (Russell et al., 2024), Bennett's wallaby

(*Notamacropus rufogriseus*) (Russell et al., 2024), and sandy inland mouse (*Pseudomys hermannsburgensis*) (Knox et al., 2025). Sarcoptic mange is enzootic in some of these species (e.g., bare-nosed wombat), and in others it appears as sporadic cases or occasional outbreaks (e.g., southern hairy-nosed wombat, agile wallaby, swamp wallaby, ringtail possum, dingo, Tasmanian devil, Bennett's wallaby, and sandy inland mouse) (Holz et al., 2011, McLelland and Youl, 2005, Hoyte, 1961, Fraser et al., 2016). In some species, *S. scabiei* may be becoming enzootic (e.g., koala and quenda) (Young et al., 2024, Botten et al., 2022). Wombats and koalas have been documented to experience some of the most severe pathology associated with *S. scabiei*, known as crusted mange, which has caused welfare and conservation concerns (Martin et al., 2018, Young et al., 2024).

Sarcoptic mange causes broadly similar pathology across host species, with disease severity shaped by species-specific immunopathological responses and disease expression the result of an exaggerated immune response to the mite infection (Næsborg-Nielsen et al., 2022). Infected (infested) hosts can express ordinary or crusted mange signs, generally reflecting Type I or Type IV hypersensitivity responses respectively (Næsborg-Nielsen et al., 2022), although aspects of each hypersensitivity response can be observed in either clinical outcome. A Type I hypersensitivity reaction (e.g. immediate antibody-mediated immune response) is typified by the milder form of mange, ordinary mange, exhibited as pruritis and alopecia. Type IV hypersensitivity reaction (e.g. delayed cell-mediated immune response) generally causes the severe form, crusted mange, and is characterised by alopecia and hyperkeratosis.

Crusted mange is the clinical form that affects wombats and koalas, and the clinical signs include varying degrees of pruritis (itching), alopecia (hair loss), erythema (skin redness), hyperkeratosis (skin thickening), excoriation, and fissuring of parakeratotic scale (cracking of the abnormal retained skin cells) (Skerratt, 2003). These clinical signs leave wombats and koalas susceptible to secondary infections and can affect the animal's behaviour, ability to forage and thermoregulate, thus often resulting in emaciation and eventual death (Skerratt et al., 2004, Martin et al., 2018, Skerratt, 2003).

1.5 SARCOPTIC MANGE IN KOALAS

The first documented case of sarcoptic mange in koalas occurred in Victoria in 1974 and consisted of one hand-reared juvenile koala which had been raised with a bare-nosed wombat, so it was believed to have been infected from the wombat (Barker, 1974). In 1980, there was an outbreak of sarcoptic mange in a captive koala population in Queensland with the arrival of a wild koala (Brown et al., 1982). Soon after, in 1983, a study of diseases in koalas found two out of 55 deceased koalas submitted for necropsy were affected by sarcoptic mange (Obendorf, 1983). Sarcoptic mange has been recently reported in a number of geographically dispersed populations across Victoria and South Australia, with the significance for conservation not well understood (Speight et al., 2017, Speight et al., 2018). Speight et al. (2017) provided the first detailed case series of sarcoptic mange in free-ranging koalas and documented the clinical presentation and histopathological changes associated with mange infection. These studies report clinical signs of severe hyperkeratosis and deep fissuring particularly on the forelimbs and ventral mandible (see Figure 1.2). Their findings suggest that mange can be severe in koalas and has unique presentation to the species. Since then sarcoptic mange has been documented as a comorbidity or cause of death in health monitoring studies (Speight et al., 2018, Wilson et al., 2025). While sporadically reported over a long period of time, there are fundamental knowledge gaps surrounding the individual and population level impacts and disease dynamics of mange in koalas.

Attempts have been made to treat and cure mange affected koalas on the individual level. In 1974 a single mange affected koala was treated via full immersion in malathion, which resulted in recovery (Barker, 1974). Later in 1982, amitraz was used (via partial immersion) to treat affected koalas in a colony (the total number of koalas affected or treated was not documented), with a follow up treatment required after 10 days (Brown et al., 1982). The koalas that were asymptomatic of mange but in close contact with the diseased koalas were given two prophylactic treatments of amitraz also 10 days apart (Brown et al., 1982). Most of the koalas recovered from infection but some needed further follow up treatment to achieve clinical resolution. Speight et al. (2017) unsuccessfully treated a severely diseased koala with ivermectin via injection, and the koala died three days later. It is notable that some of the described

treatment approaches are currently less desirable (e.g. immersion), and with koalas currently being listed as Vulnerable to extinction by the IUCN Red List of Threatened Species (Woinarski and Burbidge, 2020), a need exists for improved capacity to effectively treat free-ranging koalas for sarcoptic mange in care and *in situ*. Therefore, assessment of safe, less invasive and efficacious chemotherapeutic agent options is required for use in treating sarcoptic mange in koalas.



Figure 1.2. Images of sarcoptic mange affected koala (deceased). A) muzzle, B) fore paw. Photos credit: Dutch Thunder Wildlife Shelter.

1.6 SARCOPTIC MANGE IN BARE-NOSED WOMBATS

The earliest records of sarcoptic mange in wombats date back to 1818 at a museum in Paris, however it is unclear whether this bare-nosed wombat was infected prior to leaving Australia or en route to Paris (Gray, 1937, Skerratt et al., 1998). The earliest record of mange in Australia comes from New South Wales in 1937 where an outbreak resulted in population decline (Gray, 1937). Sarcoptic mange has become established and endemic in bare-nosed wombats across most of their geographic distribution, which extends from southeastern Queensland, eastern New South Wales, Victoria and Tasmania (including Bass Strait Islands) (Martin et al., 1998, Ringwaldt et al., 2025, Ringwaldt et al., 2023). Since mange is so widespread and the clinical signs

conspicuous, bare-nosed wombats have been the subject to a more focussed research effort over time, relative to koalas, on individual and population level impacts, as well as the treatment and disease management (Martin et al., 2019b, Martin et al., 2018, Skerratt et al., 1998, Simpson et al., 2016, Beeton et al., 2019). Bare-nosed wombats suffering from sarcoptic mange present with highly visual clinical signs, including alopecia and hyperkeratosis, typically affecting their flanks, and the disease results in changes to their foraging behaviours, declines in body condition, and survival (Skerratt et al., 1999, Skerratt et al., 2004; Figure 1.3).

Research on chemotherapeutic disease control in wombats (bare-nosed wombats and southern hairy nosed wombats are both listed as Least Concern by the IUCN Red List of Threatened Species (Woinarski et al., 2025, Old et al., 2025)) have focussed on both individual and population scales, which are conducted by research teams, government bodies, wildlife rehabilitation and animal welfare organisations. In mange affected wombats, treatments include the macrocyclic lactone moxidectin via transdermal application (Leary et al., 2025, Death et al., 2011, Martin et al., 2019b), and injectable ivermectin (Skerratt, 2003, Ruykys et al., 2013). Both of these therapeutic options are efficacious, although there are also limitations, including a lack of research around effective dose determination relative to disease severity, limited duration of action, effectiveness against all mite life stages, (not ovicidal), requirement of re-treatment, and logistics around treating, (and re-treating), free-ranging wildlife across landscapes. Recently fluralaner (Bravecto® MSD Animal Health) has also been documented to be an effective treatment for sarcoptic mange in wombats, with novel benefits being the long duration of action and wide safety margin (Wilkinson et al., 2021). With this longer acting efficacious treatment option, research has also focussed on enhanced drug delivery in the field (Wilkinson et al., 2024).

A range of research on bare-nosed wombats has focussed on understanding disease impacts at population and landscape levels. Mechanistic modelling suggests that *S. scabiei* can cause a range of epidemiological outcomes, including pathogen extirpation, endemic disease, and epizootics with host population decline (Beeton et al., 2019). Accumulating empirical evidence supports these findings, particularly showing that endemic disease (5-15% prevalence) with relatively stable wombat populations appears the most common outcome (Driessen et al., 2021, Carver et al., 2021, Burgess et al., 2023, Stannard et al., 2020), with occasional localised outbreaks

causing population collapse (Martin et al., 2018, Carver et al., 2023). Research has also focussed on the landscape epidemiology of sarcoptic mange in wombats, reflecting the importance of environmental transmission for the species (Browne et al., 2021, Browne et al., 2022). The results from landscape epidemiology studies demonstrate temporal and spatial variation in sarcoptic mange occurrence, and variability in the relationships between environmental and climatic variables, such as mean annual precipitation and land cover type (Ringwaldt et al., 2025, Ringwaldt et al., 2023, Stannard et al., 2020, Burgess et al., 2023). While wombats have received more research than other Australian host species, there remain important knowledge gaps around how changing environmental disturbance regimes, such as fire, can affect mange occurrence or how the environmental associations with mange vary across latitudes.



Figure 1.3. Camera trap image of sarcoptic mange affected bare-nosed wombat. Photo credit: Southern Ark program.

1.7 OVERVIEW AND FOCUS OF THESIS

While sarcoptic mange is a known threat to wombats and koalas, fundamental knowledge gaps still exist, particularly around: the impacts and management of mange as an emerging disease in koalas; and the landscape epidemiology in bare-nosed wombats, within populations and across different latitudes (Astorga et al., 2018). These questions are integral for advancing our understanding of this globally significant pathogen, and to inform adaptive interventions for the management of sarcoptic mange in these species.

The overall objective of this thesis is to advance our understanding of sarcoptic mange in its two iconic Australian hosts, wombats and koalas, and bridge key knowledge gaps in the literature. To address this objective, I present four core chapters in the form of independent publishable units (Table 1.1) bound by a general introduction and a general discussion (Chapter 6) (Figure 1.4).

Sarcoptic mange clinical presentation and population level epidemiology is highly variable between host species, and not well characterised in koalas. In Chapter 2 I explore the epidemiology of a persistent sarcoptic mange outbreak in a koala population in northern Victoria using detailed wildlife carer records (Young et al., 2024). I address knowledge gaps around clinical signs, disease characteristics (distribution across the body), and prevalence including seasonal and interannual patterns. From this I found that mange can be severe in koalas, however degree of alopecia is often low potentially limiting early detection of mange in free-ranging individuals and, as a result, most koalas were at advanced stages of infection when reported and had to be euthanised. We documented strong seasonal and inter-annual patterns of koalas coming into wildlife care with mange and that males may be important in driving mange dynamics in koalas. These findings may be of use in the development of monitoring and disease control programs.

Reflecting a need for improved disease management options in koalas, in Chapter 3 I test the safety and pharmacokinetics of fluralaner. Fluralaner is currently a widely used treatment for sarcoptic mange in bare-nosed wombats (Wilkinson et al., 2021). This study utilised a healthy, captive population of koalas to determine the safety and correct dose rate for treatment of sarcoptic mange in the koalas. From this study I found that fluralaner was well tolerated by koalas and there were no deleterious effects

from the transdermal application of fluralaner at a dose of 85mg/kg. I also document that fluralaner was present in plasma at concentrations which have been shown to be efficacious against *S. scabiei* mites in bare-nosed wombats. This provides a valuable reference for wildlife carers land managers and wildlife veterinarians to safely and effectively treat sarcoptic mange in koalas and improve welfare outcomes.

Table 1.1. Schematic overview of my thesis structure, showing the spatial scale, study type, methods and host-disease system used for each chapter.

Chapter	Spatial Scale	Study type	Methods	Host species	Title	Published
2	Regional	Population level case study	Wildlife carer records	Koala	A retrospective epidemiological study of sarcoptic mange in koalas (<i>Phascolarctos cinereus</i>) using wildlife carer admission records	Yes
3	Nationwide	Experimental treatment solution	Clinical trial	Koala	Pharmacokinetics and safety of topical fluralaner in koalas (<i>Phascolarctos cinereus</i>)	Yes
4	Landscape	Landscape level analysis	Camera trapping and wildlife carer reports	Bare-nosed wombat	Megafires and wildlife disease: Did the Australian Black Summer bushfires impact sarcoptic mange in bare-nosed wombats?	In preparation
5	Regional	Population level analysis	Camera trapping	Bare-nosed wombat	Advancing landscape epidemiology of sarcoptic mange bare-nosed wombats	In preparation

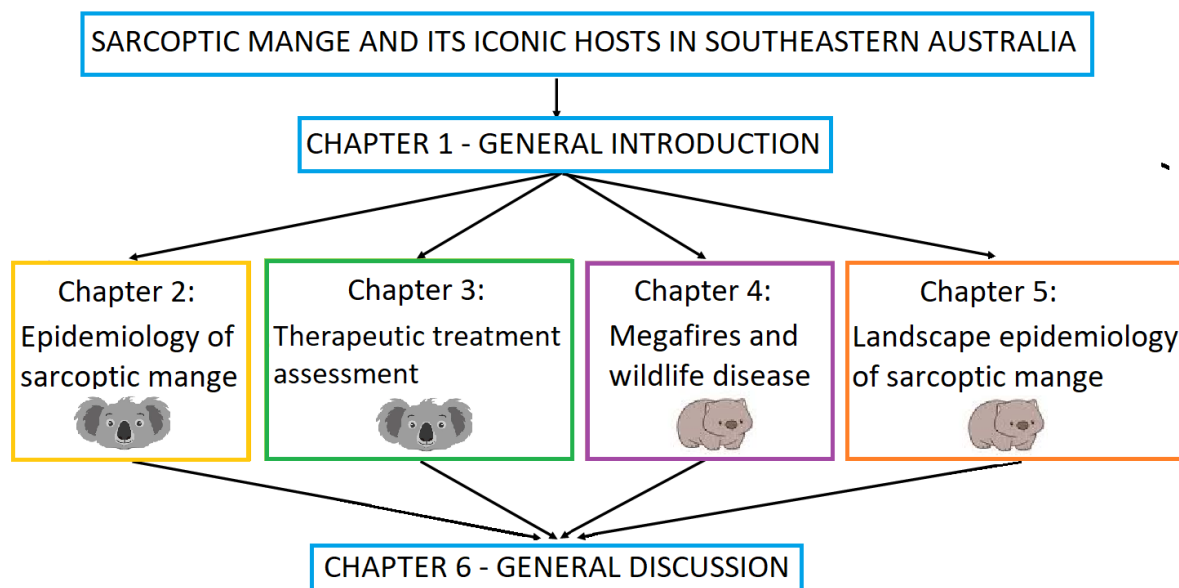


Figure 1.4. Thesis overview flowchart

Stochastic disturbance events such as bushfires directly impact wildlife survival, but there are often also indirect effects which are not as well understood, and one of these indirect effects can be wildlife disease (Albery et al., 2021). In Chapter 4, I explore the epidemiological consequence of bushfire on sarcoptic mange in wombats. In this Chapter I utilise a combination of wildlife carer records from WIRES (Wildlife Information, Rescue and Education service), and New South Wales Parks and Wildlife Service long-term WildCount dataset (2012-2021) (New South Wales National Parks and Wildlife Service (NSW NPWS), 2020) to investigate how the Black Summer 2019/2020 bushfires influenced patterns of mange disease. The findings from this research suggest that wildfire can have short-term effects on mange, mediated by the extent and severity of the local landscape burned. Our research has shed new light on how megafires can potentially have impacts on infectious diseases of wildlife.

Finally in Chapter 5 I consider the effects of fire and other environmental and bioclimatic variables on wombat mange occurrence and prevalence. I explore the landscape epidemiology of sarcoptic mange in wombats in far-east Victoria using the Southern Ark long-term camera monitoring program (Department of Energy Environment and Climate Action, 2024). I also compare my findings against those reported by two comparable landscape-scale sarcoptic mange epidemiology studies

in wombats in more southerly and northerly geographic distributions (Ringwaldt et al., 2025, Ringwaldt et al., 2023). I found that there were four key drivers of mange across this region which were year, topographic roughness, mean annual precipitation and distance to freshwater. Mange was consistently higher at low mean annual precipitation, and two variables, mean annual temperature and temperature annual range demonstrated a latitudinal switch from positive to neutral to negative relationships. There were also environmental variables which had no discernible patterns across studies and therefore may have more locally relevant effects on mange occurrence. This highlights how mange in wombat populations is subject to location specific drivers of mange.

In my final chapter (General discussion), I synthesise the key findings of my thesis, I discuss the implications for management from each research chapter, discuss the broader applications of this research and identify areas for future research.

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CHAPTER 2.

A RETROSPECTIVE EPIDEMIOLOGICAL STUDY OF SARCOPTIC MANGE IN KOALAS (*PHASCOLARCTOS CINEREUS*) USING WILDLIFE CARER ADMISSION RECORDS



Image credit: Ellyssia Young

CHAPTER 2.0: A RETROSPECTIVE EPIDEMIOLOGICAL STUDY OF SARCOPTIC MANGE IN KOALAS (*PHASCOLARCTOS CINEREUS*) USING WILDLIFE CARER ADMISSION RECORDS

Young, E.T., Phalen, D., Greenville, A.C., Donkers, K., Carver, S., 2024. A retrospective epidemiological study of sarcoptic mange in koalas (*Phascolarctos cinereus*) using wildlife carer admission records. *International Journal of Parasitology: Parasites and Wildlife*, 24, 100955. [https://doi.org/ 10.1016/j.ijppaw.2024.100955](https://doi.org/10.1016/j.ijppaw.2024.100955).

Abstract

Outbreaks of sarcoptic mange are sporadically reported in koala populations across Australia, but disease characteristics (e.g. distribution across the body) remain poorly understood. In an area of Northern Victoria regular cases coming into care suggest mange may have become enzootic, and here we characterise those koala mange admission records. In 18% (n=10) of mange affected koala reports that had a recorded outcome (n=55), the animals died before the carers could locate the animals, and of the remaining 45 koalas that were alive on arrival, 80% (n=36) had to be euthanised due to severe mange. The number of admissions varied among years (highest observed in 2019), and 2/3 of affected koalas were male. Male admissions peaked in spring and again in late summer-autumn (mating and birthing seasons), with female admissions only exhibiting the latter peak (birthing season). Fissuring on the front paws occurred in 100% of admitted koalas, with 70% showing these signs somewhere on ventral surfaces or limbs. Only male koalas had signs of mange on the chest and face, and only female koalas had signs of mange on their back. Collectively, this study suggests sarcoptic mange can be severe in koalas, and that male koalas may play an important role in seasonal transmission dynamics. We discuss how these findings may help inform intervention strategies.

Keywords: koala; *Phascolarctos cinereus*; sarcoptic mange; *Sarcoptes scabiei*; wildlife carer

INTRODUCTION

Sarcoptic mange is a highly contagious skin disease, caused by the *Sarcoptes scabiei* mite, and has been documented to infect a wide range of mammalian host species worldwide (Escobar et al., 2022). Within Australia, *S. scabiei* has become geographically widespread, but historically was only reported in a limited number of mammal species (Fraser et al., 2016). Thought to have been introduced to Australia by humans and their domestic animals, sarcoptic mange is now one of the most significant parasitic diseases of Australian marsupials (Martin et al., 2017). Sarcoptic mange is an enzootic disease in many bare-nosed wombat (*Vombatus ursinus*) populations across Australia (Martin et al., 2017), and occasional outbreaks have been reported in southern hairy nosed wombats (*Lasiorhinus latifrons*), wallabies (*Wallabia bicolor* and *Notamacropus agilis*) (McLelland and Youl, 2005, Holz et al., 2011), southern-brown bandicoots and quenda (*Isoodon obesulus* and *Isoodon fusciventer*) (Botten et al., 2022, Wicks et al., 2007), and koalas (*Phascolarctos cinereus*) (Speight et al., 2017). Furthermore, there is evidence to suggest that sarcoptic mange may be becoming endemic in some of these species as well, such as koalas (Speight et al., 2017), and quenda (Botten et al., 2022).

The first recorded case of sarcoptic mange in koalas occurred in Victoria in 1974 and consisted of one hand-reared juvenile koala which had been raised with a bare-nosed wombat, so it was believed to have acquired sarcoptic mange from the wombat (Barker, 1974). In 1980, there was an outbreak of sarcoptic mange in a captive koala population in Queensland which was linked with the arrival of a wild koala (Brown et al., 1982). In 1983 a study of diseases in koalas found two out of 55 deceased koalas submitted for necropsy were affected by sarcoptic mange (3.6%) (Obendorf, 1983). Sarcoptic mange has been recently reported in a number of geographically dispersed populations across Victoria and South Australia, with the significance for conservation not well understood (Speight et al., 2017, Speight et al., 2018).

Sarcoptic mange in koalas presents as crusted mange which is characterised as intense pruritis leading to hyperkeratotic lesions particularly on the face, chin, stomach, limbs and paws of affected individuals, which can result in thickening of the skin, fissuring, emaciation and death possibly owing to secondary infections (Speight et al., 2017). Sarcoptic mange can lead to significant welfare concerns, however it is

a treatable disease, with currently the most effective long-lasting treatment in wombats being fluralaner (Wilkinson et al., 2021) and this may also be therapeutic in infected koalas (Young et al., 2024). Very little is known about the epidemiological characteristics of mange outbreaks in koalas (Speight et al., 2017). For effective management of a disease, it is imperative to understand the characteristics of the infection.

Koalas are not currently listed as of conservation concern in Victoria, but are listed as Endangered in NSW, QLD and the ACT under the Environment Protection and Biodiversity Conservation Act (1999) (Department of the Environment, 2022), due to the species vulnerability to environmental and anthropogenic pressures (McAlpine et al., 2015). Along with the ecological value of this species, the visible effects of the disease on an iconic species such as the koalas means there is social demand to intervene and find solutions (Carver, 2016). In this study we utilize wildlife carer records to retrospectively investigate sarcoptic mange in free-ranging koalas from Northern Victoria, describing the characteristics and seasonal patterns of sarcoptic mange. We aim to contribute to the understanding of sarcoptic mange in koalas and advance understanding on areas where disease management could potentially be improved.

MATERIAL AND METHODS

Study area

Koala admission records used for this study came from Dutch Thunder Wildlife Shelter in Northern Victoria, which acts with a license issued under the Wildlife Act 1975 (Wildlife Shelter license number 14321755). This license permits the wildlife carer to undertake activities such as possess, pursue, capture, and/or euthanise and release protected wildlife in compliance with the standards outlined in the Act. Because this study consisted of analysis of existing data, it did not require Animal Ethics approval.

Records were available from October 2017 to May 2022. Admission data included variables: date, location found, weight, sex, clinical presentation, and outcome. Most koalas were found in remnant riverine woodland (Figure 2.1). All sarcoptic mange diagnosis were made by the experienced wildlife carer with vet nurse training using

clinical signs and where there was uncertainty, microscopic observation of skin scrapings were used to confirm diagnosis. Age was assigned based on visual observation of the whole animal, including body size and overall condition. Koalas were classified as either dependent joey, sub-adult, adult or geriatric, and if the animal was euthanized tooth wear was used to confirm age. However, for the purposes of this study koala age was simplified as dependent joey or adult (sub-adult, adult and geriatric). There was some variation in the extent of information among admission records and consequently sample sizes for descriptive analysis. For a subset of the records (n=12/82) a detailed assessment of the distribution and severity of mange over a koala's body could be made. To assess distribution and severity of mange the koala's body was divided into 13 segments and scored for presence or absence of mange signs (e.g., evidence of pruritis, alopecia, hyperkeratosis, and skin fissuring). Other clinical signs of ill health, such as diarrhea and emaciation were not included in severity scoring or distribution of mange assessment. A percentage of body affected was calculated based on how many segments of the body had mange signs present (e.g., 2/13 segments affected equates to 15% of body affected by mange). More detailed severity scoring was not possible from the data available. Body segments were chest, stomach, scrotum or pouch, upper hind legs, lower hind legs, hind paw, chin, front paw, lower front leg, upper front leg, ears, head and back. This scoring was then used to determine the percentage of the body affected by sarcoptic mange and indicate the severity of the sarcoptic mange signs in each animal. To evaluate koala mange admission patterns through time, we undertook a descriptive analysis of the data among calendar months, austral seasons and surrounding the koala breeding seasons (mating season August to November, birthing season December to March, non-breeding season April to July (Smith, 1980a, McLean and Handasyde, 2006)).

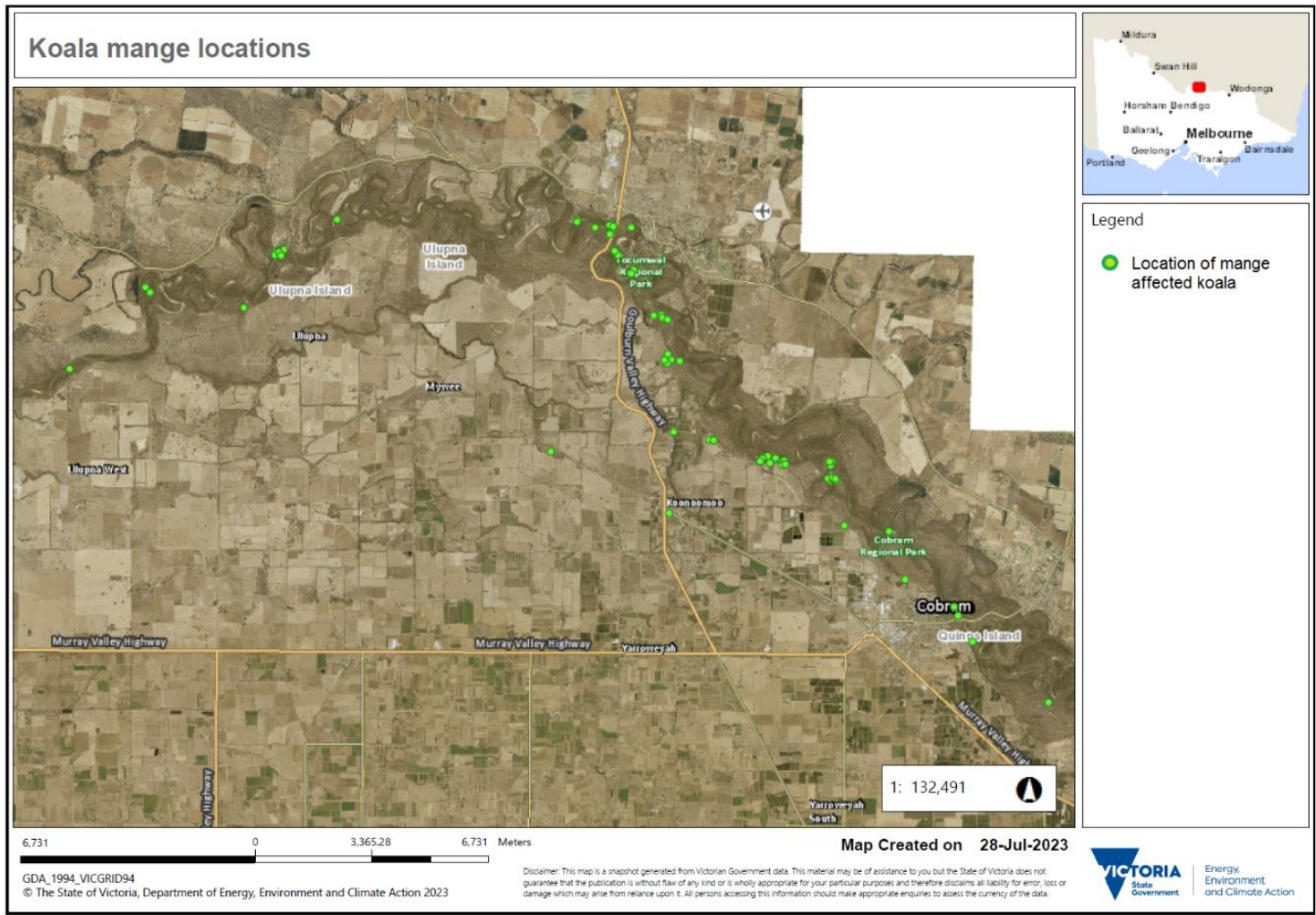


Figure 2.1. Map of locations of koalas (*Phascolarctos cinereus*) admitted with sarcoptic mange.

RESULTS

Characteristics of koala mange admissions

We undertook a descriptive analysis of koala admission records and describe patterns in the numbers and characteristics of koalas admitted with sarcoptic mange. The records consisted of 82 koala admissions with sarcoptic mange and occurred between October 2017 and May 2022 (Figure 2.2). Of the total 82 admission records 55 (67%, n=82) had a recorded outcome. The admission records with no outcome (n=27) were only excluded from analysis of outcome. Of these 55 with recorded outcomes, 10 (18%, n=55) koalas died prior to the carer's arrival, and 45 (82%, n=55) were alive upon the carer's arrival (Figure 2.2). Out of the total koala mange admissions (n=82), 45 of the koalas that were alive on the carer's arrival, of which 36 (80%, n=45) were euthanised on site by an experienced wildlife carer due to advanced stages of disease and decline in welfare state, 7 (16%, n=45) died after admission to the rehabilitation centre and 2 (4%, n=45) koalas were treated for sarcoptic mange. Of the 2 koalas that were treated, both received supportive therapies upon admission as deemed appropriate by the wildlife carer, one male koala recovered following treatment with Cydectin® pour on (Virbac Australia) (5 g/L moxidectin), 3mls per treatment weekly for 4 weeks, and was successfully released. The second male koala was a severe case of sarcoptic mange with a questionable chance of recovery. After much consultation with a wildlife veterinary specialist, treatment with a single dose of Bravecto® Spot-on (MSD Animal Health) (136.4 g/kg fluralaner) was attempted on the severely diseased koala, but the individual died overnight. No post-mortem was completed due to the obvious advanced extent of disease, and it was assumed to have died of mange associated secondary infections. Further research should include clinical progression of infection and post-mortem analysis to further examine the pathophysiology of mortality in koalas affected by sarcoptic mange.

In 22% (n=12) of the 55 records which were alive on arrival, detailed notes were made of the state of koalas upon encounter (Figure 2.3). Of these 12 koalas, 7 (58.3%) were conscious and alert upon collection by the wildlife rehabilitator, 3 (25%) were semi-conscious, and 1 (8.3%) were unconscious and 1 (8.3%) had no conscious state recorded (Figure 2.3). Unfortunately, information on evidence of co-infections was extremely limited. One koala was recorded to have urine stains but was not tested

further, another had injuries noted and one koala was recorded to have no other health issues. The remaining 79 cases did not have any comments on co-infections or other health issues.

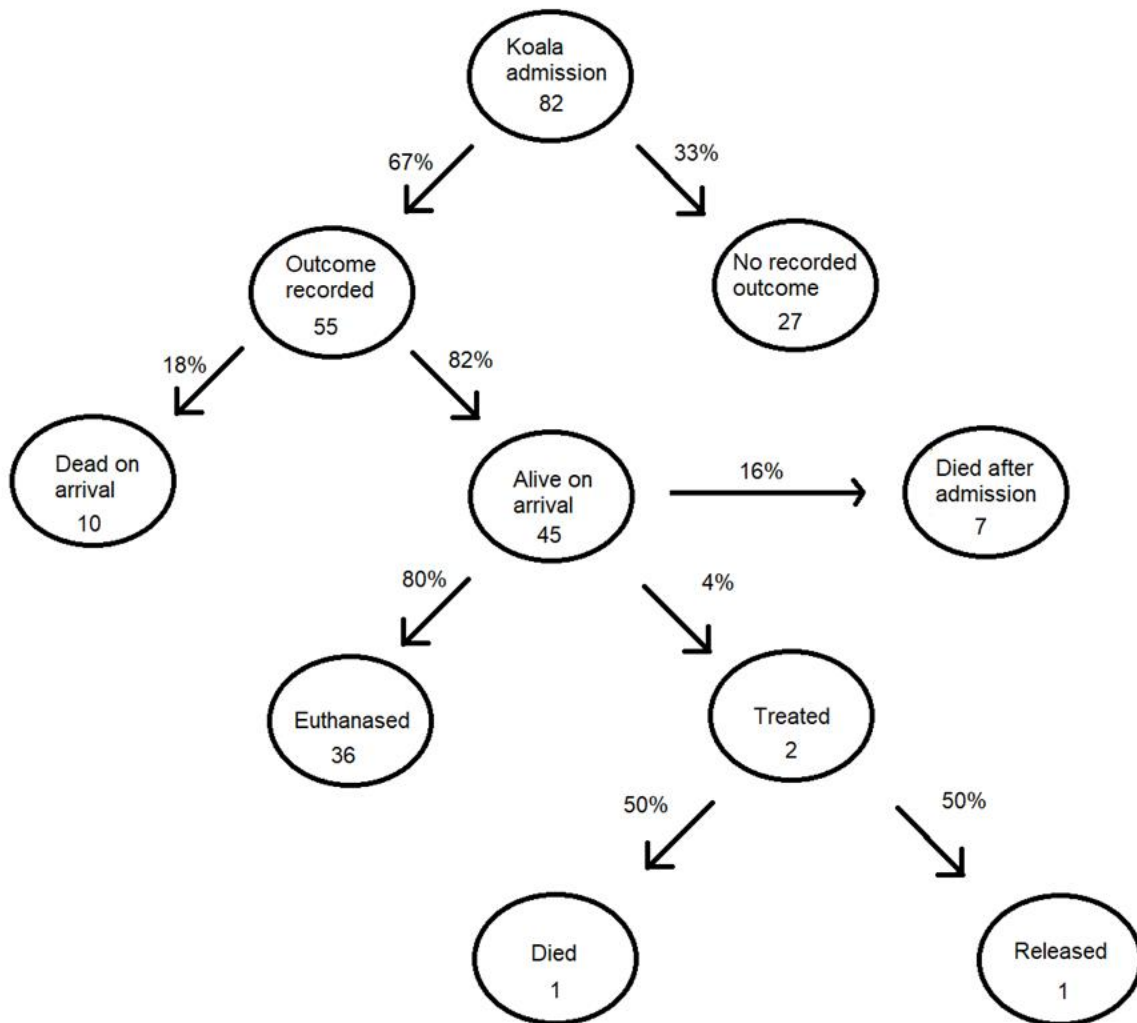


Figure 2.2. Outcome of 82 records of koala admissions with sarcoptic mange into Dutch Thunder Wildlife Shelter between October 2017 to May 2022.

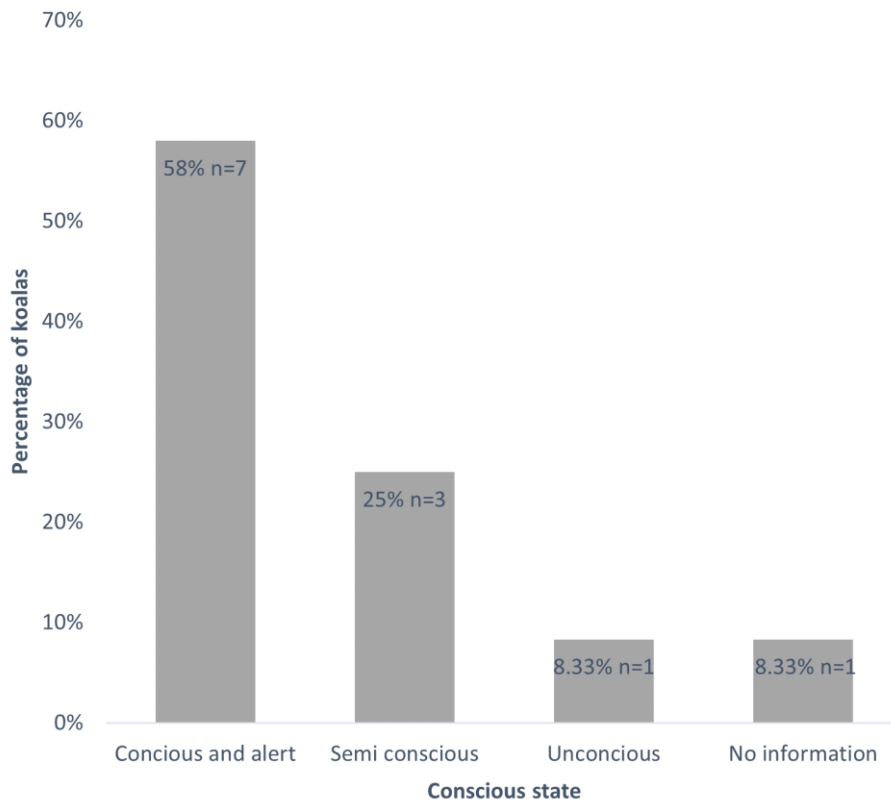


Figure 2.3. Conscious state of koalas when located by wildlife carers from 12 admission records of sarcoptic mange affected individuals from Dutch Thunder Wildlife Shelter between September 2019 to March 2020. Percentages and sample sizes are provided for each category.

Demographics and annual patterns of koala admissions

There were proportionally more male (66%, n=82) than female (28%, n=82) koalas admitted with signs of sarcoptic mange, with 6% of records having no sex listed (n=82) (Table 2.1). Of the 23 females admitted, 3 had pouch young (13%, n=23). Of these 3 pouch young, 1 was recorded to have sarcoptic mange, and 2 had no record of sarcoptic mange. The mother of the pouch young that was recorded was also recorded to have mange. We analysed the temporal patterns of koalas coming into care with sarcoptic mange. Analysis was restricted to 2018-2021 (n=75), as records for years 2017 and 2022 were incomplete. The highest number of cases of koalas coming into care suffering sarcoptic mange were documented in 2018 and 2019, with 2019 having the highest number of records (n=27) (Table 2.1). Each year there were more males

than females reported with sarcoptic mange, the largest difference was in 2018 where 83% (15 males out of 18 admissions) of the recorded sarcoptic mange affected koalas were male. Male koalas accounted for over 60% of sarcoptic mange records each year.

Table 2.1. Breakdown of admission records of koalas affected by sarcoptic mange from 2018 – 2021. Admission record data from 2017 and 2022 were incomplete and excluded from individual analysis.

	2018	2019	2020	2021	Average across years
Male	83%	63%	69%	64%	70 %
Female	6%	37%	25%	29%	24%
No record	11%	0%	6%	7%	6%
Total number of admissions	18	27	16	14	82

Monthly, seasonal and breeding cycle patterns in mange

The highest numbers of koala sarcoptic mange records were recorded in late Austral summer and early autumn (February - April) (n=75). Two distinct peaks in koala sarcoptic mange admissions were evident, from February-April and September-October (Figure 2.4a). Mange prevalence increased in males in early spring, beginning in September and peaking in October and again in February/March (Figure 2.4a,b). In contrast the early spring peak in sarcoptic mange prevalence was not observed in females, increased admissions were observed in January and peaked in February, and cases did not reduce until May (Figure 2.4a,b). There were no records of sarcoptic mange that affected female admissions across all years in June, August, October or December, whereas the only month with no admissions of males with sarcoptic mange was July (Figure 2.4a). We then analysed the rate of koala mange admissions (n=82) per month in each breeding season (mating, birthing, non-breeding) (admissions divided by the number of months in each breeding season), with the highest rate of total admissions per month occurring in the birthing season (December to March)

(9.75, n=39), followed by the mating season (August to November) (6, n=24) and then the non-breeding season (April to July) (4.75, n=19) (Figure 2.4c). When separated by sex, the highest rate of both male and female koala admissions occurred in the birthing season (5.75, n=23 and 3.25, n=13 respectively). However, within each season (mating, birthing and non-breeding) admission rate was highest for males (5, n=20; 5.75, n=23 and 2.75, n=11 per season respectively).

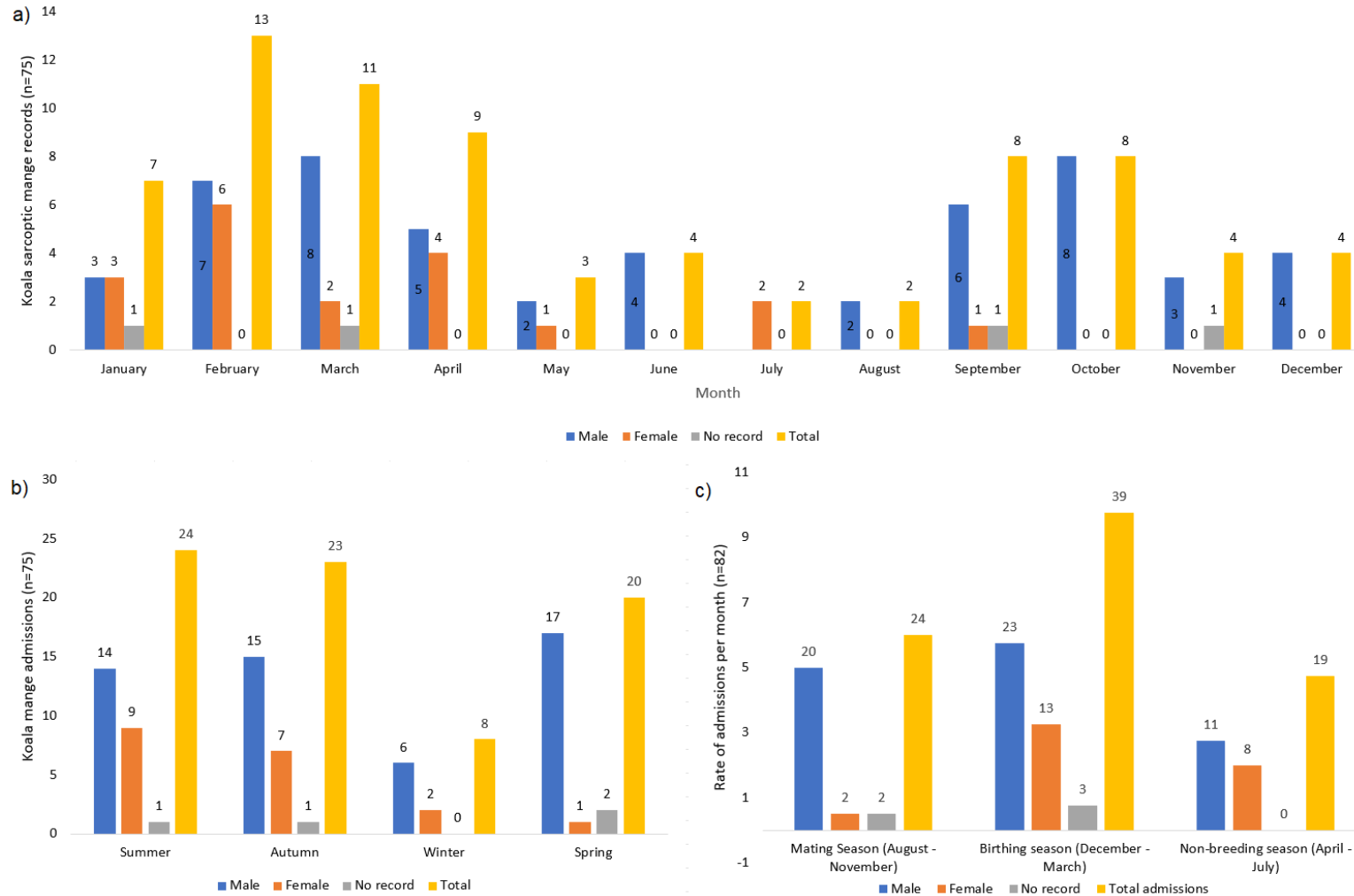


Figure 2.4. Koala sarcoptic mange admissions: a) Number of cases of koalas per month from 75 koala admission records affected by sarcoptic mange from January 2018 and December 2021. b) Number of koala sarcoptic mange admissions per season (n=75). c) Rate of koala sarcoptic mange admissions per month for each koala breeding season (n=82).

Sarcoptic mange presentation and severity

Of the 55 admission records which were alive on arrival, 12 records contained more detailed information which allowed further interrogation and were used to investigate sarcoptic mange presentation and severity (subset data). Skin fissuring was the most recorded sign of sarcoptic mange with 42% (n=12) of admissions showing these signs (Table 2.2). Another 8% (n=12) of admissions listed clinical signs as present but no specific symptom was given (Table 2.2). Emaciation and skin crusting were both recorded in 25% of admissions (n=12), with pruritis, alopecia and diarrhea recorded in only 8% (n=12) of cases. Of the 12 admissions that recorded part of body affected, the most frequently affected body region observed was the front paw with 100% (n=12) of koalas reported to show signs of sarcoptic mange on this body region. Stomach, hind paw, chin and lower fore limb were recorded to be affected by sarcoptic mange in over 70% of cases (n=12). No records noted sarcoptic mange on the pouch (females only, n=2), ears or head (both males and females, n=12). When analysed by sex, only males (n=10) were observed to have sarcoptic mange on their chest and head segments and only females (n=2) were observed to have sarcoptic mange affecting their back (Figure 2.5). Both females were recorded to have had sarcoptic mange on their stomach, upper hind legs, hind paw, front paws, and lower fore limb (100%, n=2). Whereas all males (n=10) were only reported to have sarcoptic mange on their front paws (Figure 2.5).

Table 2.2. Frequency of clinical signs recorded in 12 koalas rescued with sarcoptic mange in Northern Victoria between September 2019 and March 2020.

Clinical signs	Frequency of detection (%)
Fissuring	42%
Emaciation	25%
Hyperkeratotic lesions/skin crusting	25%
Pruritis	8%
Alopecia	8%
Diarrhea	8%
Clinical signs noted but no specific sign recorded	50%

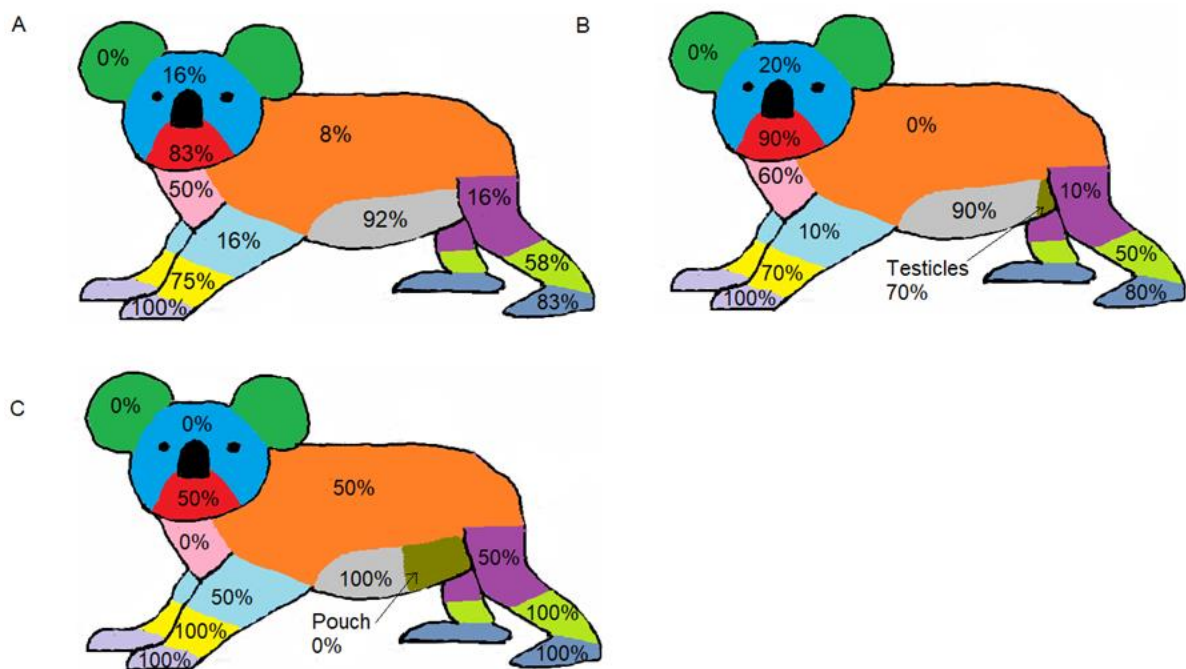


Figure 2.5. Frequency of sarcoptic mange infestation by body region recorded from 12 koalas that were reported with mange between September 2019 to March 2020. **A)** Total records. Sex specific features (pouch, scrotum) were included as the stomach region. **B)** Male records (n=10), **C)** Female records (n=2).

DISCUSSION

The epidemiology of sarcoptic mange disease can be highly variable among host species and even among populations of the same species (Browne et al., 2022). Patterns of disease may range from epizootic to enzootic (Escobar et al., 2022). The occurrence of sarcoptic mange disease in the koalas of the study area showed strong seasonal patterns. The highest numbers of koala sarcoptic mange cases were recorded in late Austral summer and early autumn (February - April). High prevalence of sarcoptic mange in males was observed in September/October (in the mating season (Smith, 1980a)) and then again in February/March (the birthing season (McLean and Handasyde, 2006)), whereas in females, high prevalence was only observed in January/February (the birthing season (McLean and Handasyde, 2006)) with cases reducing by May (non-breeding season (McLean and Handasyde, 2006)). The increase in male-male interactions during territorial disputes and competition as well as increased exposure to potentially contaminated fomites in the mating season is a likely explanation for the increase in sarcoptic mange admissions in males due to increased risk of mange transmission between individuals and the environment (Speight et al., 2017). The increased activity demonstrated by males during late winter and early spring (Watchorn and Whisson, 2019) and scent marking behaviour (which occurs throughout the year), where males rub the scent gland on their chest on trees to mark territory and communicate with other koalas (Mitchell, 1990, Smith, 1980b), may also increase their risk of acquiring sarcoptic mange mites from environmental fomites or other host species. The relative importance of fomites and specific sources of environmental reservoirs of mange mites has not yet been comprehensively investigated for koalas, but bark around the base of trees has previously been proposed as a possible exposure source to *S. scabiei*, owing to the potential of other mangy mammals (e.g. fox or wombat) to rub against these and deposit mites (Speight et al., 2017). Occurrence of mange affected females increased later in the year (the birthing season). Sarcoptic mange progression in koalas is not currently well understood, particularly relative to some other important disease issues this species faces (Robbins et al., 2020). Based on what is known about mange progression in the closely related wombat (Skerratt, 2003), it is feasible that female koalas exposed to sarcoptic mites during the mating season (from August to November) may develop visual signs of sarcoptic mange by January/February. The high rate of female koala

admissions in the birthing season and the low numbers of dependent joeys (3 out of 13 females had joeys recorded) warrants further research.

Alopecia is a commonly occurring symptom of sarcoptic mange in many affected species with varying degrees of alopecia observed between hosts, and generally quantified as an observable proportion of hair loss (Montecino-Latorre et al., 2020, Martin et al., 2018, Van Wick and Hashem, 2019, Botten et al., 2022). The koalas in this study displayed low levels of alopecia in comparison to many other impacted species, either closely or distantly related (Martin et al., 2018, Lewin et al., 2023). In this study, fissuring, skin crusting, and emaciation were more commonly observed signs of mange in koalas. This is not unique to koalas, for example Iberian lynx (*Lynx pardinus*) exhibit limited alopecia associated with sarcoptic mange (Oleaga et al., 2019). This lack of a highly visual clinical sign can have implications for detection and management of mange within free-ranging koalas. More research is needed to understand how best to assess mange among free-ranging koalas, and if the persistence and severity of sarcoptic mange are associated with anthropogenic land use, host specific physiological characteristics, comorbidities or immunological naivety.

Consistently there were higher numbers of male koalas admitted with sarcoptic mange across all years, suggesting that male koalas are more likely to be infested with sarcoptic mange. A higher proportion of male koalas affected by mange has also been observed in a previous study (Speight et al., 2018). Early in the breeding season male koalas are more active looking for females and often engage in territorial behaviour which may involve close contact (Jiang et al., 2022). Due to increased activity early in the breeding season (Austral spring) (Ryan et al., 2013), male koalas may have greater susceptibility and exposure to mange via direct transmission or to environmental fomites harbouring mange mites through activities such as scent marking. This along with the effect of anthropogenic and environmental stressors that koalas face (Narayan, 2019) may reduce immune defence mechanisms against parasitic disease. The presence of other diseases may increase the animal's risk of developing mange once exposed to the *S. scabiei* mites for example koala retrovirus, however specific comorbidities of mange infection is still unknown (Tarlinton et al., 2005, Greenwood et al., 2023). It would be valuable to have a feasible mechanism

that would support wildlife carers to collect and submit samples that could be used for further analyses. The increase in male interactions at the start of the breeding season in spring could be an important driver in sarcoptic mange dynamics and persistence in this population and warrants further investigation.

Interestingly, there were sex-specific patterns of clinical signs observed within the koala admissions with sarcoptic mange. These findings are inherently tentative, owing to the small number of females for which this information was available. All males were reported to have signs on their chest and face. Since mange signs spread from the site of infection as the disease progresses (Skerratt, 2003), this supports the idea that interactions between males during the mating season when males may be exhibiting scent marking behaviour and increased male-male interactions, is likely a key method of transmission. Both females were recorded to have sarcoptic mange signs on their backs, and disease progression characteristics as well as the increase in female sarcoptic mange cases in summer suggests that mating may be a key form of transmission in female koalas. Other studies have documented sexual differences in the prevalence and visible effects of sarcoptic mange infection (López-Olvera et al., 2015, Pence and Windberg, 1994, Speight et al., 2017), so is not unique to koala, and may help to understand transmission dynamics in this species. Currently it is believed that another important mode of transmission of sarcoptic mange among koalas is the persistence of *S. scabiei* mites from environmental fomites (Speight et al., 2017), and this is supported by this study as 100% of the koala admissions exhibited mange signs on their front paws, however sources and dynamics of environmental contamination requires further research in koalas. One case where a female with a joey both presented with mange signs suggests that vertical transmission between mothers and offspring also plays a role in transmission dynamics, but the relative importance cannot be fully understood from this study due to scarcity of applicable admission records. While conclusions from this study are tentative due to the small number of females represented, the patterns of sarcoptic mange signs observed suggest that transmission is complex and multi-factorial, and behaviours associated with mating including male-male aggression and mating itself may be important methods of transmission of infection in this population.

The highly visual nature of alopecia is commonly the observation used to identify sarcoptic mange affected animals for disease management programs and welfare assessments (Sannö et al., 2021, Montecino-Latorre et al., 2020, Simpson et al., 2016). The form of disease presentation observed in koalas with characteristic low levels of alopecia makes spotting sick individuals difficult. Indeed, the reclusive and arboreal nature of koalas makes spotting and assessing them for disease intrinsically harder. Consequently, by the time affected koalas are identified and admitted into care they may be at an advanced stage of disease, so treatment success may be more challenging. A recent study shows how koalas can have variable and often elevated stress levels during rehabilitation (Charalambous et al., 2021) and this along with more advanced stages of sarcoptic mange and the associated physical effects of this disease may hinder a koala's ability to recover following treatment. Specific population level factors such as genetic diversity and inbreeding may predispose koalas to have higher stress responses as well as disease susceptibility (Phillips, 2018). This, along with potential disease induced stress (Perez et al., 2019) may further complicate decisions as to whether it is ethical to bring koalas into care to undergo treatment, or alternatively euthanise the animal and prevent further welfare compromise.

It is possible that the COVID pandemic may have influenced koala admissions throughout the years of this study. However, it is unclear if this would have increased or decreased admissions and is deemed outside the scope of this study.

CONCLUSIONS

This study sought to investigate the epidemiology of sarcoptic mange disease in a population of koalas in Northern Victoria retrospectively using detailed carer admission records. We observed strong seasonal patterns in the prevalence of sarcoptic mange affected koalas admitted into care. Males showed two distinct peaks in mange prevalence, in the mating season (September/October) and then again later in the birthing season (February/March), whereas cases of females with sarcoptic mange only peaked in the birthing season (January/February). There were different patterns of sarcoptic mange signs between male and female koalas. The seasonal patterns and sex-specific distributions of sarcoptic mange suggest that males may be important drivers of sarcoptic mange dynamics within this population and that sarcoptic mange

may have become enzootic in this population of koalas. Our findings demonstrate how severe mange can be in koalas and may help to inform disease management plans. This study was limited in sample size due to access to suitable admission records provided by wildlife carers and requires further investigation in other koala populations to gain a more complete understanding of koala sarcoptic mange epidemiology and immunology. There is a need to advance our understanding of what severity of mange can be effectively treated and requires an active survey of koalas to detect sick animals at earlier stages of disease. The amount of time for different mange severities to develop within an individual and within the population is currently unknown in this species so future directions should aim to further our understanding of clinical progression of mange signs and the fine-scale landscape epidemiology of mange in koalas.

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AUTHOR CONTRIBUTIONS

Ellyssia T. Young: writing – review & editing, writing – original draft, visualization, resources, project administration, methodology, investigation, formal analysis, data curation, conceptualization. David Phalen: writing – review & editing, supervision, conceptualization. Aaron C. Greenville: writing – review & editing, supervision. Kylie Donkers: data curation. Scott Carver: writing – review & editing, supervision, conceptualization.

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CHAPTER 3

PHARMACOKINETICS AND SAFETY OF TOPICAL FLURALANER IN KOALAS (*PHASCOLARCTOS CINEREUS*)



Image credit: Ellyssia Young

CHAPTER 3.0: PHARMACOKINETICS AND SAFETY OF TOPICAL FLURALANER IN KOALAS (*PHASCOLARCTOS CINEREUS*)

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Abstract

Sarcoptic mange (etiological agent *Sarcoptes scabiei*) is among the most important parasitic diseases of some marsupial species and has been an emerging disease of koalas, causing welfare and conservation implications. Fluralaner (Bravecto® MSD Animal Health), an ectoparasiticide of the isoxazoline class, has been demonstrated as a long-lasting and efficacious chemotherapeutic agent against sarcoptic mange in multiple mammal species and may also be beneficial for impacted koalas. Here, we evaluated the pharmacokinetics and clinical safety of fluralaner in koalas. Healthy captive individuals were treated topically with 85mg/kg fluralaner administered to the interscapular epidermis. Following treatment, fluralaner was detected in plasma using ultra-performance liquid chromatography and tandem mass-spectrometry over a 12-week period. The mean maximum plasma concentration (C_{max}) was 66.4ng/mL; mean time was C_{max} of 2.71 days; plasma elimination half-life ($T_{1/2}$) was 30.91 days; and mean residence time (MRT) was 27.38 days. Haematological, blood biochemical, animal husbandry and clinical observations, over the same time period, demonstrated fluralaner was well tolerated. Overall, this research suggests fluralaner is a safe and long-lasting chemotherapeutic agent that may be efficacious against *S. scabiei* in koalas. Further research focussed on quantifying efficacy in captive and field settings, and across a range of disease severities would be valuable.

Keywords: Fluralaner, Sarcoptic mange, *Sarcoptes scabiei*, Koala, Safety, Pharmacokinetics, *Phascolarctos cinereus*

INTRODUCTION

Most therapeutics used on wildlife are developed in domestic animals. Accordingly, pharmacokinetic and safety (as measured by haematology, serum biochemistry and clinical observation) trials are important in determining the relative value of therapeutic agents being repurposed for use in wildlife disease management (Toutain et al., 2010). The value of pharmacokinetic and safety studies is particularly notable for marsupials, as there is comparatively little known about the pharmacokinetics or safety of many pharmaceutical agents used, and inferences are often drawn from studies on eutherian mammals (Martin et al., 2019, Kinzer, 1983, León-Vizcaíno et al., 2001). However, due to species-specific variation in physiological processes of absorption, distribution, metabolism and excretion, such extrapolation from domestic eutherian mammals to marsupials may have limitations (Death et al., 2011, Mayer et al., 2006). Therefore establishing species-specific safety and pharmacology of chemotherapeutic drugs is an integral part of developing safe and effective disease management.

Sarcoptic mange (etiological agent *Sarcoptes scabiei*) is one of the most important parasitic diseases affecting some Australian marsupials (Skerratt et al., 1999) and mammals more generally (Escobar et al., 2022). It is generally accepted that sarcoptic mange was introduced to Australia via humans and their domestic animals (dogs and possibly also agricultural and invasive animals) during European colonialism and has likely resulted in multiple spillover events, spreading over a wide geographic area (Fraser et al., 2017). In some native marsupials, sarcoptic mange is enzootic (e.g., bare-nosed wombat (*Vombatus ursinus*), and in others it appears as sporadic cases or occasional outbreaks (e.g., southern hairy-nosed wombat *Lasiorhinus latifrons*, wallabies *Wallabia bicolor* and *Notamacropus agilis*, and possums *Pseudocheirus peregrinus*) (Holz et al., 2011, McLelland and Youl, 2005, Botten et al., 2022, Wicks et al., 2007, Domrow, 1992). In koalas (*Phascolarctos cinereus*) and the western brown bandicoot (*Isodon fusciventer*) sarcoptic mange may be regionally becoming endemic (Botten et al., 2022, Speight et al., 2017). Clinical presentation varies among host species, but common signs include alopecia, hyperkeratosis, pruritis, and erythema (Bornstein et al., 2001, Pence and Ueckermann, 2002, Escobar et al., 2022). Species-specific behavioural ecology is a key contributor to disease occurrence and

persistence (Browne et al., 2022). Furthermore, *S. scabiei* is treatable using a range of pharmacological agents, including ivermectin (Skerratt, 2003), moxidectin (Death et al., 2011) and fluralaner (Wilkinson et al., 2021, Van Wick and Hashem, 2019).

Koalas are facing numerous threats and are currently classified by the IUCN as Vulnerable (Woinarski and Burbidge, 2020). Among the emerging threats is sarcoptic mange (Tompkins et al., 2015). Koalas develop the more severe disease form termed crusted mange (Næsberg-Nielsen et al., 2022, Speight et al., 2017), characterised by severe parakeratotic lesions on the feet and lower regions of the limbs which can extend onto the face and ventral side of the animal, with significant crusting and fissuring of the epidermis resulting in open wounds, increased risk of secondary infections, loss of body condition, and death (Speight et al., 2017). Sarcoptic mange has been documented in koalas sporadically across their range in Victoria, South Australia and New South Wales (Speight et al., 2017, Speight et al., 2018, Obendorf, 1983, Canfield, 1987), but there is limited knowledge about epizootiology. Since mange causes severe disease that results in significant and prolonged welfare impact in koalas, there is an urgent need to find solutions to this issue (Carver et al., 2022).

Fluralaner (Bravecto® MSD Animal Health) is a new class of antiparasitic compound originally developed as a flea and tick treatment for dogs and cats (Kilp et al., 2016), and has been shown to be an efficacious and long-lasting treatment against sarcoptic mange in domestic animals and wildlife, such as wombats (*Vombatus ursinus*) (Wilkinson et al., 2021), rabbits (*Oryctolagus cuniculus*) (Singh et al., 2022) and black bears (*Ursus americanus*) (Van Wick and Hashem, 2019). Key properties of fluralaner as a treatment for sarcoptic mange are a rapid onset of effect (Kilp et al., 2016), extended half-life and duration of action (e.g., 1-3 months in bare-nosed wombats) relative to other therapeutic options (Wilkinson et al., 2021). Owing to the rapid onset and extended duration of action, fluralaner also has potential to interrupt the 11-14 day lifecycle of *S. scabiei* on the host (Bornstein et al., 2001) and prevent reinfestation of individuals from environmental sources and other animals (Browne et al., 2022, Wilkinson et al., 2021, Rowe et al., 2019). Thus, use of fluralaner has potential to minimise the number of drug administrations for achieving parasite control and clinical resolution. Importantly, fluralaner has also been reported to have a wide safety margin in domestic and free-ranging animals (Gassel et al., 2014, Van Wick and Hashem,

2019, Prohaczik et al., 2017, Walther et al., 2014, Fisara et al., 2018). However, the pharmacokinetic profile of fluralaner has been demonstrated to vary among species (Kilp et al., 2016, Wilkinson et al., 2021), suggesting species-specific metabolic and body fat effects are possible (Martin et al., 2018, Wilkinson et al., 2021).

In this study, we sought to establish the pharmacokinetic profile and safety of fluralaner in koalas. Because wildlife are more likely to be treated due to presence of mange signs, rather than as preventative medicine as in domestic animals, so a higher dose is often necessary, but within safety limits (Wilkinson et al., 2021, Taenzler et al., 2016). Thus we focussed on the pharmacokinetics and safety of fluralaner in koalas at a single topical dose of 85mg/kg, comparable to what has been investigated in bare-nosed wombats (Wilkinson et al., 2021). Overall, we find fluralaner to be long-lasting and well tolerated in koalas.

METHODS

Trial and sample collection

Five clinically healthy adult koalas (2 males and 3 females, ages ranging from 4-7 years) were enlisted in this study. All were residents at the Phillip Island Nature Park and were housed in free-range enclosures. This study was approved by University of Sydney Animal Ethics Committee and Phillip Island Nature Park Animal Ethics (AEC Project number 7.2021). Average body weight of female koalas was 8.5kg and 10.4kg for males. Based on a recent fluralaner pharmacokinetic study in bare-nosed wombats (Wilkinson et al., 2021, Wilkinson et al., 2024) an 85mg/kg dosage was adopted, and spot-on application was chosen as the most feasible commercially available (Bravecto®) method of administering fluralaner to captive held koalas. All procedures were conducted by a wildlife veterinarian (L. Wicker) between March and June 2022. Daily monitoring by park staff included faecal output, appetite and demeanour monitoring, weekly health assessments by park staff and the supervising veterinarian, including pre-examination assessment of movement and demeanour, measurement of body weight, physical examination under manual restraint, and collection of blood for assessment of haematological and blood biochemical parameters.

On day 0 of this trial, each animal was anaesthetised for a thorough assessment of animal health (including body weight, body condition score, and full physical examination) collection of blood via cephalic venipuncture, and topical application of 85mg/kg fluralaner (Bravecto® Spot-on) to the interscapular epidermis. Anaesthesia of the first koala was induced via intramuscular injection of alfaxalone 2 mg kg⁻¹ and medetomidine 40 µg kg⁻¹, reversed with atipamezole at 0.16mg kg⁻¹ at the end of the procedure, as described by Downey et al. (2020). For the remaining four koalas, anaesthesia was induced via mask delivery of 5% isoflurane in oxygen and maintained via mask delivery of 1-2% isoflurane in oxygen, a regimen which resulted in more stable anaesthesia and more rapid recovery following the cessation of the procedure. Additional blood samples were collected from each koala while they were manually restrained on day 0 at times 0, 1, and 4 hours after treatment and on days 1, 2, 4, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77 and 85.

Each blood sample was aliquoted into 1ml subsamples in separate ethylenediaminetetraacetic (EDTA) blood collection tubes for pharmacokinetic and haematological analyses, a lithium heparin tube for biochemical analysis, and a fresh blood smear made within 1 hour of blood collection to support haematological evaluation. Aliquots intended for biochemical analysis were stored at 4°C until analysis within 24 hours of blood collection. Aliquots intended for pharmacokinetic analysis were centrifuged to extract the plasma from the whole blood sample, and plasma was stored at -20°C until submission to the laboratory.

Ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) using a Waters Acquity H-class UPLC system (Waters Corporation, Milford, MA) was undertaken to determine the fluralaner concentration in plasma (Appendix A - S3.1 and Table S3.1). Plasma samples underwent liquid-liquid extraction and protein precipitation prior to the analysis of solvent extracts as described by Wilkinson et al. (2021) with the following modification. For quantitative determination, stable isotope dilution was employed utilising the addition of ¹³C₄,²H₃-Fluralaner (Alsachim, France) to each plasma sample prior to extraction (41.6 µg per sample). Chromatography was performed using an Acquity BEH C18 VanGuard pre-column (5.0 x 2.1 mm, 1.7 µm) and an Acquity BEH C18 column (2.1 x 100 mm x 1.7 µm) (Waters Corporation). The UPLC was operated with a mobile phase consisting of 0.1% (v/v) Formic acid (Solvent

A) and Acetonitrile (Solvent B). Elution was using a gradient. Initial conditions were 20% B before a gradient to 95% B over 4 min, which was held for 2 min. The system was returned to initial conditions at 6.5 min and re-equilibrated for 3 min. The flow rate was 0.35 ml/min and the column was held at 45°C. Injection volume was 2 µl. The UPLC was coupled to a Waters Xevo TQ triple quadrupole mass spectrometer (Waters Corporation). Analyses were undertaken using multiple reaction monitoring (MRM) in negative electrospray ionisation mode, with 2 MRM Transitions monitored for Fluralaner as described by Wilkinson et al. (2021). Electrospray ionisation was performed with a capillary voltage of 2.5 kV, and individual cone voltages and collision energies for each MRM transition, as described below. The desolvation temperature was 450°C, nebulising gas was nitrogen at 950 l/h and cone gas was nitrogen at 100 l/h. MRM transition dwell times were 120 msec. Quantitation was undertaken using matrix matched (koala plasma) external calibration standards as described by Wilkinson et al. (2021).

Plasma biochemistry and electrolyte analysis was conducted in house using a VetScan VS2 Chemistry analyser, Comprehensive Diagnostic Profile (Zoetis Inc. Australia) and full haematological analysis (automated cell count and blood smear evaluation) were conducted at Gribbles Veterinary Pathology, Australia. Biochemical and haematological results were clinically interpreted against expected values for the species and analysed against published species reference ranges to provide a clinical assessment of animal health, identify changes in values for each parameter over time, and interrogated to assess for signs of drug toxicity.

Data Analyses

All statistical analyses were undertaken using R (R version 4.2.2).

Non-compartmental methods (R packages 'pk' and 'linpk' (Jaki and Wolfsegger, 2011, Rich, 2022)) were also used to calculate fluralaner pharmacokinetic values, maximum recorded plasma concentrations (C_{max}), time to C_{max} (T_{max}), area under curve (AUC), plasma elimination half-life ($t_{1/2}$) and mean residence time (MRT).

The dynamics of individual haematology variables, biochemistry values, and body weight in relation to time and fluralaner concentration were assessed using generalised additive mixed models (GAMMs) using the R package 'mgcv' (Wood, 2011), with koala ID as the random effect. GAMMs were used because it enabled non-linear changes in the response variables over time to be modelled as a spline function. Finally, the relative 'health relevance' of haematology and biochemistry values were also conservatively assessed in relation to available reference range information, acknowledging current published reference ranges are based on small numbers of geographically restricted koalas (Speight et al., 2014, Canfield et al., 1989, Fabijan et al., 2020, Pye et al., 2012), and assessed against values for each parameter listed in the Zoo Information Management System (ZIMS) (Species360 Zoological Information Management System (ZIMS), 2022).

RESULTS

The five koalas were assessed as being clinically healthy based on thorough physical examination and the results of blood biochemistry and haematology obtained on Day 0. Topical fluralaner was administered to the interscapular region at a dosage of 85mg/kg and monitored continuously for 12 weeks. There were no deleterious changes in gross behaviour, appetite, faecal production or body mass observed. All koalas remained alert, normally responsive, maintained appetite and with normal faecal output for the duration of the study. Body mass exhibited a slight increase throughout the study period in relation to time, but not fluralaner concentration (time – $F=3.212$, $P=0.08$; fluralaner plasma concentration – $t=0.483$, $P=0.631$) (see Appendix A – S3.2 Figure S3.1).

Pharmacokinetics

After topical administration, fluralaner was detected in the plasma, initially exhibiting a rapid rise in concentration and then slow decay over the 12-week period (Figure 3.1, $F=16.36$, $P<0.001$). Pharmacokinetic calculations showed fluralaner had an average: C_{max} 66.4ng/mL, T_{max} 2.71 days, AUC 1270.87 day*ng/ml, $T_{1/2}$ 30.91 days, and MRT 27.38 days.

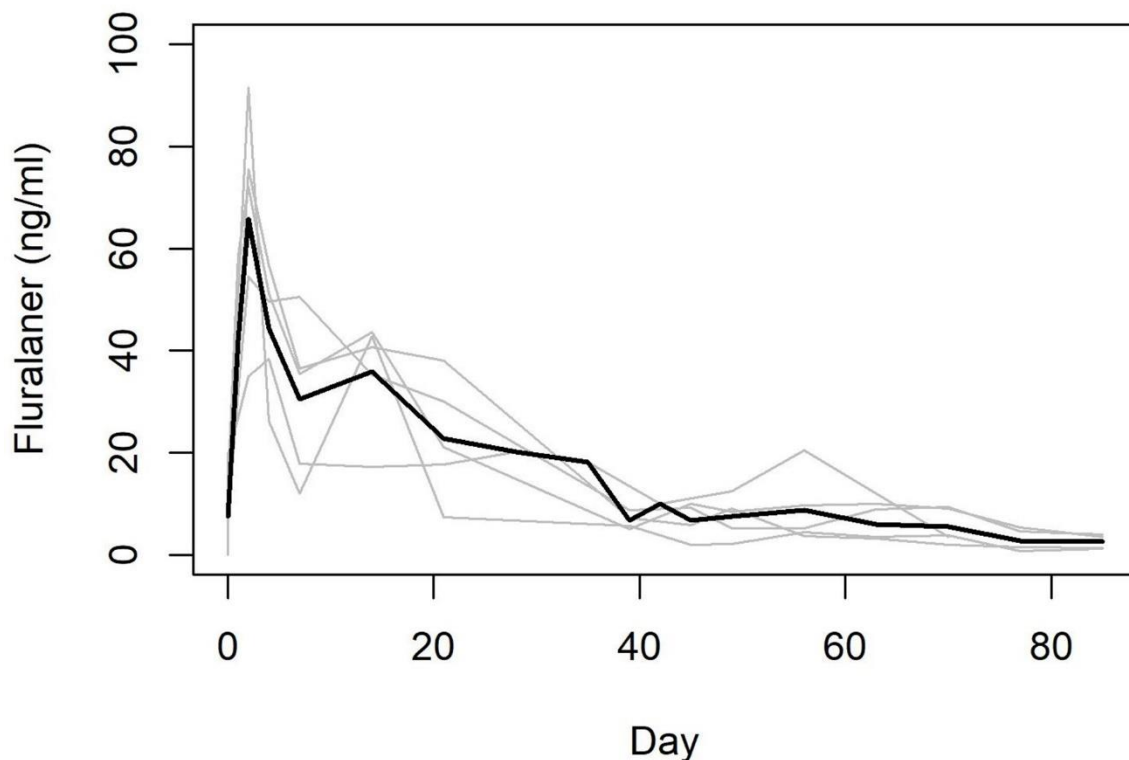


Figure 3.1. Plasma concentrations of fluralaner in five koalas given a single topical dose of 85mg/kg. Black line is the mean for each time point, and grey lines represent the profile of individual koalas.

Safety

Overall, no deleterious drug induced effects of fluralaner on haematology and biochemistry values were evident over the 12-week trial (Tables 3.1 and 3.2). Increases over time, predominantly within reference range information (Tables 3.3 and 3.4, Appendix A – S3.2 Figures S3.2-3.5), were documented for total protein, globulin, haematocrit, and mean corpuscular volume, with only a slight increase observed in albumin. Significant decreases over time, again predominantly within reference range information were observed for glucose, alkaline phosphatase, platelets and mean corpuscular haemoglobin concentration.

While some changes in these variables over time were observed, we also looked at whether these variables changed in relation to fluralaner concentration (Tables 3.3 and 3.4, Appendix A – S3.2 Figures S3.6-3.7). Changes in haematology and blood biochemistry was observed in relation to fluralaner plasma concentration (Tables 3.3

and 3.4, Appendix A – S3.2 Figures S3.6-3.7). A decrease in reticulocytes was observed whereas albumin, lymphocyte count, neutrophil count, and white blood cells increased, and reticulocytes ratio showed high variability with an overall increase with increased plasma fluralaner concentration.

Table 3.1. Plasma biochemistry reference intervals for healthy koalas (*Phascolarctos cinereus*) used in this study relative to the mean, minimum and maximum values obtained from koalas over the duration of this trial (n=5).

Biochemical parameter	Reference interval*	Trial (n=5)	
		Average	Min-Max
Sodium (mmol/l)	132-147	143.8	134-157
Calcium (mmol/l)	1.47-5.47	3	1.0-3.9
Phosphorus (mmol/l)	0.12-2.02	1.3	0.71-1.81
Glucose (mmol/l)	0.1-8.35	4.9	3.0-6.6
Blood urea nitrogen (mmol/l)	0.8-32.6	3.4	0.9-6.0
Creatinine (µmol/l)	4.0-3249	74.6	38-113
Alanine aminotransferase (u/l)	0-983	12.5	5.0-24
Alkaline phosphatase (u/l)	36-219	102.9	38-161
Total Bilirubin (µmol/l)	0-13.5	4.2	3.0-9.0
Total protein (g/l)	15.9-85.4	71.6	58-84
Albumin (g/l)	16.3-56	42.8	36-49
Globulin (g/l)	15-45	28.3	20.0-41.0

*(Pye et al., 2012, Canfield et al., 1989, Speight et al., 2014, Species360 Zoological Information Management System (ZIMS), 2022)

Table 3.2. Haematological reference intervals from healthy koalas (*Phascolarctos cinereus*), relative to the mean, minimum and maximum values obtained from koalas over the duration of this trial (n=5) located at Phillip Island Nature Park, Victoria, Australia.

Haematological parameter	Reference interval*	Trial (n=5)	
		Average	Min-Max
RBC (10 ¹² cells/l)	2.29-4.43	3.78	3.16-4.09
HGB (g/l)	77-140	125.53	106-133
HCT (ratio)	0.31-0.45	0.42	0.34-0.51
MCV (fl)	84.7-139.5	108.11	99-115
MCH (pg)	28.9-41.6	33.34	30.8-35.7
MCHC (g/l)	289-373	308.83	299-344
Reticulocytes (10 ⁹ cells/l)	Insufficient data	53	19-131
Reticulocytes (ratio)	Insufficient data	1.39	0.4-3.2
Nucleated red blood cells (/100WBCs)	0.0-36	7.91	1.0-12
Platelets (10 ⁹ cells/l)	0.05-0.38	21.92	0.12-219
WBC (10 ⁹ cells/l)	2.4-9.84	7.51	4.5-12.2
Lymphocyte count (10 ⁹ cells/l)	0.17-7.77	4.13	2.8-6.7
Monocyte count (10 ⁹ cells/l)	0.00-1.08	0.34	0.1-0.8
Neutrophil count (10 ⁹ cells/l)	0.7-6.62	2.01	0.8-5.5
Band count (10 ⁹ cells/l)	0-0.281	0	0-0
Eosinophil count (10 ⁹ cells/l)	0.0-1.1	0.9	0-3.4
Basophil count (10 ⁹ cells/l)	0.00-0.11	0.02	0-0.1
Lymphocyte (%)	10.0-92.2	55.68	33-70
Monocyte (%)	0.0-10.0	4.68	1.0-8.0
Neutrophil (%)	4.4-86.1	27.58	11.0-60
Band (%)	0.0-5.0	0	0-0
Eosinophil (%)	0-4.00	10.89	0-33
Basophil (%)	0.0-3.00	0.16	0.0-1.0

*(Fabijan et al., 2020, Canfield et al., 1989, Species360 Zoological Information Management System (ZIMS), 2022)

The remaining parameters did not change significantly over time or fluralaner concentration and were mostly within reference intervals (Tables 3.1 and 3.2). Some koalas showed occasional variation in biochemical parameters outside of reference ranges (see Appendix A – S3.2 Figures S3.2-S3.7) but were generally concluded to be not clinically indicative of drug toxicity or were within the expected error range of measurements. Some parameters were omitted from analysis due to a high number of suspected measurement errors (potassium) and band count was omitted from modelling as none were detected in any of the koalas.

Table 3.3. Generalised additive mixed model outputs for biochemical parameters for all koalas (*Phascolarctos cinereus*) (n=5) involved in the study and located at Phillip Island Nature Park, Victoria, Australia.

Biochemical parameter	Time		Fluralaner concentration	
	F	P	t	P
Sodium (mmol/l)	1.575	0.284	0.171	0.865
Creatinine (µmol/l)	3.648	0.063	0.491	0.626
Calcium (mmol/l)	2.013	0.164	1.528	0.134
Blood urea nitrogen (mmol/l)	0.183	0.671	0.877	0.386
Glucose (mmol/l)	7.834	0.008	0.685	0.497
Total protein (g/l)	17.69	<0.001	2.139	0.385
Alanine aminotransferase (u/l)	0.392	0.534	0.864	0.393
Alkaline phosphatase (u/l)	5.918	0.003	1.923	0.062
Total Bilirubin (µmol/l)	0.003	0.96	0.171	0.865
Albumin (g/l)	10.32	0.003	2.142	0.038
Globulin (g/l)	10.55	0.002	1.805	0.078

Table 3.4. Generalised additive mixed model outputs for haematological parameters for all koalas (*Phascolarctos cinereus*) (n=5) involved in the study and located at Phillip Island Nature Park, Victoria, Australia.

Haematological parameter	Time		Fluralaner concentration	
	F	P	t	P
Haemoglobin (g/l)	4.29	0.06	2.08	0.06
Haematocrit (ratio)	8.51	0.01	0.01	0.99
Red blood cells (10 ¹² cells/l)	2.41	0.14	1.72	0.11
Mean corpuscular volume (fl)	6.68	0.01	1.46	0.17
Mean corpuscular haemoglobin (pg)	0.20	0.66	0.34	0.74
Mean corpuscular haemoglobin concentration (g/l)	4.59	0.05	0.78	0.45
Reticulocytes (10 ⁹ cells/l)	6.49	0.03	2.84	0.02
Reticulocytes (ratio)	14.23	0.01	3.68	0.01
Nucleated red blood (/WBCs)	4.78	0.07	1.04	0.34
Platelets (10 ⁹ cells/l)	5.44	0.01	0.56	0.59
White blood cells (10 ⁹ cells/l)	2.23	0.16	4.47	<0.01
Lymphocyte count (10 ⁹ cells/l)	0.55	0.39	1.98	0.07
Monocyte count (10 ⁹ cells/l)	0.91	0.36	0.70	0.50
Neutrophil count (10 ⁹ cells/l)	1.55	0.15	2.12	0.05
Eosinophil count (10 ⁹ cells/l)	1.84	0.20	1.30	0.21
Lymphocytes (%)	0.14	0.71	2.20	0.04
Monocytes (%)	0.64	0.44	0.50	0.62
Neutrophils (%)	0.96	0.34	0.89	0.39
Eosinophils (%)	2.92	0.11	0.78	0.45

DISCUSSION

Wildlife medicines are largely derived from those developed for domestic animals, with pharmacokinetic properties and safety assumed by extrapolation (Martin et al., 2019, Kinzer, 1983, León-Vizcaíno et al., 2001, Govendir, 2018). However, differences in drug dynamics and safety occur among species, as well as conditions of use (e.g., prophylactic vs. therapeutic application) (Takano et al., 2023, Toutain et al., 2010). Here we undertook an analysis of the pharmacokinetics and safety of the ectoparasiticide fluralaner in five healthy captive adult koalas. Our key findings are: *i*). fluralaner was detectable in plasma at concentrations that have been shown to be therapeutic against *S. scabiei* mites in related species the wombat, *ii*). fluralaner persisted in plasma at quantifiable levels for at least 12 weeks post treatment, and *iii*) a single topical 85 mg/kg dose of fluralaner was safely tolerated in the koalas treated (as assessed by complete cell counts, repeated blood biochemistries, and clinical observation). These results suggest fluralaner may be a viable long-lasting treatment for sarcoptic mange in koalas.

The pharmacokinetic profile of fluralaner in koalas demonstrated a relatively long duration of action for an ectoparasiticide. In comparison, other therapeutic agents that have been used to treat koalas for sarcoptic mange typically exhibit shorter durations of protection and often require repeated treatments, including malathion (Barker, 1974), amitraz (Brown et al., 1982), ivermectin (Speight et al., 2017), and moxidectin (Chapter 2; Young et al., 2024). Our findings of a relatively long $T_{1/2}$ and MRT for fluralaner are broadly consistent for trials of isooxazolines in other mammal species (Wilkinson et al., 2021, Kilp et al., 2016), and may confer distinct therapeutic advantages for treated koalas, relative to the aforementioned therapeutic agents. We recognised three key areas where fluralaner may provide significant value for koalas: 1) fluralaner is known to induce rapid killing of mites on the host (Wilkinson et al., 2021, Beugnet et al., 2015); 2) diminished requirements to re-treat mange affected koalas in care, resulting in less need for handling and restraint associated with treatment, and thus less stress to the individual; and 3) fluralaner has a high potential for breaking the transmission cycle of the mite both on the host and in the environment owing to this long duration of protection.

The pharmacokinetics of fluralaner in koalas was similar to other mammal species, in that it is longer lasting than most macrocyclic lactones (Wilkinson et al., 2021). Our data along with that of others demonstrates that pharmacokinetic parameters should be expected to vary significantly between species (Kilp et al., 2016, Wilkinson et al., 2021). In the current study, the mean C_{max} was nearly 6 times higher in koalas (66.4ng/ml) as compared to wombats given the same dose rate (16.4 ng/ml), but was still substantially lower than the C_{max} determined in dogs (1698 ng/ml) and cats (2399 ng/ml) (Kilp et al., 2016, Wilkinson et al., 2021). Additionally, the $T_{1/2}$ was shorter in koalas (31 days) than in wombats (166.5 days, but note $n=2$), but longer than observed in cats (12 days at 80mg/kg dose) and dogs (17 days at 50mg/kg dose) (Kilp et al., 2016, Wilkinson et al., 2021). Fluralaner demonstrated long systemic persistence in koalas, and the previous studies mentioned above observed parasite extermination and efficacy for 3 months or longer, with shorter fluralaner half-life. So, it is likely that this extended duration of action would also be observed in koalas.

Overall, the pharmacokinetic profiles of topical fluralaner in koalas was similar to that reported in wombats (Wilkinson et al., 2021), a closely related species. This is somewhat expected as wombat and koala diets, habitats and adaptations to survive in Australia has shaped their physiological processes (Govendir, 2018). The underlying explanations for the smaller differences in pharmacokinetics are not yet clear, but they may relate to species specific differences in absorption, distribution, metabolism and excretion processes (Toutain et al., 2010). Our pharmacokinetic calculations suggest that measurable amounts of fluralaner persists in plasma for at least 85 days. The duration of protection against *S. scabiei* remains to be understood, and further research clarifying drug efficacy is warranted. Further studies into the absorption and distribution of fluralaner into other tissues, particularly adipose tissue since fluralaner is lipophilic, is required to gain a more complete understanding of fluralaner pharmacokinetics in koalas.

Koalas have a specialist folivorous diet primarily composed of eucalyptus foliage (Moore and Foley, 2005). Eucalyptus leaves have comparatively low nutrient content (Cork and Sanson, 1991) and contain plant secondary metabolites (PSMs), including some which are cytotoxic (Moore and Foley, 2005). Koalas behaviour and physiology has co-evolved to cope with this specialist diet and use considerable amounts of their

energy managing the absorption and excretion of these compounds (Krockenberger et al., 1998, Freeland and Janzen, 1974). Koalas have processes that limit the absorption and accelerate elimination of PSMs, xenobiotic compounds and toxins (Govendir, 2018), such as rapid hepatic metabolism (Govendir, 2018), and energy conservation strategies including low metabolism (Degabriele and Dawson, 1979, Nagy and Martin, 1985). These processes can reduce the efficacy of some therapeutic medicines (Govendir, 2018), and since fluralaner undergoes some metabolism in the liver (Kilp et al., 2016) these adaptations may influence how koalas absorb, distribute, metabolise and excrete potential toxins as well as PSMs, and can help to understand the pharmacokinetic profile of fluralaner observed in this study.

Previous studies on a taxonomically wide variety of species have shown that fluralaner has a wide safety margin in vertebrate species both wild and domestic (Van Wick and Hashem, 2019, Prohaczik et al., 2017, Walther et al., 2014, Fisara et al., 2018), with few side effects documented in mammals (reviewed in Jiang and Old, 2025). In this study, koalas also tolerated fluralaner application at higher dosage than that recommended for domestic animals (Taenzler et al., 2016). Some changes in biochemical and haematological parameters were observed in the treated koalas, but never were the changes considered to be outside of the normal ranges for this species, nor were behavioural changes observed in these koalas, so we found no indication that fluralaner administered at the dosage used in this study had a negative impact on the treated animals. It is also important to note that haematology and blood biochemistry reference range information is limited in koalas (Fabijan et al., 2020, Canfield et al., 1989, Speight et al., 2014, Pye et al., 2012), and many wildlife species. Thus, the time zero data from our study provides valuable additional reference range information for koalas, and we recommend further reference range data collection would be useful for this threatened wildlife species.

Fluralaner is lipophilic in nature and therefore will distribute into fatty tissue (Committee for Medicinal Products for Veterinary Use, 2016). Koalas have low amounts of body fat (0.87-3.72%), relative to domestic species, such as dogs and cats (15-25% and up to 65%, respectively) (Laflamme, 1997, Toll et al., 2010, Witzel et al., 2014). The distribution of fluralaner into the adipose tissue can extend duration of action, as has been seen in dogs (Queiroga et al., 2021). While there were no clinical, physical or

behavioural signs of toxicity demonstrated by the koalas in this study, monitoring is still needed to exhibit care regarding overdosing risks. Further, the sequestration of fluralaner into adipose tissue may explain differences in circulating fluralaner and persistence in the body.

Although our study cohort was limited to adult koalas that were not reproducing, fluralaner had no impact on reproductive behaviour or success in dogs (Committee for Medicinal Products for Veterinary Use, 2016), or juvenile (Walther et al., 2014), or maternally dependent bare-nosed wombats (Wilkinson et al., 2021). Research has identified that fluralaner can be transmitted into the environment and non-target animals, and there may be possible environmental impacts as a result (Diepens et al., 2023) which requires further eco-toxicological study.

CONCLUSION

Here we demonstrate that healthy koalas safely tolerated transdermal application of fluralaner at a dose of 85mg/kg and suggest that this drug may be efficacious in the treatment of sarcoptic mange in affected koalas for multiple weeks.

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AUTHOR CONTRIBUTIONS

Ellyssia T. Young: writing – original draft, reviewing and editing, formal analysis, data curation. Jessica McKelson: writing – review & editing, resources, project administration, methodology. Daniel Kalstrom: investigation. Lachlan Siphthorp: investigation. Leanne Wicker: writing – review & editing, validation, methodology, investigation, data curation. Damien Higgins: funding acquisition, conceptualization. Caroline Marschner: project administration, funding acquisition. David S. Nichols: methodology, investigation. David Phalen: writing – review & editing, supervision,

resources, funding acquisition, conceptualization. Aaron C. Greenville: writing – review & editing, supervision. Scott Carver: writing – review & editing, visualization, supervision, funding acquisition, formal analysis, conceptualization.

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ETHICS APPROVAL

Ethics approval was obtained from the University of Sydney animal ethics committee and Phillip Island Nature Park animal ethics committee (AEC Project number 7.2021).

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CHAPTER 4.

MEGAFIRES AND WILDLIFE DISEASE: DID THE AUSTRALIAN BLACK SUMMER BUSHFIRES IMPACT SARCOPTIC MANGE IN BARE-NOSED WOMBATS?

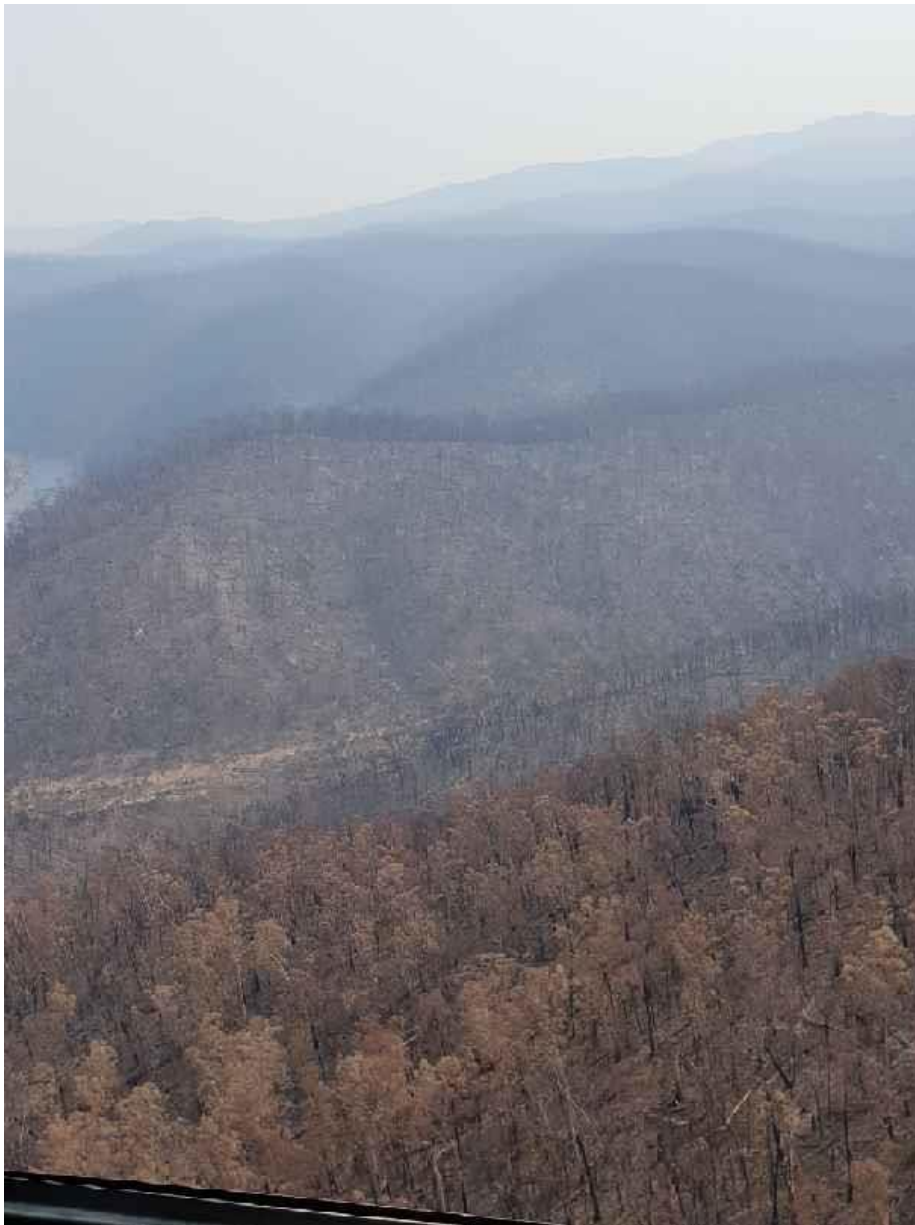


Image credit: Ellyssia Young

CHAPTER 4.0: MEGAFIRES AND WILDLIFE DISEASE: DID THE AUSTRALIAN BLACK SUMMER BUSHFIRES IMPACT SARCOPTIC MANGE IN BARE-NOSED WOMBATS?

Young, E.T., Carver, S., de Montfort, M., Cuneo, J., Harris, T., Mills, D., Zhou, A., Birnbaum, A., Saunders, G., Noll, S., Phalen, D., Greenville, A.C. Megafires and wildlife disease: did the Australian Black Summer bushfires impact sarcoptic mange in bare-nosed wombats? (In prep.). *Landscape Ecology*.

Abstract

Large, high intensity wildfires can have dramatic impacts on wildlife, but how fires shape wildlife disease is virtually unknown. The Australian Black Summer bushfires (a megafire) were unprecedented in scale and impacts, large areas of the bare-nosed wombat (*Vombatus ursinus*) range were impacted, and sarcoptic mange (caused by *Sarcoptes scabiei*) is a disease commonly occurring in wombats within these areas. We sought to investigate the impact of the megafire on the dynamics of wombat mange. Using two independent lines of evidence in a Before After Control Impact (BACI) design, we first tested whether the occurrence of the megafire influenced the probability of wombats having mange, finding no evidence of effect. We then investigated the influence of burn severity on sarcoptic mange, showing that signs of disease increased, associated with greater local landcover impacted by more severe burn categories. This effect was transient, and did not persist beyond the year of the wildfire. Our results suggest that transmission of *S. scabiei* temporarily increases as a function of the extent of landcover impacted by severe fire conditions, possibly owing to wombats aggregating around spatially restricted food resources following the fire, and effects this has on local environmental transmission. Taken together, our research suggests wildfire can have short-term effects on wildlife disease, mediated by the extent and severity of the local landscape burned.

Key words: Bare-nosed wombat, megafire, *Sarcoptes scabiei*, sarcoptic mange, *Vombatus ursinus*, wildfire

INTRODUCTION

The frequency and scale of natural environmental disturbances (e.g., floods, wildfires) are increasing under anthropogenic induced climactic changes (IPCC, 2023), and have the potential to impact infectious diseases (Albery et al., 2021). The recent megafires in Australia, Siberia, the Amazon, and California have contributed to development of the concept of the 'Pyrocene' to reflect this new phase of earths history (Pyne, 2020). Megafires - high intensity fires exceeding 10,000 hectares - have both direct and indirect impacts on free-ranging animal populations. Direct mortality and indirect effects through resource changes (e.g., habitat coverage, retreat sites) are relatively conspicuous. However, potentially less obvious indirect impacts, such as infectious diseases, remain poorly understood (Albery et al., 2021).

The Australian Black Summer bushfires in late 2019 – early 2020 collectively burned through 10.3 million hectares and included the largest ever documented area to be burned at high severity (Collins et al., 2021, Linley et al., 2022). Many ecosystems were affected including woodlands, heathlands, grasslands, farmlands, and rainforests that typically do not burn (Nolan et al., 2020). Extensive impacts on wildlife have been documented (Ward et al., 2020, Driscoll et al., 2024). At least 832 species of native vertebrate occupy the impacted landscape many of which were already in decline due to drought, disease, habitat modification and introduced species (Kearney et al., 2019, Ward, 2019). While Driscoll et al. (2024) documented an overall negative effect of the megafires on species abundance and occurrence, of the 1648 effects studied, 44% were positive effects on species. Indicating that the megafires were not a disaster for all species, and that there are complex and context-specific impacts from wildfires including fire severity, local fire regime, and land use (Miritis et al., 2024, Driscoll et al., 2024). This megafire event also had an underappreciated indirect health impact on wildlife through stress, pollution, habitat modification and altered movement patterns (Albery et al., 2021). However the extent to which this event has affected disease causing pathogens in wildlife is currently unknown.

A number of important pathogens impact wildlife across south-eastern Australia, with a notable example being *Sarcoptes scabiei* in bare-nosed wombats (*Vombatus ursinus*). Bare-nosed wombats are large herbivorous fossorial marsupials that occur widely across south eastern Australia (Carver et al., 2024), coinciding with the

distribution of the Australian megafires. Infestation by the *S. scabiei* mite, causing sarcoptic mange, occurs across the range of bare-nosed wombats (hereafter referred to as wombats), and more generally *S. scabiei* is among the most burdensome of mammalian parasites worldwide (Escobar et al., 2022). In wombats *S. scabiei* infection causes pruritis, hyperkeratosis and fissuring of the skin due to the mites penetrating the hosts epidermal layer, which can lead to secondary infections, emaciation and death (Skerratt, 2003). Sarcoptic mange also changes the nutritional requirements (time spent drinking, slower feeding rate, thermoregulation) and behavioural patterns (time spent active outside of burrow, time spent walking) of infected wombats (Simpson et al., 2016). Sarcoptic mange transmission among wombats is understood to be environmental via burrows, which they share asynchronously, as individuals are solitary and rarely come into direct contact outside of breeding (Skerratt et al., 1998, Burgess et al., 2023, Ringwaldt et al., 2023).

The impacts of the 2019-20 Australian megafires on sarcoptic mange in wombats have not previously been investigated. It is possible that the megafires could either enhance or decrease the occurrence of sarcoptic mange (Figure 4.1). Megafires could lead to an increase in the probability of wombats having mange. This may result from fires causing individuals to aggregate more at spatially confined resources, increasing use of and exposure to mites in local burrows (Linley et al., 2024). It is also possible that the megafires may have little impact on sarcoptic mange in bare-nosed wombats, whereby wombats may be resilient to the impacts of fire. This could result from wombats sheltering in burrows and being able to sustain through temporary resource restrictions owing to their low metabolic rates (Evans et al., 2003) and stable burrow environments (Morris et al., 2024, Browne et al., 2021), supporting stable transmission of *S. scabiei* mites. Conversely, the megafires could have a beneficial disease outcome for wombats, either through heat and smoke induced environmental sterilization (i.e. rapid dessication of mites in burrow environments) or reduced wombat encounters with burrows containing mites owing to fire-induced wombat mortality. For this study, we only investigated environmental factors.

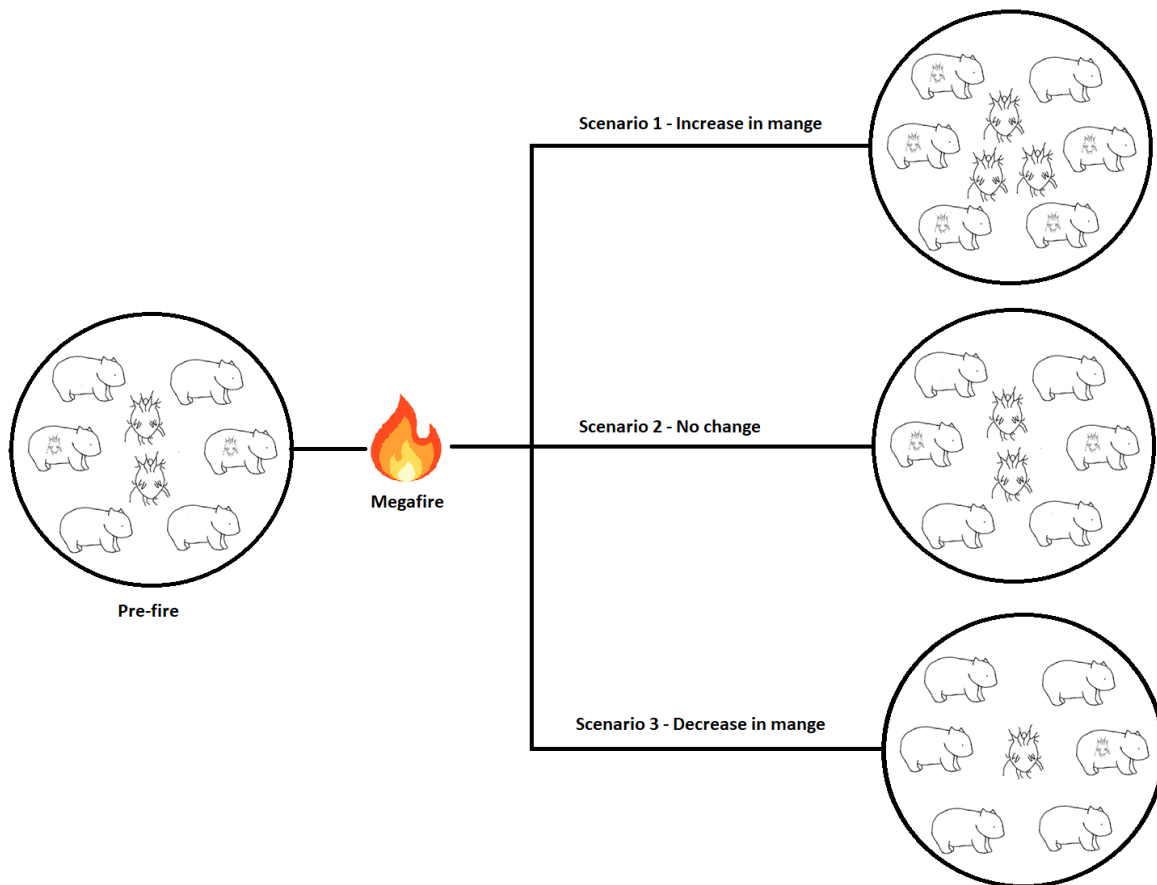


Figure 4.1. Three scenarios for how the effect of the Australian Black Summer megafire event could impact sarcoptic mange dynamics in bare-nosed wombats. Scenario 1: There is an increase in the probability of wombats having mange. Potentially due to aggregation around spatially confined resources, increased exposure to mites in burrows (burrow use as refuge from fire), and/or increased direct contact between wombats. Scenario 2: Fires have little impact on the probability of wombats having mange due to wombats having resilience against the impacts of the fire by retreating to burrows and can sustain through temporary resource limitations which supports ongoing transmission of mites. Scenario 3: There is a decrease in the probability of wombats having mange due to heat and smoke induced environmental sterilisation (i.e. rapid desiccation of mites in burrow environments, and/or reduced wombat encounters and transmission from burrows containing mites owing to fire induced wombat mortality).

Here we investigate evidence relating to contrasting scenarios for epidemiological consequence of the Australian Black Summer megafires on sarcoptic mange in bare-nosed wombats (Figure 4.1). Our specific aims are to: 1) quantify the impact of the megafire event on the occurrence of wombat mange; and 2) assess the effect of fire severity extent on mange in burned areas. We use two independent datasets spanning 2019-2021 that allow us to adopt a before-after-control-impact (BACI) study design: the New South Wales (NSW) camera trapping data set WildCount (State Government of NSW and NSW Department of Climate Change Energy the Environment and Water, 2025); and wildlife carer records from the Wildlife Information, Rescue and Education Service (WIRES, 2023). WildCount data represents passive systematic data collection, whereas WIRES data represents targeted wombat disease management efforts. Analysis of both datasets, thus, provide lines of evidence on mange disease in wombats from two nuanced perspectives, and collectively, a robust evidence base from which to assess how wildfire impacts this wildlife disease.

METHODS AND STATISTICAL ANALYSIS

The Australian Black Summer bushfires refers to the series of megafires which started in September 2019 until February 2020 and burnt through 10.3 million hectares of eastern and south-eastern Australia (Boer et al., 2020, Collins et al., 2021). Much of south-eastern Australia is temperate broadleaf and mixed forest dominated by the genus *Eucalyptus* and are considered fire-prone landscapes, however typically only small percentages of this biome are burned annually (Olson et al., 2001, Boer et al., 2020). Bare-nosed wombats occupy much of this landscape; Ward et al. (2020) estimated that 13% of the total bare-nose wombat distribution was impacted by this fire event.

Aim 1: Quantify the impact of the megafire event on the occurrence of wombat mange

We sourced data sets which are intended to portray an overall picture of sarcoptic mange in wombats before and after the fires across the broader landscape and land tenures in NSW (Figure 4.2). To do this we used two unique data sources which

together can provide information on the broadscale patterns surrounding sarcoptic mange dynamics in wombats.

WIRES wildlife carer records

WIRES records were accessed from across NSW between 2019 and 2021 (Figure 4.2). Wildlife carer records of bare-nosed wombats from affiliated members of the public were reported to WIRES and included date of encounter, encounter type, locality, animal condition. The case criteria to capture wombat records of sarcoptic mange included filtering records by encounter type, with external parasite, disease-mange, and disease being filtered and then filtered again via animal condition category to only include records listed under parasite infestation, skin problem and fur problem. Locality was confirmed as the geographic area in which the animal was located, as specific geographic location such as grid references, were not recorded in this database, but this still provided sufficient resolution to align with the geographic occurrence of the Black Summer bushfires. Localities were broadly classified as either burned or unburned using a Fire Extent and Severity mapping layer (State Government of NSW and NSW Department of Climate Change Energy the Environment and Water, 2020).

WildCount monitoring

The WildCount long-term monitoring program was a monitoring program consisting of 204 sites across eastern New South Wales National Parks and reserves (Figure 4.2) – with sites originally selected using stratified random sampling (New South Wales National Parks and Wildlife Service (NSW NPWS), 2025). Detailed program information, site selection, and survey design can be found in (Barry, 2011, Porter et al., 2013, MacKenzie, 2011, New South Wales National Parks and Wildlife Service (NSW NPWS), 2020). In brief, four Reconyx PC800 cameras were installed at each site and located in a 500 x 500 metre grid (Figure 4.2). Cameras were secured 1 metre above the ground, and a lure was placed 2 metres from the camera. Each site was surveyed for a minimum of 14 consecutive days per year, and cameras were programmed to capture 3 images per trigger to enhance chances of species identification. Species or species groups were identified from images and recorded in the exif data for each image by the NPWS WildCount Team along with other important

information including confidence level of species identification survey time and bait station status (New South Wales National Parks and Wildlife Service (NSW NPWS), 2020).

All images from 2019 to 2021 identified to have captured a wombat were included in this study. Camera trap images were tagged and sorted using DigiKam software (KDE, 2021). Images were tagged as positive or negative for mange based on visual diagnosis utilising established methods (Simpson et al., 2016, Ringwaldt et al., 2023) and written into the exif data of each image file (jpeg). Image series were only scored and included in the analysis if the whole length of the body was clearly visible, excluding series where the wombat was too far away or blurry to accurately score mange. If a female wombat with a joey at foot was observed and either of them had mange, then the pair were recorded as one individual with mange. Lesions observed but considered inconsistent with clinical signs of *S. scabiei* infection, were recorded as undetermined. To extract exif data from the images for further analysis we utilised the R package *camtrapR* vs 2.3.0 (Niedballa J et al., 2016). To ensure independence of events and to reduce the risk of scoring the same individual multiple times, a 3-minute interval was used when extracting the exif data from the images. This has previously been shown to be adequate to assume independence between trapping events (Noll, 2021), and is a common method in camera trap studies (Greenville et al., 2014, Spencer et al., 2022). To classify if a site was burned or unburned we placed a 500m buffer around the four camera locations at each WildCount site and dissolved the buffers together to create a single buffered polygon for each site representing potential wombat home range totalling 20 hectares for each site (Evans, 2008) (Figure 4.2). These polygons were then used to extract fire severity information for each site using a Fire Extent and Severity mapping layer (State Government of NSW and NSW Department of Climate Change Energy the Environment and Water, 2020).

Aim 1 analyses

We used a BACI design for both datasets (WIRES wildlife carer records and WildCount camera trap monitoring). The year 2019 was classified as the year before the fire as almost all data from 2019 occurred before the extent of fire was observed since access to the fire affected landscape was limited during and post-fire; 2020 was classified as

the year of fire impacts; and 2021 was the year after fire impacts. Therefore, year was included as a factor in our analyses. Localities/sites where the habitat was burned in 2020, were classified as burn sites (impact), distinguishing them from sites where the habitat did not burn in the 2020 wildfires (control). Predictor variables in our analyses were thus, year, treatment (burned or unburned) and their interaction, and the response variable was sarcoptic mange presence/absence. Sites that were burned in 2020 were classed as burned in 2019 also to serve as a comparison. We used a binomial generalised linear mixed effects models (R package glmmTMB vs 1.1.10) (Brooks et al., 2017) for both datasets, with locality/site included as a mixed effect to account for the repeated measures design.

Aim 2: Assess the effect of fire severity extent on mange in burned areas

Focusing on the WildCount camera trap data, where we could precisely assign fire condition associated with sites where wombats were observed, we further investigated the effect of fire severity on the probability of mange occurrence. We generated a 500m buffer around the four camera locations at each WildCount site (Figure 2) and extracted fire severity extent information for each site using a Fire Extent and Severity mapping layer (State Government of NSW and NSW Department of Climate Change Energy the Environment and Water, 2020). Fire severity included grid-cell (100m²) classifications of unburned, low severity, medium severity, high severity and extreme severity. For the purposes of this Aim, we condensed fire severity into three 'proportion of pixels' categories: unburned, low/medium severity, and high/extreme severity. We also focussed on the year of the fire (2020) and year following the fire (2021), as these were the years of fire impact. We used ArcGIS Pro vs 3.1.0 (ESRI, 2023) geoprocessing function zonal histogram to extract the proportion of pixels in the buffer covered by each of these fire severity categories for each study site. Thus, the predictor variables were year, the proportion of each fire severity class (low/medium, high/extreme), their interaction, and the response variable was mange presence/absence. Following preliminary data analyses, we excluded the unburned category from this analysis due to negative collinearity with both the proportion of low/medium and high/severe. Binomial GLMMs (R package glmmTMB vs 1.1.10) (Brooks et al., 2017) were used, with site as a random effect.

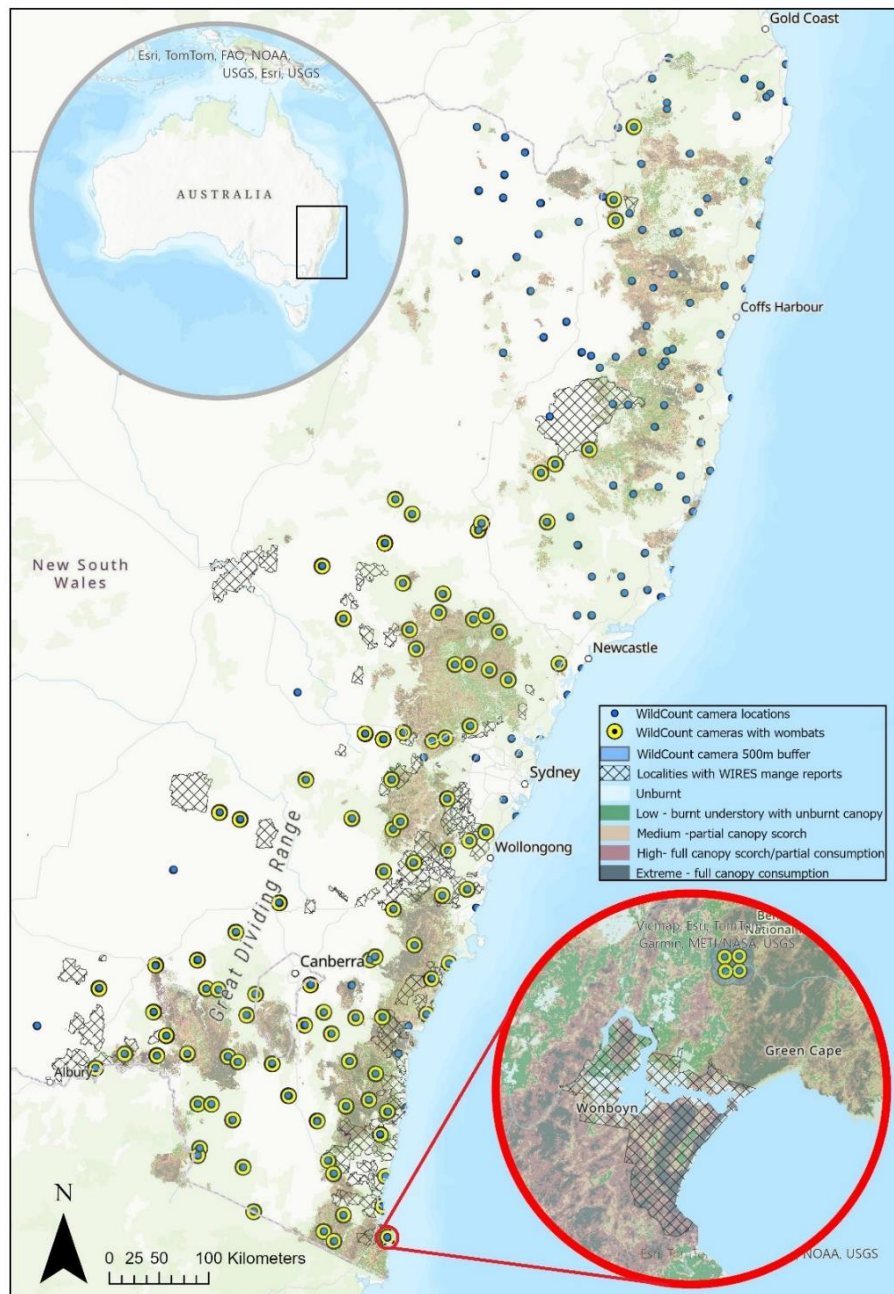


Figure 4.2. Study area extent including WildCount camera locations (blue circles), buffered areas around cameras (blue polygons, inset map only), localities with WIREs records of wombats with range (black polygons), and black summer fire extent and severity classes (see legend). Inset map shows study site including four camera layout 500 metres apart and 500m buffer around each camera dissolved to form 1 polygon representing minimum wombat home range (5ha). This was conducted for each site in the WildCount program.

All statistical analyses were undertaken using R (version 4.4.1) (R Core Team, 2022). Prior to GLMMs, temporal autocorrelation analysis was undertaken using the ACF function in the tseries package v0.10-58 (Trapletti and Hornik, 2024) to check for correlation among years; none were detected.

RESULTS

Aim 1: Quantify the impact of the megafire event on the occurrence of wombat mange

A total of 1431 WIRES wombat records were used in this study. Of this, 434 records fit the case criteria and were included in our analyses as reports of wombats with mange. These were across 153 localities, spanning 54 postcodes (Figure 4.2). Of the 153 localities, 71 were fire affected (burned) and 82 were not burned (unburned) (Table 4.1). There were 79 mange reports across 47 localities in 2019, 79 mange reports across 54 localities in 2020 and 95 mange reports across 49 localities in 2021 (Table 4.1). Prevalence of mange reports varied minorly between years with 36% in 2019, 23% in 2020, and 31% in 2021 (Table 4.1). There was no significant interaction between fire treatment (burned and unburned sites) and year (2019, 2020, or 2021) on the probability of wombats being documented with sarcoptic mange, however there was a trend in the WIRES data for unburned sites to have slightly higher probability of mange in 2021 than in 2019 (Table 4.2, Figure 4.3), which appeared to cause an overall trend independent of the year (Table 4.2, Figure 4.3).

A total of 32,957 images of wombats from the WildCount programme were assessed with 1063 independent wombat presences identified. Wombats were observed at 75 sites in 2019, 69 sites in 2020 and 41 sites in 2021 (Table 4.1). 212 independent images series (wombat presences) were recorded as mange positive, varying between 86 mange positive wombats out of 599 wombats in 2019 across 75 sites (14% prevalence), 104 mange positive wombats out of 326 wombats in 2020 across 69 sites (32% prevalence), and 22 mange positive wombats out of 138 wombats observed in 2021 across 41 sites (16% mange prevalence) (Table 4.1). Wombats with mange were observed at 13 to 29 sites (32-42% of wombat sites documented as mange positive) across the years of this study. A total of 500 image series across the

years were excluded where they did not fit our inclusion criteria (see Methods). For the WildCount data, wombats have a significantly greater probability of being documented with sarcoptic mange in 2020 than in 2019, although this was unrelated to any relationships with the wildfire (Table 4.2, Figure 4.3).

Table 4.1. Summary of burned and unburned sites, numbers of wombats and sarcoptic mange per fire treatment per year for WIRES reports and WildCount datasets.

Data set	Site status	Year	Number of sites	Number of wombat presences	Number of scorable wombat presences	Proportion of mange
WIRES						
	Burned	2019	71	129	129	37%
	Unburned	2019	82	91	91	34%
	Burned	2020	71	165	165	23%
	Unburned	2020	82	178	178	23%
	Burned	2021	71	135	135	33%
	Unburned	2021	82	167	167	29%
WildCount						
	Burned	2019	44	246	245	17%
	Unburned	2019	102	252	250	11%
	Burned	2020	82	412	212	31%
	Unburned	2020	52	214	114	33%
	Burned	2021	36	191	78	14%
	Unburned	2021	42	144	60	18%

Table 4.2. The effect of the Black Summer wildfires on sarcoptic mange in wombats as assessed using binomial generalised linear mixed effects models. Significant results in bold and trending results ($P < 0.1$) marked with *.

Variable	Coef. (SE)	Z	P
WIRES			
Treatment Unburned Habitat	-0.48 (0.28)	-1.74	0.08*
Year 2020	-0.23 (0.22)	-1.08	0.28
Year 2021	-0.06 (0.21)	-0.31	0.76
Treatment Unburned Habitat: 2020	0.51 (0.32)	1.59	0.11
Treatment Unburned Habitat: 2021	0.54 (0.31)	1.76	0.08*
WildCount			
Treatment Unburned Habitat	-0.18 (0.45)	-0.40	0.69
Year 2020	1.04 (0.28)	3.72	<0.01
Year 2021	0.27 (0.45)	0.61	0.54
Treatment Unburned Habitat: Year 2020	0.19 (0.44)	0.44	0.66
Treatment Unburned Habitat: Year 2021	0.53 (0.65)	0.81	0.42

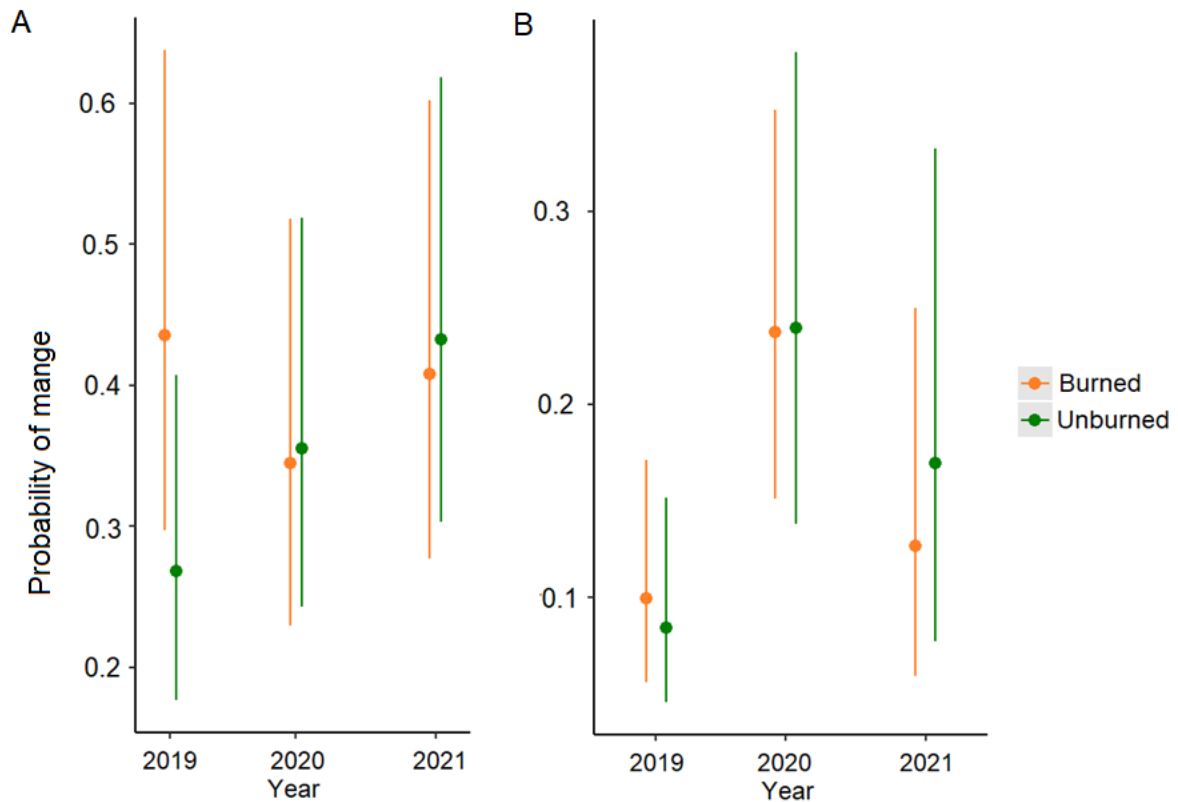


Figure 4.3. The probability of wombats being classified as having sarcoptic mange before the Black Summer Wildfires (2019), the year of the fire impact (2020), and the year after the fires (2021) from (A) WIRES wildlife carer records and (B) the WildCount camera trap program.

Aim 2: Assess the effect of fire severity extent on mange in the areas burned

When assessing the influence of fire severity on mange, there were significant interaction effects of the extent of burning and year on the probability of wombats being documented with sarcoptic mange (Table 4.3, Figure 4.4). Wombats had a higher probability of being documented with mange as the proportion of burning increased in the wildfire year (2020), with the relationship being transient, not observed in the year after the wildfires (2021) (Figure 4.4). This interaction effect was observed for both the proportion of area burned by low/medium and high/extreme fire severities (Figure 4.4).

Table 4.3. The effect of the Black Summer wildfires on the probability of wombats being classified as having sarcoptic mange between fire severity classes, from binomial generalised linear mixed effects models. Significant results in bold and trending results ($P < 0.1$) marked with *.

Variable	Coef. (SE)	Z	P
Proportion low/medium	0.03 (0.01)	1.83	0.07*
Proportion high/extreme	0.02 (0.13)	1.35	0.18
Year 2021	1.95 (1.35)	1.45	0.15
Proportion low/medium: Year 2021	-0.04 (0.02)	-1.93	0.05
Proportion high/extreme: Year 2021	-0.03 (0.02)	-2.03	0.04

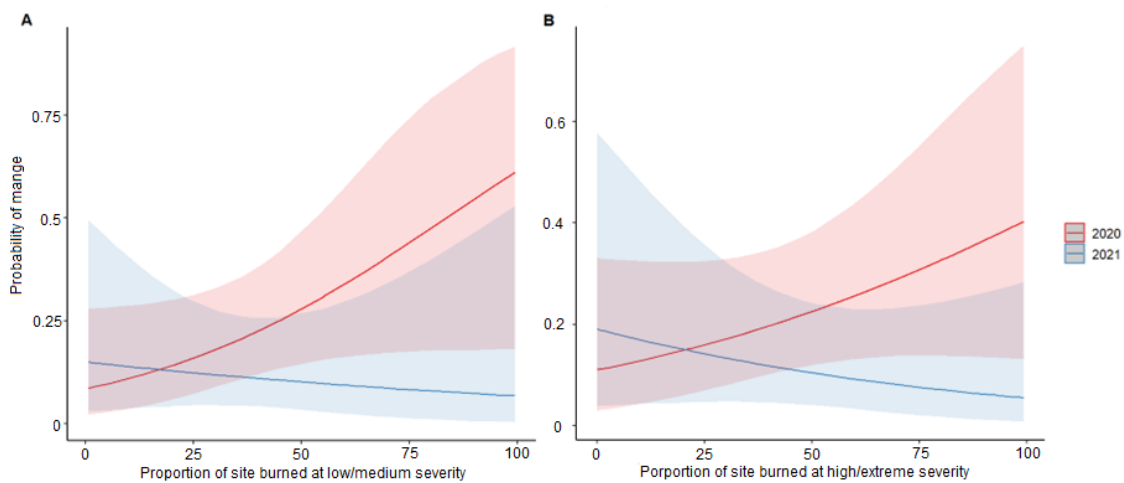


Figure 4.4. The influence of the extent of (A) Low/Medium and (B) High/Extreme wildfire landscape burn severity in the year of the wildfires (2020, red) and year following (2021, blue) on the probability of mange in wombats from the WildCount images.

DISCUSSION

How wildfires can influence infectious diseases of wildlife is poorly understood. We used two independent lines of evidence in a BACI design to test the impact of the 2019-2020 Australian megafires on the dynamics of sarcoptic mange in bare-nosed wombats. Our first aim tested the influence of the megafire on the probability of wombats having mange, finding no evidence of fire on disease occurrence. In our second aim, we investigated the influence of landscape burn severity on sarcoptic mange, showing that signs of disease increased in the year of the wildfire as more local landcover was impacted by low/medium or high/severe burned categories. This effect was transient, not persisting into the year following the wildfire. Taken together, our research suggests wildfire can have temporary effects on wildlife disease, mediated by the extent and severity of the local landscape burned.

Prior to commencing this investigation we considered it equally plausible that sarcoptic mange in wombats could increase, decrease, or be unaffected by wildfires, based on host-parasite-environment interactions. Our research suggests that transmission mechanisms underscoring dynamics of *S. scabiei* are resilient to the presence of wildfire, but can be influenced by fire extent. Indeed, our research shows mange affected wombats and their mites survived the megafire, most likely through refuge in their burrows. There are several mechanisms which could explain why greater extent of local landcover impacted by wildfire temporarily increases the occurrence of mange in wombats. Perhaps most likely is that wildfire is known to influence wombat foraging behaviours (Green et al., 2015, Heaton et al., 2022), and this could influence how individuals aggregate around more spatially confined foraging resources and competition over nearby burrows containing environmental fomites. Because wombats impacted by sarcoptic mange also spend a greater amount of time foraging (Simpson et al., 2016), this transmission mechanism may be particularly amplified. It is also possible that fire may influence wombat susceptibility to *S. scabiei* infestation through various immune suppressing factors (e.g., stress, nutrition related immune depletion, smoke inhalation). However, there is currently little evidence that wombats mount effective immune responses against sarcoptic mange (Skerratt, 2003, Næsberg-Nielsen et al., 2022), so we cannot ascertain if immune factors are a likely explanation in this scenario but may warrant further study. Research investigating how wombat

spatial ecology and burrow usage is influenced by local fire extent would be valuable to improve understanding of mechanisms governing transient increases in sarcoptic mange following fire. Our results also suggest that reactive efforts to mitigate sarcoptic mange impacts – an area of public interest in Australia (Rowe et al., 2019) – following wildfire are best targeted in areas with greater local fire extents.

The transient effect of local fire extent on sarcoptic mange in wombats suggests that disruptions to transmission mechanisms were short lived. Transient effects are perhaps a reflection of the resilience of wombats to fire in the landscape (Heaton et al., 2022), including megafire events (Miritis et al., 2024). The insulative qualities of burrows are ideal to enable high wombat survival during fires (Green et al., 2015, Keith et al., 2002, Mikac et al., 2023) and also *S. scabiei* mites in the environment (Browne et al., 2021, Ross et al., 2021). While the effect of the fire was transformative upon the vegetation landscape in southeastern Australia (Turner, 2010, Ward et al., 2020), being obligate grazers with low metabolic rates means wombats are advantaged by being able to both sustain through periods of resource depletion and then take advantage of the earliest of plant regrowth and recolonisation (e.g., grasses and forbes) (Evans et al., 2003, Guy and Kirkpatrick, 2021, Carver et al., 2024). Indeed, grazing herbivores often benefit from the increase in nutritional quality of plants following fire (Leigh et al., 1991, Van Dyke and Darragh, 2006, Heaton et al., 2022). Increased primary productivity and nutritional quality of forage for wombats may be particularly valuable for restoring transmission to background levels, given increased nutritional demands on mange affected individuals (Simpson et al., 2016, Martin et al., 2018). Indeed, high rainfall across eastern Australia occurred in 2020 (Bureau of Meteorology, 2021) likely restoring forage and water resources for wombats (Linley et al., 2024). Thus, in context of existing literature, our study suggests that mechanisms promoting increased transmission in areas more impacted by the megafire, (see above discussion of mechanisms), rapidly reverted to their pre-fire state.

It is also worth noting that the occurrence of fire is a normal condition across the Australian landscape in which bare-nosed wombats are distributed (Nimmo et al., 2021, Carver et al., 2024). Wombats are thus adapted to avoid and survive fire events, primarily through use of their burrow refuge. Combined with knowledge that sarcoptic mange occurs across the distribution of bare-nosed wombats (Martin et al., 1998) it is

perhaps unsurprising that this host-pathogen system has demonstrated such resilience to the disruptions of the 2019-2020 Australian megafires. While we looked at the presence and severity of fire on mange dynamics, we also recognise that there are complex interactions between mange dynamics in wombats and environmental variables including vegetation type, host habitat suitability, annual precipitation, sources of freshwater, and land use (i.e farmland vs native habitats) (Ringwaldt et al., 2023). All of these environmental variables are documented to have relationships with occurrence of sarcoptic mange in wombats, although research by Ringwaldt et al. (2023) did not investigate the effects of fire. It would be valuable for research to further investigate the relative importance of fire activity and severity, in context of other environmental variables, on sarcoptic mange dynamics in wombats.

Our research has shed new light on how megafires can potentially have impacts on infectious diseases of wildlife. We focussed on wombats reflecting the conspicuous nature of sarcoptic mange in this widespread marsupial in southeastern Australia and also availability of different data sources from which to test if this wildlife disease changed in association with fire impacts. While our focus was on mange in wombats, we note that other species also use wombat burrows and could potentially be exposed to *S. scabiei* mites, particularly if burrow usage increases when fire occurs in the landscape. Wombat burrows have an important ecological role as refuge for other species, including fire and temperature extremes, as well as sub-terranean water sources (Linley et al., 2024). A study by Linley et al. (2024), identified that sites with wombat burrows had a greater species richness than sites without wombat burrows, and that this relationship strengthened immediately following the Black Summer bushfires in 2019-2020. Marsupials, such as swamp wallabies and ringtailed possums, are occasionally recorded investigating wombat burrows, and sarcoptic mange has been occasionally documented to affect these species (McLelland and Youl, 2005, Skerratt et al., 1998), which could further influence sarcoptic mange dynamics. It is plausible, therefore, that increased use of wombat burrows by other species could contribute to mange transmission, (multi-species transmission), when the local extent of fire impacts are high. However, whether other species use burrow regions that lead to increased mite encounters is unknown, as is whether they are dead-end hosts, and we note that increases in mange occurrence in other species have not been reported in association with fire. Presently, the role of other species in the transmission of *S.*

scabiei to wombats is more conjecture than evidence based and, thus, we interpret our findings as most relevant for interpretation in a single-species transmission context.

CONCLUSION

Our findings suggest that transmission of *S. scabiei* temporarily increases as a function of the extent of landscape impacted by higher severity fire, potentially resulting from wombats aggregating around spatially restricted food and water resources following the fire, and effects this has on local environmental transmission. The influence of fire on range dynamics in wombats is not as simple as our hypothesised scenarios, with scenario 1 (an increase in range affected wombats) being observed in the short term, but in the longer-term scenario 2 prevails with no overall change in range dynamics. Taken together, this research suggests wildfire can have transient effects on wildlife disease, which may be mediated by the extent and severity of the local habitat burned.

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AUTHOR CONTRIBUTIONS

Ellyssia T. Young: conceptualization, visualisation, methodology, data curation, analysis, writing – original draft, reviewing and editing. Aaron C. Greenville and Scott Carver: conceptualisation, methodology, supervision and writing – reviewing and editing. Madeleine de Montfort, Jessica Cuneo, Teresa Harris, Doug Mills, Annie Zhou, Adam Birnbaum and Gary Saunders were involved in the WildCount program

development, implementation and data curation. The WIRES team including wildlife carer network: data curation. Stella Noll and David Phalen: data curation. All authors reviewed and approved the final manuscript.

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CHAPTER 5.

ADVANCING THE LANDSCAPE EPIDEMIOLOGY OF SARCOPTIC MANGE IN BARE-NOSED WOMBATS



Image credit: Southern Ark (DEECA)

CHAPTER 5: ADVANCING THE LANDSCAPE EPIDEMIOLOGY OF SARCOPTIC MANGE IN BARE-NOSED WOMBATS

Young, E.T., Carver, S. and Greenville, A.C. Advancing the landscape epidemiology of sarcoptic mange in bare-nosed wombats. (In prep.). *Transboundary and Emerging Diseases*.

Abstract

Understanding spatial dynamics and drivers of wildlife pathogens is often challenged by sampling logistics, but pathogens causing visually apparent clinical signs present an opportunity to overcome such problems. We investigated the landscape epidemiology of visually apparent sarcoptic mange (etiological agent *Sarcoptes scabiei* mite) in the iconic Australian bare-nosed wombat (*Vombatus ursinus*). We examined 188,982 remote camera trap images of bare-nosed wombats from 720 sites across ca. 9,000km² of far east Victoria, Australia, collected from 2016-2022. We found wombats exhibiting signs of sarcoptic mange were widespread across the region, with a mean prevalence of 8% (235/2977). The probability of mange occurrence in wombats was associated with year, was higher in areas with more topographic roughness, lower annual mean precipitation and with increasing distance to freshwater sources. Contrasting our investigation with landscape epidemiology studies from Tasmania and New South Wales – regions south and north of our study area – we found annual precipitation was a consistent negative predictor of mange, mean annual temperature and temperature annual range relationships exhibited a latitudinal switch from positive to negative, and other predictor variables were more local in their associations. This study adds to a growing body of research advancing the landscape epidemiology of wildlife disease by using pathogens with visually apparent clinical signs and helps to clarify distribution-wide and local-scale factors associated with mange occurrence in wombats.

Keywords: Bare-nosed wombat, landscape epidemiology, *Sarcoptes scabiei*, sarcoptic mange, *Vombatus ursinus*

INTRODUCTION

Landscape epidemiology is a potentially valuable disciplinary framework from which to understand the dynamics and guide management of wildlife disease issues. Broadly, landscape epidemiology seeks to understand environmental drivers of spatiotemporal patterns in the incidence and risk of infectious diseases (Ostfeld et al., 2005, Manlove et al., 2022). While of particular value for wildlife disease issues, landscape scale epidemiological studies are commonly limited by complex challenges associated with sampling hosts and their pathogens (MacDonald et al., 2022). This critical challenge is particularly significant for introduced pathogens that impact animal welfare and conservation. However, in cases where pathogens cause visually apparent clinical signs, it may be possible to use contemporary surveillance technologies to achieve assessments of disease drivers at the landscape level.

The use of remote camera traps has become a valuable tool for surveying wildlife across large areas (Harvey et al., 2021, Wearn and Glover-Kapfer, 2019, Ringwaldt et al., 2023), and has more recently been applied to facilitate assessment of visually apparent disease issues (Barroso and Palencia, 2025). For example, Muneza et al. (2019) used camera traps to determine the presence and severity of giraffe skin disease. The emerging body of literature focussed on use of camera trapping for assessment of pathogens that cause visually apparent clinical signs suggests significant potential for overcoming historical limitations and advancing the field of landscape epidemiology for a subset of wildlife pathogen issues. Most notably, landscape epidemiological assessments may assist managers to determine where management actions are most critical, and how wildlife disease issues will be affected under different climate scenarios (MacDonald et al., 2022).

The parasitic mite, *Sarcoptes scabiei*, causes sarcoptic mange in a wide range of mammalian host species, inducing visually apparent clinical signs of skin disease in many affected species (Escobar et al., 2022). Sarcoptic mange is among the most geographically widespread of mammalian parasitic diseases and has been reported in 11 Australian mammal species (Fraser et al., 2016, Martin et al., 2018, Skerratt et al., 2004, McLelland and Youl, 2005, Botten et al., 2022, Hoyte, 1961, Holz et al., 2011, Young et al., 2024, Russell et al., 2024, Ruykys et al., 2009, Knox et al., 2025). Bare-nosed wombats (*Vombatus ursinus*, hereafter referred to as wombats) are severely

affected by sarcoptic mange, with the parasite present throughout their geographic distribution, ranging from endemic low-level disease to sporadic epizootics that cause localized population declines (Carver et al., 2023, Martin et al., 2018, Stannard et al., 2020, Martin et al., 1998). Sarcoptic mange is, thus, both a conservation concern and an animal welfare issue in wombats (Pence and Windberg, 1994, Martin et al., 2018). Clinical signs of sarcoptic mange include pruritis, alopecia, parakeratotic lesions, emaciation, secondary infections and death (Næsborg-Nielsen et al., 2022, Pence and Ueckermann, 2002). Since the transmission of *S. scabiei* is environmental, fomite survival is shaped by environmental conditions, and patterns of mange occurrence differ among wombat populations, there is a fair rationale that environmental factors likely play a role in mange epidemiology across landscapes (Beeton et al., 2019, Burgess et al., 2023).

In this project we aim to advance understanding of the landscape epidemiology of sarcoptic mange in wombats. We have three objectives: 1) assess the occurrence of wombats and sarcoptic mange across our ca. 9,000 km² study area of far east Victoria, Australia; 2) determine landscape and environmental factors associated with sarcoptic mange; and 3) contrast findings from this research with other landscape epidemiological studies of mange in wombats to develop a more general understanding of landscape epidemiology of mange across the range of bare-nosed wombats.

METHODOLOGY

Objective 1: Occurrence of wombats and sarcoptic mange

We utilised a large-scale (ca. 9,000km²) camera trapping dataset from the Southern Ark program across far east Victoria (Department of Energy, Environment and Climate Action) (Figure 5.1). This monitoring program was originally set up in 1995 to support suppression of introduced red fox (*Vulpes vulpes*) populations to aid the recovery of native animals including the Long-footed and Long-nosed potoroo (*Potorous tridactylus*, *Potorous longipes*), Southern Brown bandicoot (*Isodon obesulus*), and Southern Brush-tailed rock wallaby (*Petrogale penicillata*). Naturally, other wildlife were also observed on the camera traps, including wombats. The project

extends across multiple land tenures, with 720 camera trap sites across State Forest, National Parks and private land from the Snowy River to Cape Howe. Imagery utilised in this study is from a 6-year period, from 2016 – 2022 (excluding 2018). The number of camera trap sites varied between years (2016 – 480 sites, 2017 – 427 sites, 2019 – 234 sites, 2020 – 249 sites, 2021 – 902 sites, 2022 – 685 sites). In some years, multiple rounds of camera trap deployments were undertaken, so some sites may have been sampled in different seasons. In 2019/2020 there were reduced camera traps due to Black Summer fires. Camera trap sites consisted of a patch cleared of vegetation for a two-metre radius and a bait station in the centre (Figure 5.1). Each camera was placed on a metal stake 40 centimetres above the ground and facing due south towards the bait station (containing either herbivore or predator lure) (Figure 5.1).

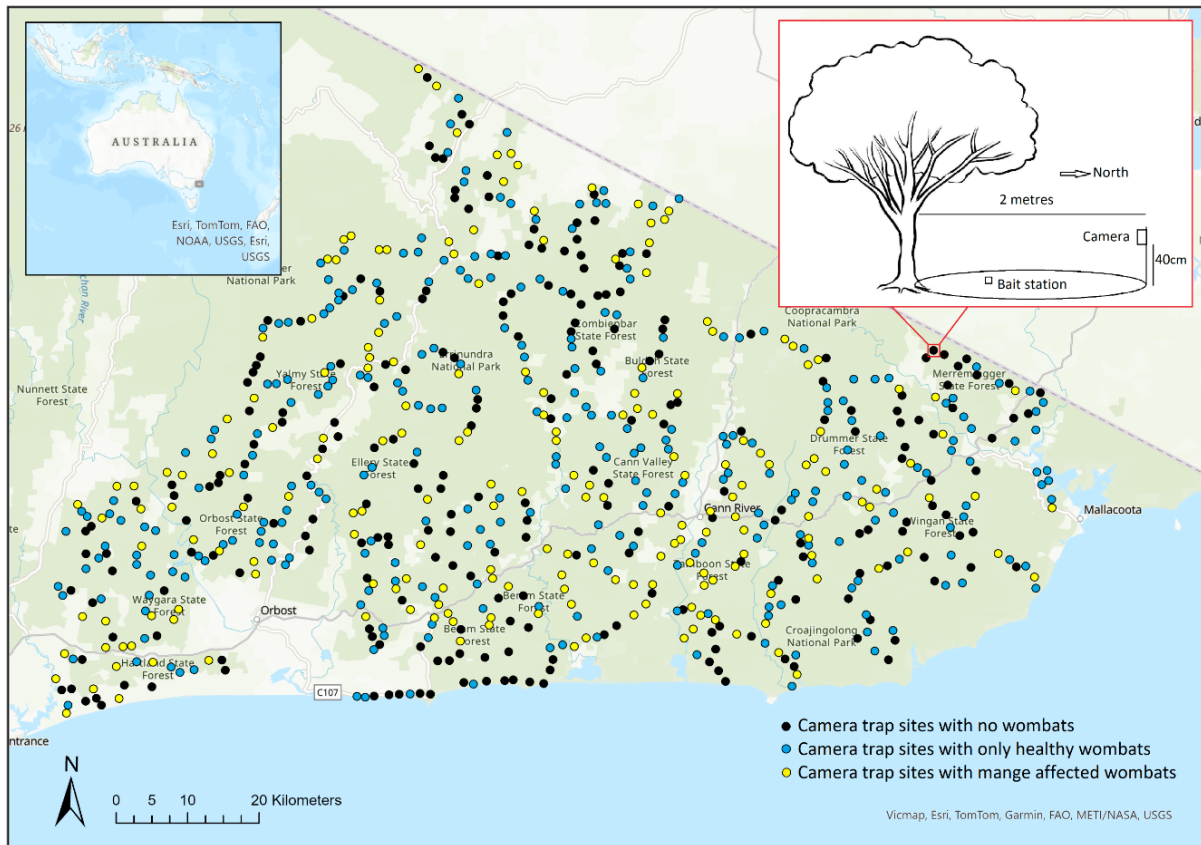


Figure 5.1. Extent of camera trap sites (all years) (black, blue and yellow points, n = 720) with camera trap sites with no wombats detected between 2016 and 2022 (black points, n = 152) and camera trap sites with wombats detected (blue and yellow points, n=568). Camera trap sites with only healthy wombats detected between 2016 and 2022 (blue points, n = 287) and sarcoptic mange presence between 2016 and 2022 (yellow, n = 195) across far east Victoria, Australia used for landscape scale analysis of sarcoptic mange (*Sarcoptes scabiei*) in bare-nosed wombats (*Vombatus ursinus*). Southern Ark camera layout.

For each camera trap site (referred to as site), we recorded the presence or absence of wombats, and the presence or absence of mange signs. Every wombat presence was scored for the visual signs of sarcoptic mange using standard scoring methods of clinical signs by the same observer (Chapter 4; Young et al., 2025, Simpson et al., 2016, Ringwaldt et al., 2023). Clinical signs in wombats include skin reddening, skin thickening and hair loss which are often able to be observed from 4 weeks after

infection (Simpson et al., 2016). In severe cases physiological and behavioural changes lead to a decline in body condition and typically results in death (Martin et al., 2018, Pence and Ueckermann, 2002). Mange scores (healthy, mange affected, undetermined), were assigned if a wombat's whole body was clear in the image and was also dry (wet fur separates and it can be difficult to distinguish mange signs). Images were also interrogated for clinical signs which may be unrelated to sarcoptic mange, (e.g., wounds, hair loss from other causes), and if in doubt, these were listed as undetermined and excluded from analysis. When extracting the exif data from the images, a 3-minute time interval between wombat image series was applied to ensure independence of events, to reduce the risk of scoring the same individual multiple times. This has been demonstrated to be appropriate to assume independence between image series in wombats (Noll, 2021), and is a commonly utilised method remote camera trap studies (Greenville et al., 2014, Spencer et al., 2022).

We then determined eleven environmental, climatic and landscape predictors for each site that could be important for influencing patterns of *S. scabiei* in wombats (see Appendix B – Table S5.1 for data source and methodology). These were: annual mean temperature (°C), temperature annual range (maximum temperature of the warmest month minus minimum temperature of the coldest month), annual mean precipitation (mm), seasonal precipitation (co-efficient of variation), land use (classified into 4 major land uses: production native forests, nature conservation, roads, grazing modified pasture), distance to pasture (straight-line distance from camera site to closest point of modified pasture), vegetation (four classifications of major biologically relevant vegetation types: forests, rainforests, heathlands and shrublands), distance to freshwater (straight-line distance of each camera to a freshwater source - river, stream or dam), elevation (meters above sea-level, masl), topographic roughness (variability in elevation), and fire history (time since fire - prescribed fire or wildfire simplified into 4 time since fire groups: 1-4 years, 5-9 years, 10-19 years and greater than 20 years). Site and year were included as predictor variables to capture spatial and temporal patterns of mange. Therefore, 13 predictor variables were used for statistical analysis. All mapping and spatial analysis was undertaken in ArcGIS Pro version vs 3.1.0 (ESRI, 2023) and R (version 4.4.1) (R Core Team, 2022).

Objective 2: Determine the landscape and environmental factors associated with sarcoptic mange

Utilising the dataset resulting from Objective 1, we then employed Random Forest (RF) analyses to assess landscape and environmental factors associated with sarcoptic mange. This analysis was used to account for non-linearity of predictor variables. All statistical analyses were undertaken in R (version 4.4.1) (R Core Team, 2022) using the randomForest package version 4.7-1.2 (Liaw and Wiener, 2002). Prior to analysis all variables were checked for multi-collinearity, with exclusions of highly correlated variables ($r \geq 0.7$). The predictor variables elevation and annual mean temperature were highly positively correlated ($r = 0.99$), and we retained annual mean temperature due to existing literature on how temperature influences mite survival and wombat distribution (Carver et al., 2021, Arlian et al., 1984, Browne et al., 2021). The remaining 12 predictor variables were only minimally correlated with maximum linear correlation of $r = 0.6$.

We trained and bootstrapped Random Forest models 10 times, recording specificity, sensitivity, Cohen's kappa and true-skill statistic for each resample. Specificity measures the model's ability to correctly identify true negative cases, and sensitivity is the models' ability to identify true positive cases (Cutler et al., 2007). The kappa statistic measures the agreement beyond that expected by chance and can be used to assess how much better the models predictions are compared to a random guess, which is of value in ecological modelling because it can account for the likelihood of misclassification due to random chance (Prasad et al., 2006). Kappa values range between -1 and +1 with negative values suggesting worse than random performance, values close to 0 indicate no agreement beyond chance and +1 indicates perfect agreement. The true-skill statistic is a simple and robust measure of binary classification model accuracy in ecological models (Allouche et al., 2006). True-skill statistic values of < 0.4 indicate poor predictive capacity, 0.4-0.8 indicate useful predictions, and > 0.8 indicate good to excellent predictive capability (Zhang et al., 2015). Variable importance and partial dependence plots were also developed for all predictor variables.

Objective 3: Contrast findings from this research with other wombat-mange landscape epidemiology studies

We are aware of two other studies which have used a comparable set of environmental variables from geographically distinct regions to assess predictors of *S. scabiei* in bare-nosed wombats. These two studies encompass the southern and northern parts of the bare-nosed wombat distribution (Ringwaldt et al., 2025, Ringwaldt et al., 2023). Because our study represents a valuable middle portion of the wombat distribution, we synthesized key study findings to provide a more general understanding of how the landscape epidemiology of sarcoptic mange varies across the wombat distribution. We focus on comparing directionality of key predictor variables across the studies as there are statistical methodological differences which make calculation and comparison of effect sizes challenging.

RESULTS

Objective 1: Occurrence of wombats and sarcoptic mange

Wombat presences were recorded at least once at 568 of the 720 camera trap sites (81% of sites) across the far east Victoria from 2016 – 2022 (excluding 2018) (Figure 5.1). Wombats were observed on cameras across the elevational range of camera trap sites, from sea level to 1296 m elevation. A total of 188,982 wombat images were recorded, and all were assessed for signs of sarcoptic mange. Of the camera trap sites with wombats detected (568), the presence of mange was recorded at 195 camera trap sites (34%), with only mange affected wombats observed at 131 camera trap sites (23%), and both healthy and mange affected wombats observed at 150 camera trap sites (26%) (Figure 5.1). Only healthy wombats were observed at 287 camera trap sites (51%) across the study area. The prevalence of mange in wombats at each camera trap site was low and varied between years, with a mean prevalence of 8% (2016 – 6% (29/480), 2017 – 11% (47/427), 2019 – 7% (16/234), 2020 – 8% (20/249), 2021 – 6% (54/902), 2022 – 10% (69/685)).

Sites were unequally distributed across land use types with 18 in road reserves, 529 in production native forests, 171 in nature conservation areas and 2 on the boundary of farmland (grazing modified pasture). Vegetation type was also unevenly

represented in camera site data with 710 in forests, 2 in shrublands, 5 in heathlands and 3 in rainforests. There were 13 sites burned 1-4 years ago, 608 sites 5-9 years ago, 18 sites burned 10-19 years ago, 48 sites burned 20+ years ago and 33 sites where there were either no data or no fires ever recorded. Out of the 720 camera trap sites, an artificial water point (farm dam or fire water point) was the closest source of freshwater for 121 sites, and 599 sites were closest to a natural watercourse (stream or river etc).

Objective 2: Determine the landscape and environmental factors associated with sarcoptic mange

Analysis of landscape and environmental factors associated with occurrence of sarcoptic mange in individual wombats (image series) yielded moderate sensitivity (0.59) and high specificity (0.98) and fell into the 'useful' mange classifications category for kappa (0.65) and true-skill statistic (0.57) values (Table 5.1). Year had the largest influence on occurrence of sarcoptic mange (>65% increase in mean squared error), followed by topographic roughness, distance to freshwater and annual mean precipitation (50-60% increase in mean squared error) (Figure 5.2). Site, seasonal precipitation, distance to pasture, temperature annual range and mean annual temperature were the next most important predictors with a 30-50% increase in mean squared error. Land use, vegetation and time since fire had lesser influences on mange patterns with <20% increase in mean squared error (Figure 5.2, Appendix B – Figures S5.1 - 5.2).

Partial dependence plots demonstrated non-linearity in predictor variable relationships with wombat-mange (Figure 5.3, Appendix B – Figures S5.1 - S5.2). It is also notable that there is relatively little variation in the probability of mange occurrence associated with any of the predictor variables (Figure 5.2). For the four most important predictor variables (Figure 5.3): 1) mange was higher in 2016 then dropped between 2020 and increased again from 2021; 2) greater topographic roughness values (higher roughness values indicate greater heterogeneity of the terrain), were associated with increased occurrence of mange; 3) the occurrence of mange increased with distance from freshwater sources; and 4) the occurrence of mange was lower in areas with higher precipitation.

Table 5.1. Summary table of random forest model bootstrapping including mean and standard deviation for specificity, sensitivity, True-skill statistic, and Cohens kappa value for the 10 resampled models.

Statistic	Mean	Standard deviation
Sensitivity	0.59	0.008
Specificity	0.98	<0.001
True-skill statistic	0.57	0.008
Cohens kappa	0.65	0.008

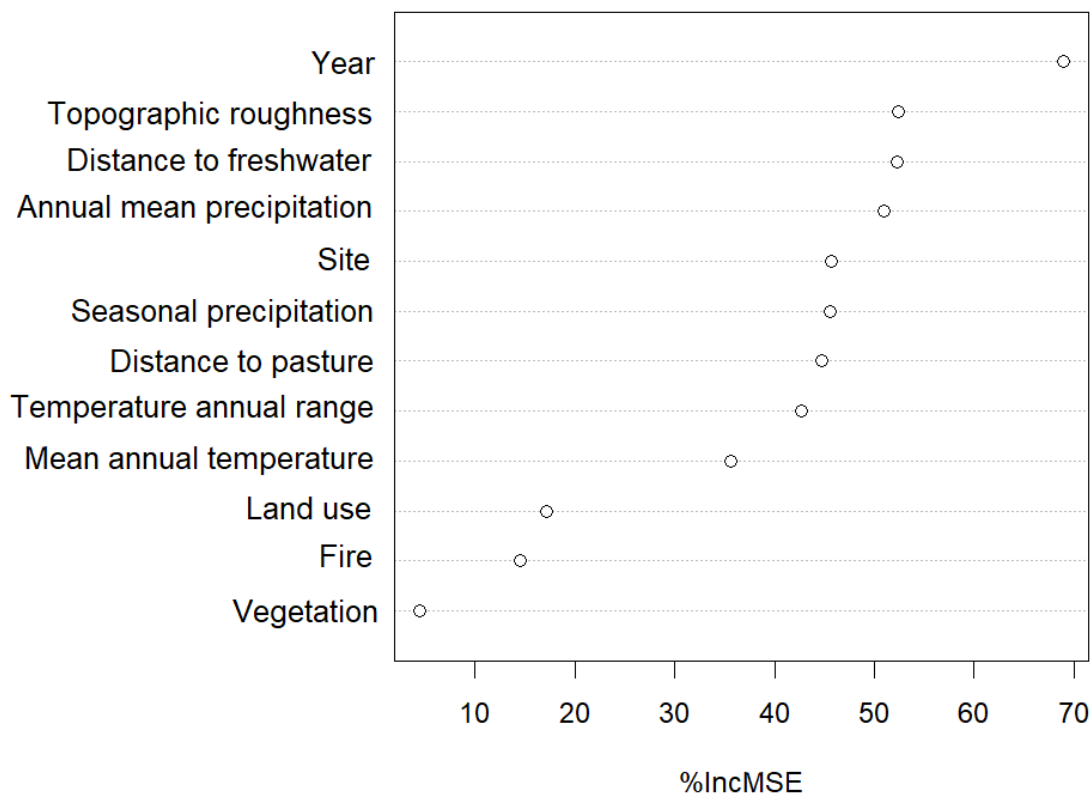


Figure 5.2. Variable importance plot for all predictor variables based off increase in mean squared error (%IncMSE). Year, topographic roughness, distance to freshwater and annual mean precipitation were the four variables with the greatest influence on range.

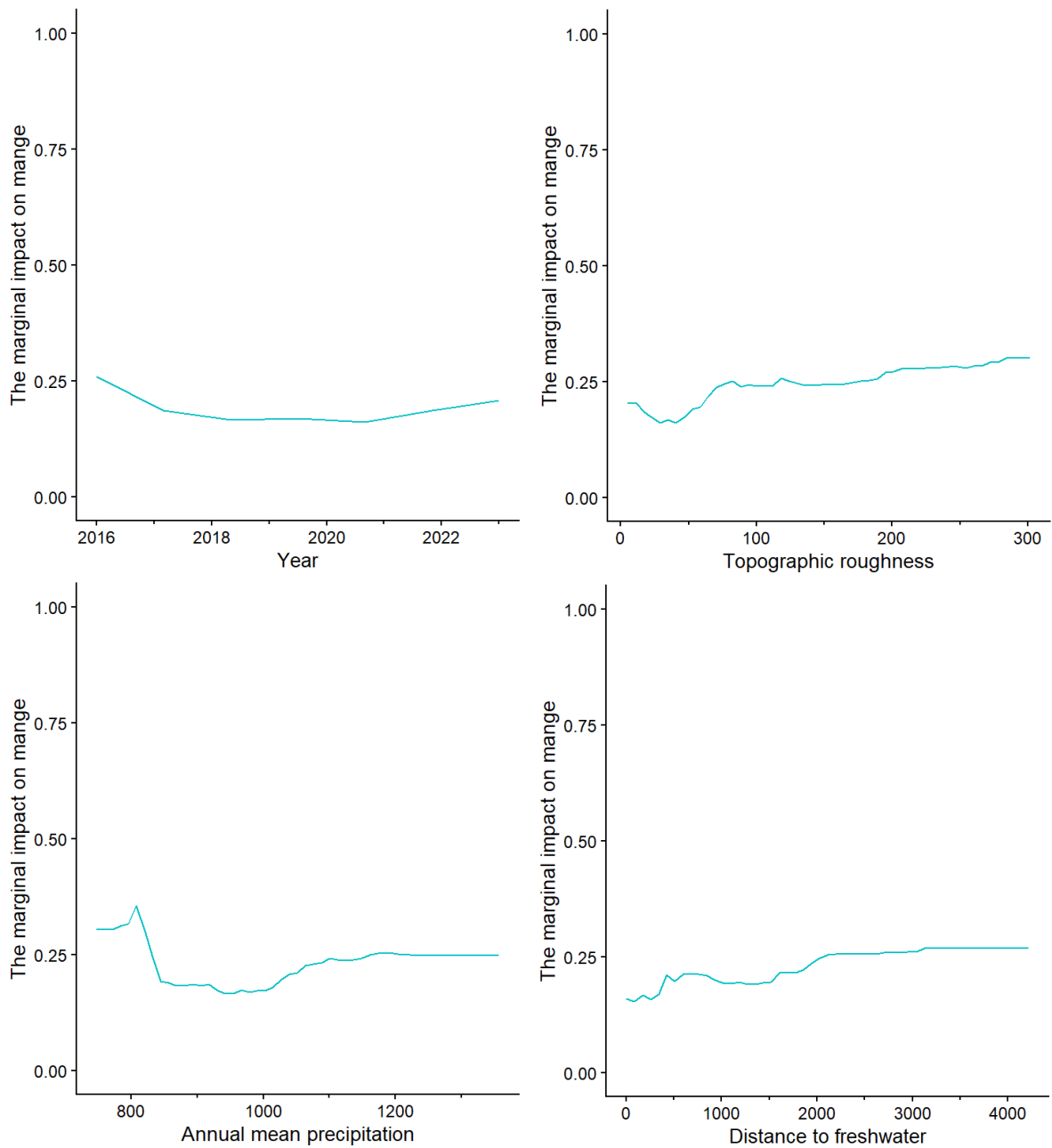


Figure 5.3. Partial dependence plots showing the effects of the four variables having the highest contributions to variation in mangle patterns predicted by the randomForest model.

Objective 3: Contrast findings from this research with other wombat-mange landscape epidemiology studies

Our contrast of landscape epidemiology studies of sarcoptic mange in wombats identified both similarities and differences among studies (Table 5.2). Annual mean precipitation was consistently negatively associated with mange across the three studies, with the strength of the relationship appearing strongest in Tasmania. We found that the importance of some variables may change with latitude. In Tasmania and our study, there was a positive relationship between temperature annual range and mange, but in NSW a negative relationship was reported. Mean annual temperature was also variable across geographic locations, with positive relationships documented in Tasmania and in our study, and negative in NSW (Table 5.2).

We also found multiple predictor variables exhibited no discernible pattern across sites. Topographic roughness was a key predictor in both Tasmania and Victoria, but the directionality differed (Table 5.2). Similarly, distance to freshwater was a key predictor of mange in both NSW and Victoria, with differing direction of association among the sites (Table 5.2).

Table 5.2. Environmental predictor relationship comparison table between three sarcoptic mange studies in bare-nosed wombats from three major Australian geographic regions.

Environmental predictors	SE Victoria (current study)	Tasmania (Ringwaldt et al., 2023)	New South Wales (Ringwaldt et al., 2025)
<i>Relationships that are similar across landscape epidemiology studies</i>			
Annual mean precipitation	-*	-*	-
<i>Relationships that indicate changing landscape epidemiology from south – north</i>			
Temperature annual range	+	+	-
Mean annual temperature	+	+	-*
<i>Cases where there is no discernible pattern</i>			
Topographic roughness	+*	-*	<i>Not tested</i>
Distance to freshwater	+*	-*	<i>Not tested</i>
Seasonal precipitation	No relationship	-	<i>Not tested</i>
Vegetation	No relationship	Vegetation dependent	Vegetation dependent *
Land use	No relationship	intensity dependent	intensity dependent
<i>Variables which were only tested in a single a study</i>			
Distance to pasture	No relationship	<i>Not tested</i>	<i>Not tested</i>
Fire	No relationship	<i>Not tested</i>	<i>Not tested</i>
Elevation	Removed due to collinearity with mean annual temperature	Removed due to collinearity with mean annual temperature	-*
Wombat density	<i>Not tested</i>	<i>Not tested</i>	-*
Wombat habitat suitability	<i>Not tested</i>	+*	<i>Not tested</i>

**Identified as a key predictor by authors*

+ Positive relationship

- Negative relationship

DISCUSSION

This study contributes both new empirical information and fills a north-south distributional gap in landscape-scale mange epidemiology studies in bare-nosed wombats. We found that mange prevalence in wombats varied from 6% - 11% across years, and that the presence of mange was most strongly predicted by year, in areas with greater topographic roughness, lower annual mean precipitation, and increasing distance to freshwater sources. Contrasting our results with other landscape epidemiology studies, we found that annual precipitation was consistently negatively associated with the occurrence of sarcoptic mange, and that the effects of temperature annual range and mean annual temperature changed from negative to positive associations with mange from south to north. These results enhance our knowledge of sarcoptic mange epidemiology in bare-nosed wombats and contribute to a growing literature on the value of visually apparent clinical signs for studying the landscape epidemiology of wildlife diseases.

Our research supports other studies, suggesting that far east Gippsland has high habitat suitability for bare-nosed wombats (Heard and Ramsey, 2020). Importantly, our research provides a high level of spatial coverage for this geographic area, not previously reported, and thus helps with both fine and broad scale understanding of wombat distribution patterns. Unique to this study, we also showed that wombats exhibited visual signs of mange across the study area, suggesting widespread suitability for disease occurrence. A common concern for mange in wombat populations is the risk of outbreaks causing population declines, and our mean prevalence estimate of 8% is generally consistent for populations with endemic diseases, rather than associated with population declines (Martin et al., 2018, Carver et al., 2023, Beeton et al., 2019).

Environmentally transmitted pathogens have potential to be highly responsive to environmental variables (Beeton et al., 2019) and, consistent with this, we found that mange was predicted by a number of environmental variables in our study area. In particular, topographic roughness, distance to freshwater, and mean annual precipitation were the most important environmental variables, after the effect of year. Interestingly, we also found that the probability of mange occurrence was only moderately responsive to our predictor variables – based on model performance

metrics and variation in marginal impact values. We attribute these moderate effects to reflect both the widespread nature of mange across our study area and stable temporal patterns of prevalence. Indeed, Ringwaldt et al. (2025), found that environmental factors were also only moderately predictive of mange in wombats from NSW. However, in contrast, there appear to be more distinct signatures of environmental variables with mange in wombats from Tasmania (Ringwaldt et al., 2023). While host variables may also play a role in mange dynamics there is not enough evidence regarding wombat specific physiological factors which may predispose an individual to mange infection (Næsborg-Nielsen et al., 2022, Skerratt, 2003). Therefore, these regional differences may suggest environmental predictors of wombat-mange are stronger in cooler (latitudinally south) geographic regions, relative to warmer northern latitudes. A longer potential survival time of environmental fomites in cooler climates (Browne et al., 2021) may be a mechanistic basis from which to understand latitudinally varying predictor variables, and further research is needed to explore such relationships.

For the environmental variables that were most predictive of mange occurrence in our study, there are a number of mechanistic explanations. In rougher landscapes and in areas at higher altitude, wombats often have larger home-ranges (Evans, 2008, Matthews and Green, 2012, Thorley and Old, 2020) and the underlying geology of the areas further influences how wombats use the landscape (Roger et al., 2007), influencing pathogen exposure. Topographically rough areas may also have fewer burrows available to wombats and, thus, increase pathogen encounter rates. We also found increased probability of mange in areas of low annual precipitation, consistent with other studies (Ringwaldt et al., 2025, Ringwaldt et al., 2023). A mechanistic basis for this relationship may be that wombat occurrence also follows this similar pattern (Ringwaldt et al., 2025). Research focussed on quantifying the probability of wombat occurrence and sarcoptic mange occurrence would be valuable to more fully understand the basis of the precipitation relationship. Lastly, increasing proximity to freshwater source – either natural (creek, river), or anthropogenic (fire water point, farm dams) – was also a top predictor of mange risk in wombats in our study. This result is somewhat counterintuitive, based on other research suggesting increased mange nearer water bodies (Ringwaldt et al., 2023). We emphasize the marginal impact associated with fresh water in this study and thus are careful not to over

interpret this result. Nonetheless, it is possible the positive relationship we found between distance to freshwater and risk of sarcoptic mange may reflect preferential selection of small water bodies, not captured by our GIS layers, (e.g., small ponds, high order streams, water sources down burrows), by wombats across our study area. If this is the case, it could hold that wombats may preferentially seek to inhabit areas with smaller water sources and therefore experience greater pathogen exposure at such sites. Further research on wombat occurrence in relation to water sources would be valuable for exploring this hypothesis.

Contrasting landscape epidemiology studies of mange in wombats presented an opportunity for a more general understanding of environmental covariates associated with this wildlife disease. A single variable, annual mean precipitation, was a consistent predictor across all three landscape scale studies, with mange risk associated with lower rainfall. This finding indicates a level of confidence in predicting mange risk over landscapes in which bare-nosed wombats occur. We also found some intriguing relationships that may indicate shifting landscape epidemiology from southern to northern areas of the wombat distributional range. We found a positive relationship for mean annual temperature and temperature annual range with mange in Tasmania and Victoria, but negative in NSW. This relationship possibly indicates a thermal performance relationship for mange risk in wombats, whereby the risk relationship goes from positive to neutral to negative along the latitudinal temperature range. Further research potentially combining data sources from Tasmania to northern NSW would be valuable to address this latitudinal switch hypothesis.

While there is some bias around capturing the same wombat at successive time points, which could influence the patterns observed, the study was conducted in remote bushland and wombats were not a target species for the programme. Hence the cameras were not set up to capture high numbers of wombats or in ideal wombat habitat and is unlikely to significantly influence the patterns of mange observed in this study.

CONCLUSIONS

Advancing our understanding of host-parasite landscape epidemiology is important for wildlife, which are typically difficult to study for this context. Our study has advanced understanding of the landscape epidemiology of sarcoptic mange in bare-nosed wombats both within Victoria and across their distributional range. Collectively this helps identify areas most likely to have wombats impacted by mange and can thus support focussed disease management efforts. Our research also identifies some potentially fruitful areas of further research, most notably focussed on how wombats utilise water sources within the landscape, and thermal performance relationships to investigate the mechanistic basis for latitudinally varying predictors of wombat-mange. Additional studies investigating the landscape epidemiology for other visually apparent diseases of wildlife will be valuable for advancing a level of general knowledge for this area of scholarship (Barroso and Palencia, 2025).

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AUTHOR CONTRIBUTIONS

Ellyssia T. Young: data curation, conceptualization, investigation, formal analysis writing – original draft, reviewing and editing. Scott Carver: conceptualisation, supervision, writing – reviewing and editing. Aaron C. Greenville: conceptualisation, supervision, writing – reviewing and editing.

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CHAPTER 6.

GENERAL DISCUSSION



Image credit: Ellyssia Young

CHAPTER 6: GENERAL DISCUSSION

6.1 THESIS OVERVIEW

Sarcoptic mange is of global concern in wildlife, it affects a wide range of mammalian species with variable impacts across species and populations (Escobar et al., 2022). *Sarcoptes scabiei*, the causative agent of sarcoptic mange, causes broadly similar pathology across host species however the relative impact of disease and the distribution of clinical signs across the body can vary (Lewin et al., 2023). There are also marked differences in prevalence and severity between hosts and populations and hence there is fundamental knowledge gaps for individual species, particularly in Australia. At least eleven species of Australian mammals have been documented to be affected by sarcoptic mange (Ruykys et al., 2009, Young et al., 2024, Martin et al., 1998, Speight et al., 2017, McLelland and Youl, 2005, Holz et al., 2011, Hoyte, 1961, Botten et al., 2022, Fraser et al., 2016, Russell et al., 2024, Knox et al., 2025), and of these, bare-nosed wombats and koalas are among the most severely and persistently affected across large geographic areas.

This thesis combined clinical trials, population epidemiology and broad-scale landscape epidemiology to address our overarching aim which was to investigate the impacts of sarcoptic mange as an invasive pathogen on some of the most iconic of hosts that it affects in Australia. To do this I had four core research objectives: firstly, in Chapter 2, I focussed on the individual and local population impacts of an outbreak of sarcoptic mange in koalas in northern Victoria by interrogating detailed wildlife carer records over a five-year time period. In Chapter 3, I tested the pharmacokinetics of topical fluralaner administered to healthy captive koalas with the intention to quantify safety in koalas. In Chapter 4, I investigated the influence of a megafire event on landscape scale mange dynamics in the eastern seaboard of New South Wales. And lastly in Chapter 5, I aimed to advance our understanding of the landscape epidemiology of sarcoptic mange in bare-nosed wombats by undertaking a broadscale camera trap analysis in a population of wombats in a previously undocumented geographic location and incorporating a measure of fire into landscape epidemiology. This research contributes to a growing body of research around sarcoptic mange in some of Australia's iconic host species, as an emerging disease in koalas and as an endemic disease in wombats.

6.2 KEY FINDINGS

I firstly focussed on an outbreak of sarcoptic mange in koalas in northern Victoria utilising detailed long-term wildlife carer records (Chapter 2). Outbreaks of mange in koalas are sporadically reported across Australia (Speight et al., 2017), however the presentation and characteristics of disease are not well documented. The findings from this study suggest that mange can be severe in koalas and mange may be becoming enzootic in this population (Table 6.1). I documented differences in the distribution of mange signs across the body between male and female koalas and discuss how this may relate to both direct and indirect modes of *S. scabiei* transmission. All koalas were documented to have mange signs on their front paws, and most had further signs elsewhere on their limbs and ventral surfaces. However, only males were recorded to have mange signs on their chest and head, and only females had signs of mange on their back. The presence of mange signs on the chest of males is interesting, and may be linked with scent marking behaviour, where males rub their scent gland located on their chest (this occurs throughout the year) (Smith, 1980, Mitchell, 1990). There were also consistently higher numbers of males admitted with sarcoptic mange across all years of the study. From these findings, and the strong seasonal patterns of mange affected koalas admitted, particularly around the mating season (September/October (September/October, McLean and Handasyde, 2006)), I conclude that males may be important drivers of mange dynamics in koalas in this population. The koalas in this study displayed less alopecia than in other species, and this may have direct implications for mange detection, monitoring, and management programs. Since mange signs in koalas are somewhat less obvious, and along with their arboreal nature, detection of mange affected koalas may not be early enough to intercept disease progression in many cases, and therefore koalas may be less likely to come into care at an infection stage which is ethical to treat.

There is currently no safe and effective treatment suitable for use for sarcoptic mange in free ranging koalas. The current treatments often require follow up treatments and have varying efficacy (Speight et al., 2017, Brown et al., 1982). Fluralaner is the treatment that has recently been adopted for use against sarcoptic mange in bare-nosed wombats with a high safety margin across a wide range of species (Gassel et al., 2014, Walther et al., 2014, Prohaczik et al., 2017, Fisara et al., 2018, Van Wick and Hashem, 2019, Wilkinson et al., 2021). I undertook a study investigating the safety

and pharmacokinetics of fluralaner in non-reproductive koalas to provide a knowledge base for wildlife carers and veterinarians to safely and responsibly treat sarcoptic mange in koalas (Chapter 3, Table 6.1). There were no deleterious effects of 85mg/kg transdermal application of fluralaner reported, and fluralaner was detected in the blood plasma at quantities which have been shown to achieve clinical resolution in bare-nosed wombats (Wilkinson et al., 2021). This is essential foundational research for this emerging disease in koalas to inform veterinarians, wildlife carers and land managers for the safe use of chemotherapeutic treatment.

Extending beyond koalas, I then focussed on how changing environmental disturbance regimes can affect wildlife disease (Chapter 4). In Australia, bushfire activity and regimes are changing, but the impact of bushfires on wildlife disease are virtually unknown, although strongly expected (Albery et al., 2021, Nimmo et al., 2021). To tackle this, I investigated how bushfires affect sarcoptic mange in bare-nosed wombats. Two independent lines of wombat mange evidence were combined with remote sensed fire mapping . Wombat data were from the New South Wales National Parks and Wildlife Service long-term remote camera monitoring program, WildCount and Wildlife Information, Rescue and Education (WIRES) wildlife carer admission and rehabilitation records. Much of NSW was heavily affected by the 2019-2020 Black Summer megafires, and the role of fire on the incidence and risk of wildlife pathogens has been suspected but not quantified. We found no evidence to suggest that the occurrence of a megafire influenced the probability of wombats having mange, but there was a transient effect of landscape burn severity, and extent of landscape burn severity, on sarcoptic mange in the year of the megafire, mange was higher where there was more landscape burned at higher severities. I conclude from this study that megafires can have a short-term effect on mange in wombats, and that this is mediated by the extent and severity of the immediate landscape burned (Table 6.1).

Chapter 5 extended beyond the bushfire focus to include other environmental and climatic variables which can influence wombat-mange dynamics. Using Southern Ark camera program data (far-east Victoria, Australia) combined with remotely sensed variables, I found that wombats and wombats with mange were widespread across the study area with a mean mange prevalence of 8%. Random forest modelling identified four key predictors of mange with year being the most important variable. There was a general increase in mange with increasing topographic roughness, higher mange at

lower rainfall areas and higher mange with increasing distance to freshwater sources (Table 6.1). Lastly, by comparing these findings with other landscape epidemiology studies of mange in bare-nosed wombats from Tasmania and NSW, I found that there was a general negative relationship between mange and mean annual rainfall across all three studies (Table 6.1). The relationships between mean annual temperature and temperature annual range both varied with latitude, where positive relationships were documented in Tasmania and Victoria (this research), but negative in NSW (Table 6.1). This potentially indicates a thermal performance relationship for sarcoptic mange risk in wombats, where the risk relationship switched from positive, to neutral, to negative across the latitudinal temperature range.

Table 6.1. Overview of key findings and practical impact from core research chapters

Chapter	Aims/Objectives	Key findings	Practical/Theoretical impact
2	1. Contribute to the understanding of sarcoptic mange in koalas and advance understanding on areas where disease management could be improved.	1. Sarcoptic mange can be a severe disease in koalas 2. Male koalas may play an important role in seasonal transmission dynamics.	Findings may help target intervention strategies
3	1. Establish the pharmacokinetic profile and safety of fluralaner in koalas	1. Fluralaner is a safe and long-lasting chemotherapeutic agent that may be efficacious against <i>S. scabiei</i> in koalas	Provided an evidence base for wildlife carers, land managers and veterinarians in the treatment options for sarcoptic mange in koalas
4	1. Quantify the impact of the megafire event on the occurrence of wombat mange 2. Assess the effect of fire severity extent on mange in the areas burned	1. Wildfire can have short-term effects on wildlife disease, mediated by the extent and severity of the local landscape burned.	Identified that wildfire can have a short-term influence on sarcoptic mange dynamics in wombats which may help inform disease management efforts
5	1. Understand the landscape epidemiology of sarcoptic mange in wombats from far east Victoria, Australia. a) Assess the occurrence of wombats and sarcoptic mange across the study area b) Determine landscape and environmental factors associated with sarcoptic mange c) Contrast findings from this research with other wombat-mange landscape epidemiology studies	1a) Wombats were widespread across study area and mange was widespread as well b) Distance to freshwater, mean annual precipitation and topographic roughness were key drivers of mange in Victoria c) Mean annual precipitation was consistently a key driver of mange across Victoria, New South Wales and Tasmania	Identified key environmental drivers of landscape scale mange dynamics in wombats which can be utilised to identify high risk populations and inform management

6.3 PRACTICAL APPLICATIONS AND MANAGEMENT IMPLICATIONS

This thesis has produced valuable findings which have direct implications for koalas and wombats, and more broadly, other *S. scabiei* hosts. Firstly, from Chapter 2, the presentation of sarcoptic mange in koalas was found to display less alopecia than in other host species. This affects early detection of infected koalas and often results in koalas being identified at advanced stage of infection, and hence beyond the point where they can be ethically or effectively treated. Therefore, targeted proactive mange treatment programs may be more important than relying on reports from members of the public. Seasonal dynamics of mange in koalas was distinct around mating and birthing seasons, which could be used to inform the timing of management programs to intercept and mitigate transmission between individuals in the lead up to the mating and birthing seasons.

The research findings from Chapter 2 emphasised how mange is emerging and severe in koalas, and there is currently no suitable chemotherapeutic treatment option. The rapid onset and extended duration of action of fluralaner in koalas documented in Chapter 3, suggests that this could be a useful chemotherapeutic option for proactive mange control programs for free-ranging koalas (with consideration of the risk of environmental contamination and impacts on non-target animals (Diepens et al., 2023)), and reactive for cases of earlier-stage disease, hence avoiding the need to bring animals into care or rely on reports of koalas at severe stages of infection. This has potential for direct positive impacts on the health and welfare of koalas in areas that are affected by sarcoptic mange.

The frequency and severity of wildfires is predicted to increase, as well as the incidence of megafires (Nimmo et al., 2021). The influence of wildfires and megafires on wildlife disease has received very little research (Albery et al., 2021). Findings from Chapter 4 suggests that the presence and extent of higher severity fire can have short term effects on wombat mange dynamics, where mange transiently increased post-fire. If fire extent and severity occur more frequently this could potentially have a greater influence on sarcoptic mange incidence. This knowledge could be used by public land managers when undertaking fuel reduction burning or bushfire suppression for targeted mange control programs in high-risk areas. Landowners, wildlife shelters

and wildlife protection organisations may also use this to target the timing of mange control programs.

Lastly, findings from Chapter 5 identified key environmental drivers of landscape scale mange dynamics in wombats and emphasised how the fundamental drivers of mange can vary greatly across populations, habitats and latitudes. Our study population in Victoria identified that sub-populations in areas with higher topographic roughness, increased distance to freshwater and lower rainfall were associated with increased occurrence of mange, although these were only moderate relationships. Mean annual precipitation was consistently a key driver of mange across all three studies, Victoria, New South Wales and Tasmania. This research can be utilised to identify high risk wombat populations across their distribution and inform the treatment programs to better manage the welfare and conservation implications of this invasive pathogen.

This research was able to utilise broadscale remote camera trapping programs to address questions around landscape epidemiology of sarcoptic mange and in the influence of disturbance events such as bushfires. There is a growing body of national and international networks of camera traps which focus mainly on ecological questions but could also be of benefit to wildlife disease monitoring and research.

6.4 FUTURE DIRECTIONS

Several key areas of research have emerged out of this PhD that warrant particular attention.

Clinical presentation and epidemiology of sarcoptic mange in koalas

From Chapter 2, I identified that koalas display less visually apparent clinical signs associated with mange infection and along with their arboreal and reclusive nature, this may pose challenges for assessing koalas for mange management programs. This warrants further research to improve detection and interception of mange infection for targeted disease mitigation. This may be through using wildlife carer records to identify areas where camera trap networks may be of use to monitor disease and further research into landscape epidemiology to identify the environmental variables associated with mange dynamics and target high risk areas and populations

prior to when we documented peak mange admissions. Lastly there is a need to advance our understanding of the clinical progression of mange in koalas, the amount of time it takes to develop different mange severities and identify the stages of disease severity which can be ethically and effectively treated.

Therapeutic assessment of fluralaner

In this thesis I quantified the safety and pharmacokinetics of fluralaner in healthy koalas which demonstrated that fluralaner should be efficacious against sarcoptic mange (Chapter 3). Further research could look at including koalas at varying reproductive stages, juvenile and maternally dependent individuals, and include quantifying the absorption and distribution of fluralaner into other tissues, particularly adipose tissue, to ensure a more comprehensive understanding of the safety and pharmacokinetics of fluralaner in koalas. Due to the study koalas being healthy individuals for safety analysis, I could not quantify clinical resolution. While it can be relatively safely assumed from this study and previous studies in bare-nosed wombats, that the circulating plasma fluralaner levels should be efficacious against *S. scabiei* infection in koalas, further research into ability of fluralaner to achieve clinical resolution in koalas, in both captive and field settings, including the treatment across a range of disease severities, would be highly valuable.

Fire and wildlife disease

We found that fire presence and severity can have some short-term influence on mange dynamics in wombats (Chapter 4). Further research investigating how local fire extent and severity influences wombat spatial ecology, foraging ecology and burrow usage would be of benefit to advance our understanding around the underlying mechanisms associated with this short-term increase in sarcoptic mange observed following fire.

Landscape epidemiology of wombat-mange

The latitudinal switch hypothesis identified in Chapter 5 warrants further investigation, which may include combining data sources from the three landscape epidemiology studies, (Tasmania, Victoria and NSW), to gain a more complete understanding. We suggested that a longer mite survival time for *S. scabiei* mites in the environment in cooler climates may be a mechanistic explanation for the relationship observed,

however, further research exploring these relationships would be of benefit, including the influence of water sources on wombat and wombat mange occurrence.

6.5 THESIS CONCLUSIONS

Firstly this thesis has advanced our understanding of the individual and population level impacts of sarcoptic mange in koalas including the clinical presentation, seasonality, transmission dynamics, prevalence of mange and interannual variation in sarcoptic mange patterns. Secondly, has identified fluralaner as a novel treatment and documented that it is safe and may be of use for sarcoptic mange treatment in koalas. Thirdly, has identified that fire can have short term impacts on sarcoptic mange occurrence in wombats, which is mediated by the extent and severity of the local landscape burned and fourthly, has advanced our understanding of the landscape epidemiology of sarcoptic mange in wombats on landscape scale and across latitudes. The findings of this thesis have direct implications and applications for sarcoptic mange management and contribute to our broader understanding of an invasive visual pathogen on iconic Australian host species, which has global significance.

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APPENDICIES

APPENDIX A: Chapter 3 supplementary material

S3.1 ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY (UPLC-MS/MS)

Sample preparation:

Plasma samples (200 µl) were extracted with 780 µl of Methanol:Acetone (1:1, v/v) to which 20 µl of Surrogate Standard ($^{13}\text{C}_4, ^2\text{H}_3$ -Fluralaner; AlsaChim, France) solution in Methanol was added at a concentration of 2.08 µg/ml (Table 5). The extraction mix was vortexed for 30 sec and placed in a freezer (-20°C) for 60 min to allow protein precipitation. Extracts were then centrifuged at 15,000 rpm for 12 min at 4°C, and the supernatant transferred to a LC vial for analysis.

Analysis:

The UPLC instrument was a Waters Acquity H-class UPLC system (Waters Corporation, Milford, MA). Chromatography was performed using an Acquity BEH C18 VanGuard pre-column (5.0 x 2.1 mm, 1.7 µm) and an Aquity BEH C18 column (2.1 x 100 mm x 1.7 µm) (Waters Corporation). The UPLC was operated with a mobile phase consisting of 0.1% (v/v) Formic acid (Solvent A) and Acetonitrile (Solvent B). Elution was using a gradient. Initial conditions were 20% B before a gradient to 95% B over 4 min, which was held for 2 min. The system was returned to initial conditions at 6.5 min and re-equilibrated for 3 min. The flow rate was 0.35 ml/min and the column was held at 45°C. Injection volume was 2 µl.

The UPLC was coupled to a Waters Xevo TQ triple quadrupole mass spectrometer (Waters Corporation). Analyses were undertaken using multiple reaction monitoring (MRM) in negative electrospray ionisation mode, with 2 MRM Transitions monitored for Fluralaner and the Surrogate Standard, respectively. Electrospray ionisation was performed with a capillary voltage of 2.5 kV, and individual cone voltages and collision energies for each MRM transition, as described below. The desolvation temperature was 450°C, nebulising gas was nitrogen at 950 l/h and cone gas was nitrogen at 100 l/h. MRM transition dwell times were 120 msec.

Table S3.1. UPLC-MS/MS multiple reaction monitoring conditions employed for the analysis of fluralaner, and stable isotope labelled surrogate standard.

Analyte	Precursor [M-H] ⁻ (m/z)	Product [M-H] ⁻ (m/z)	Cone Voltage (V)	Collision Energy (V)
Fluralaner 1	554.0	534.0	44	17
Fluralaner 2	554.0	494.0	44	23
¹³ C ₄ , ² H ₃ – Fluralaner 1	561.0	501.0	44	17
¹³ C ₄ , ² H ₃ – Fluralaner 2	561.0	541.0	44	23

S3.2 CLINICAL PATHOLOGY TIME AND FLURALANER CONCENTRATION PLOTS

See Figures S3.1-3.7.

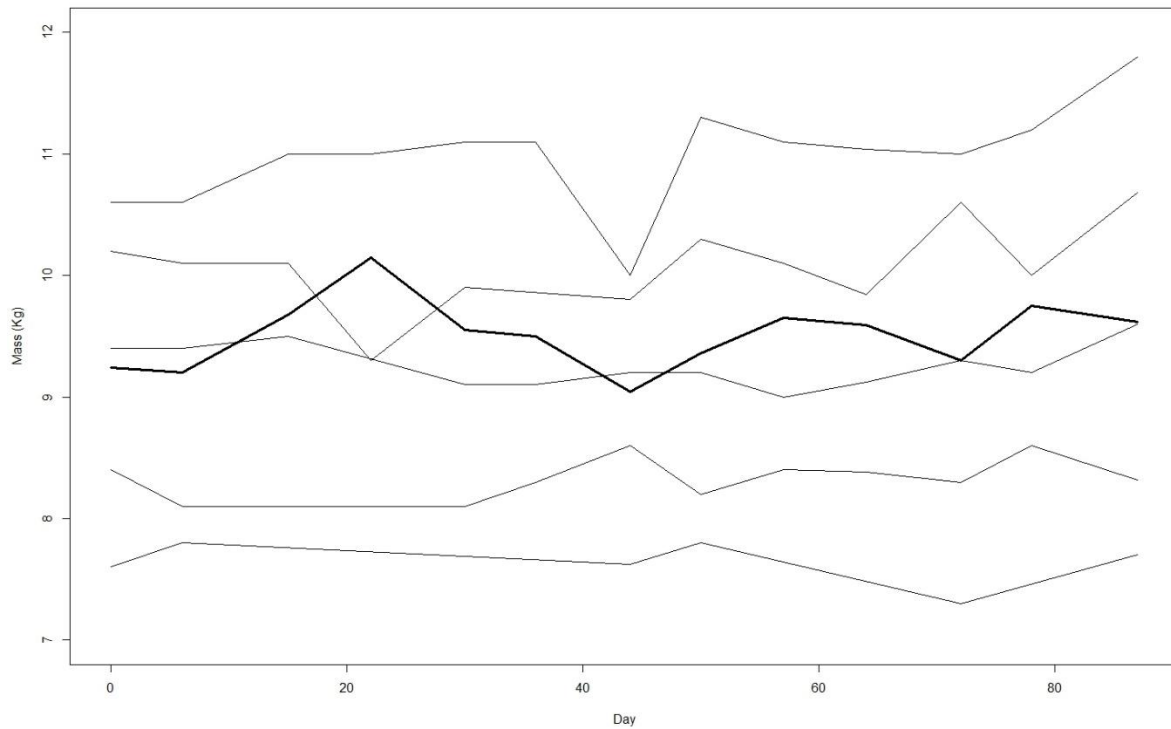


Figure S3.1. Body mass of koalas (*Phascolarctos cinereus*) (n=5) over time (day) involved in this study and located at Phillips Island Nature Park, Victoria, Australia. Thick black line represents the mean, and the thin black lines represent individual koalas.

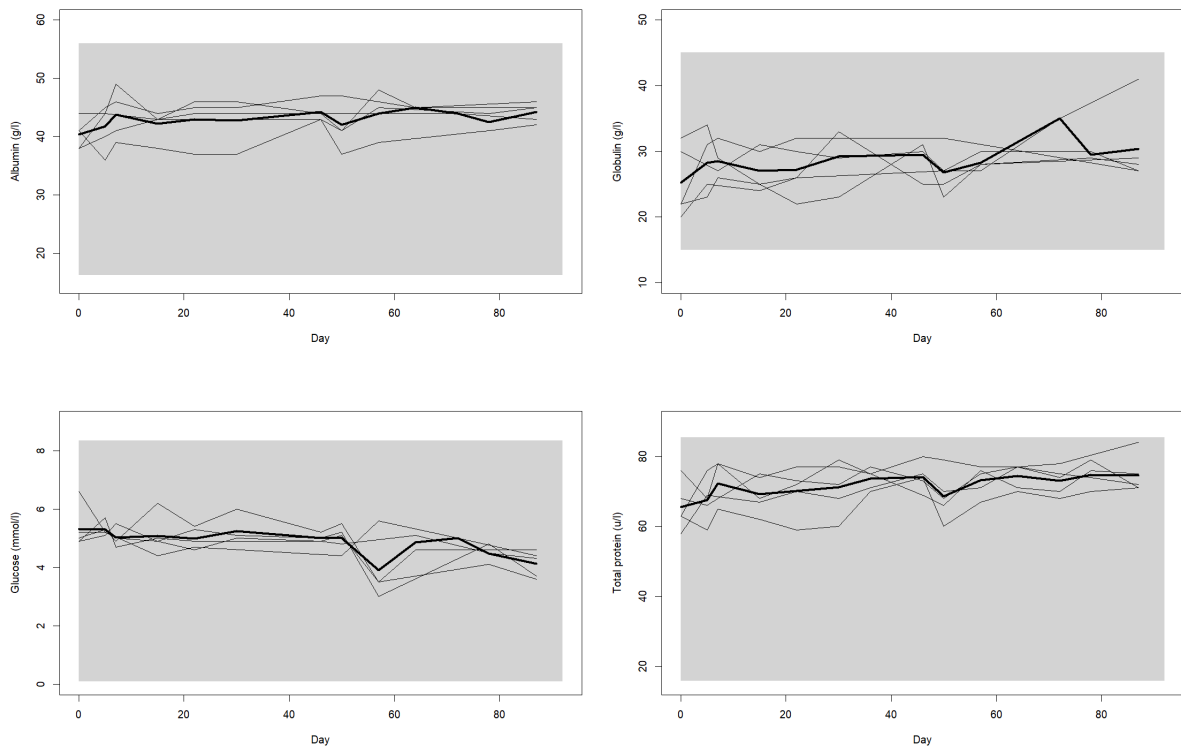


Figure S3.2. Observed values of albumin, globulin, glucose and total protein over time (day). The thick black line is the mean, the thin black lines represent individual koalas (*Phascolarctos cinereus*) (n=5) involved in this study and located at Phillip Island Nature Park, Victoria, Australia, and grey polygon indicates published reference ranges for koalas. (Canfield et al., 1989, Speight et al., 2014, Pye et al., 2012, Species360 Zoological Information Management System (ZIMS), 2022).

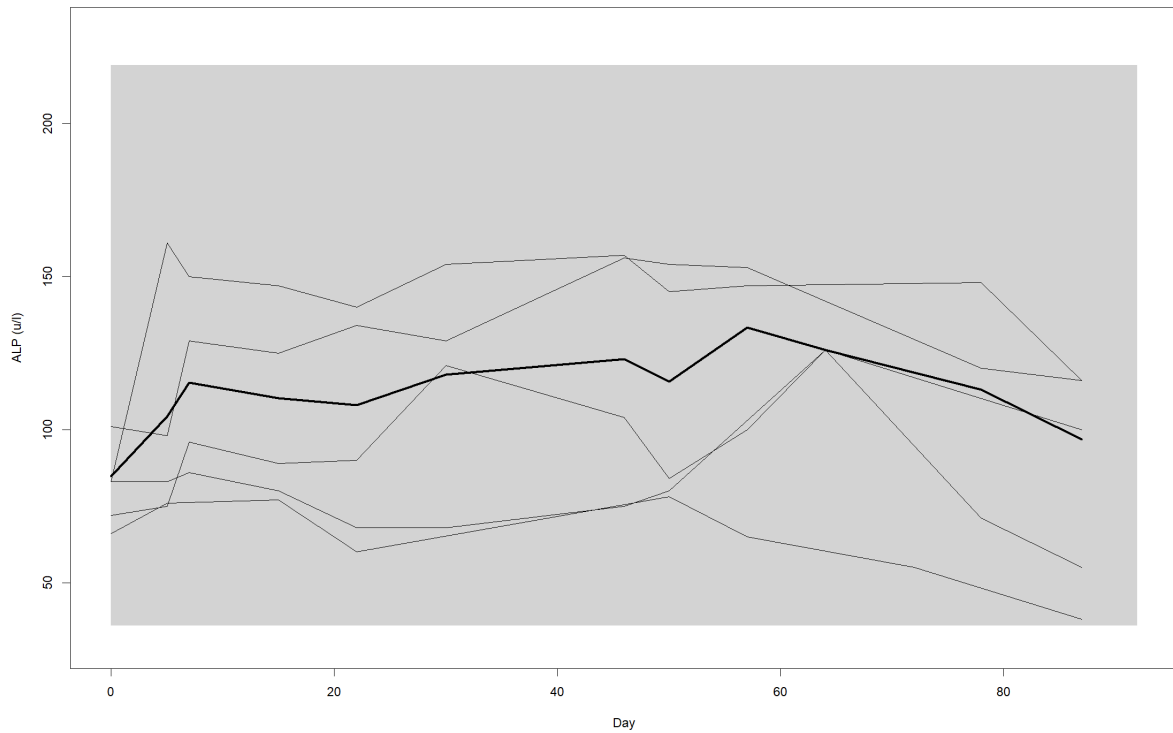


Figure S3.3. Observed values of alkaline phosphatase (ALP) over time (day). The thick black line is the mean, the thin black lines represent individual koalas (*Phascolarctos cinereus*) (n=5) involved in this study and located at Phillip Island Nature Park, Victoria, Australia, and grey polygon indicates published reference ranges for koalas. (Canfield et al., 1989, Speight et al., 2014, Pye et al., 2012, Species360 Zoological Information Management System (ZIMS), 2022).

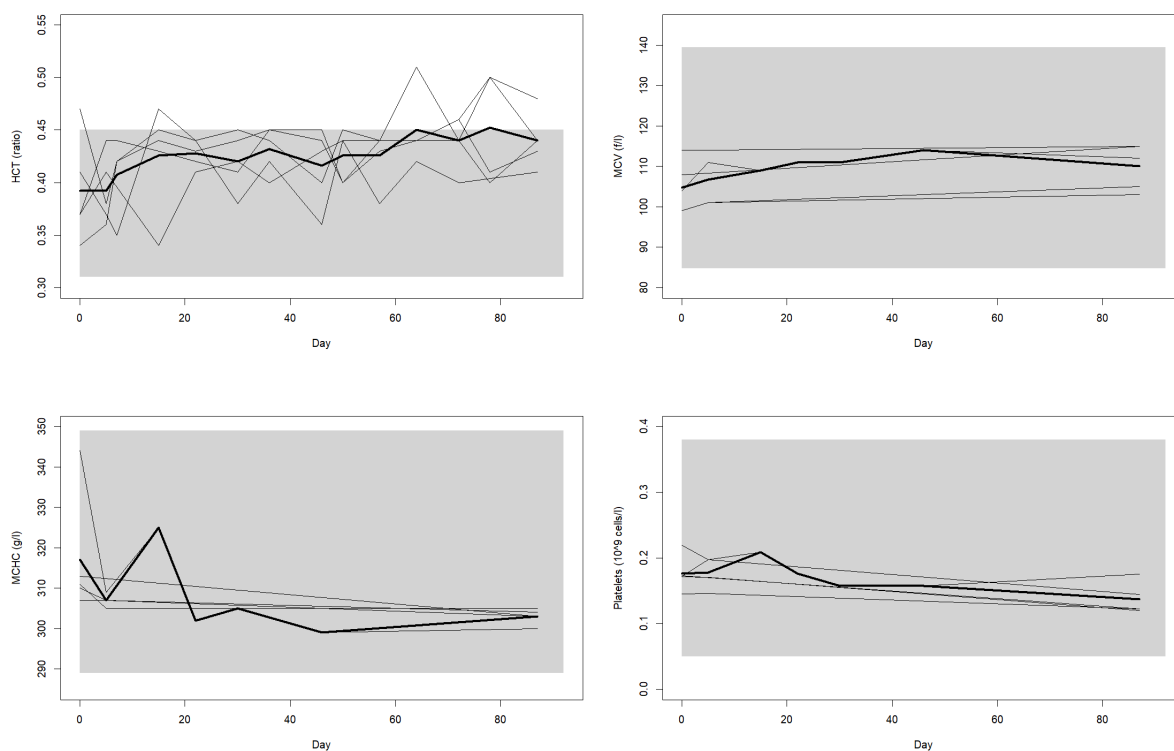


Figure S3.4. Observed values of haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and platelets over time (day). The thick black line is the mean, the thin black lines represent individual koalas (*Phascolarctos cinereus*) (n=5) involved in this study and located at Phillip Island Nature Park, Victoria, Australia, and grey polygon indicates published reference ranges for koalas. (Canfield et al., 1989, Speight et al., 2014, Pye et al., 2012, Species360 Zoological Information Management System (ZIMS), 2022).

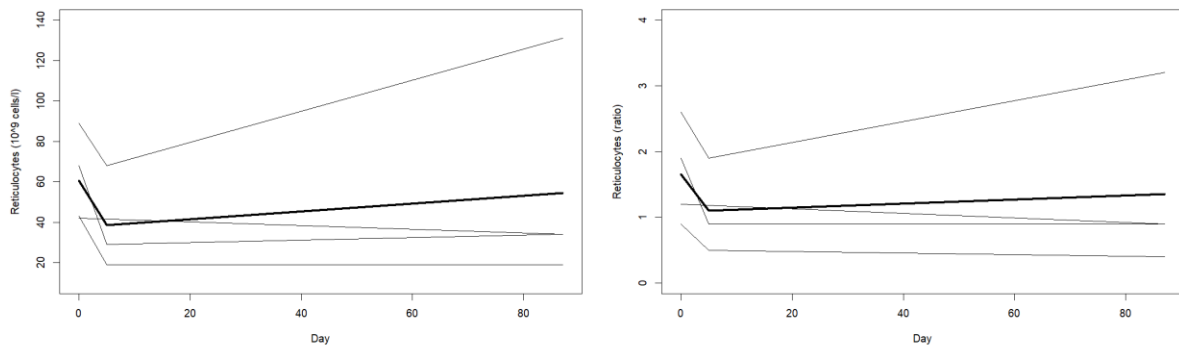


Figure S3.5. Observed values of reticulocytes and reticulocytes ratio over time (day). The thick black line is the mean, the thin black lines represent individual koalas (*Phascolarctos cinereus*) (n=5) involved in this study and located at Phillip Island Nature Park, Victoria, Australia. Reference range information was not available for these parameters. (Canfield et al., 1989, Speight et al., 2014, Pye et al., 2012, Species360 Zoological Information Management System (ZIMS), 2022).

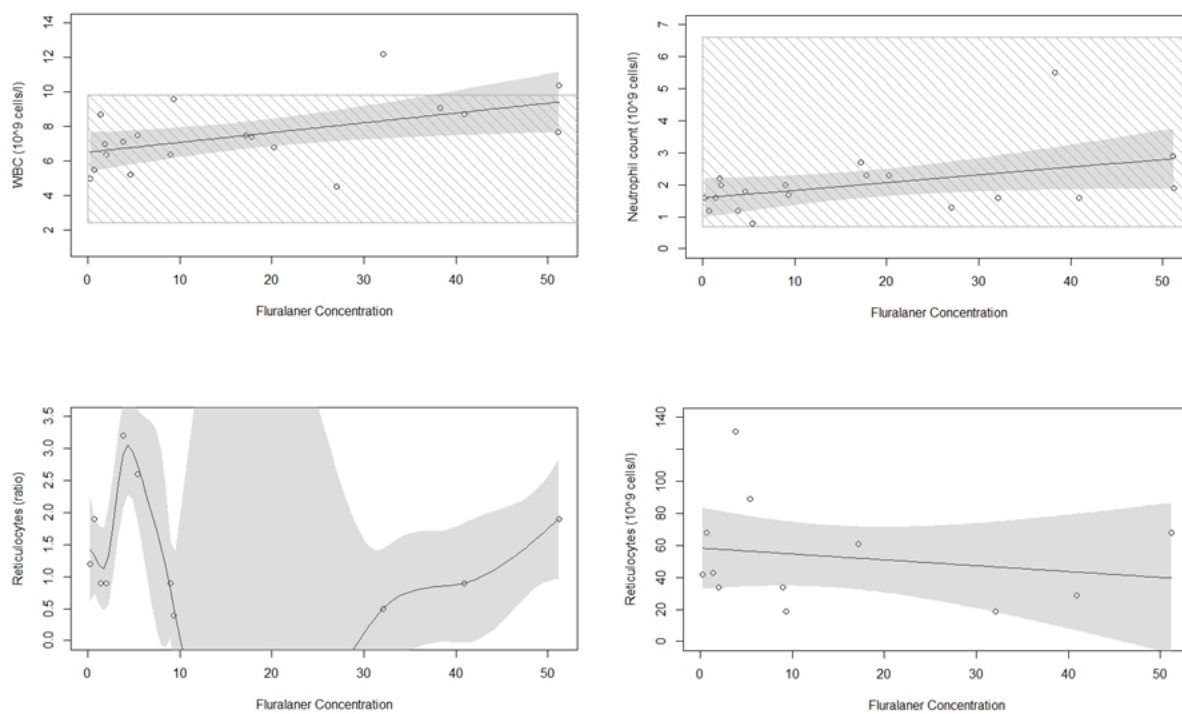


Figure S3.6. Observed values of white blood cell count (WBC), neutrophil count, reticulocyte ratio, reticulocyte count against fluralaner concentration for all koalas (*Phascolarctos cinereus*) (n=5) involved in this study and located at Phillip Island Nature Park, Victoria, Australia. Black line is the predicted trend line, grey polygon represents the standard deviation, and grey hashed polygon represents published reference ranges. Reference range information was not available for reticulocytes and reticulocytes ratio parameters. (Canfield et al., 1989, Speight et al., 2014, Pye et al., 2012, Species360 Zoological Information Management System (ZIMS), 2022).

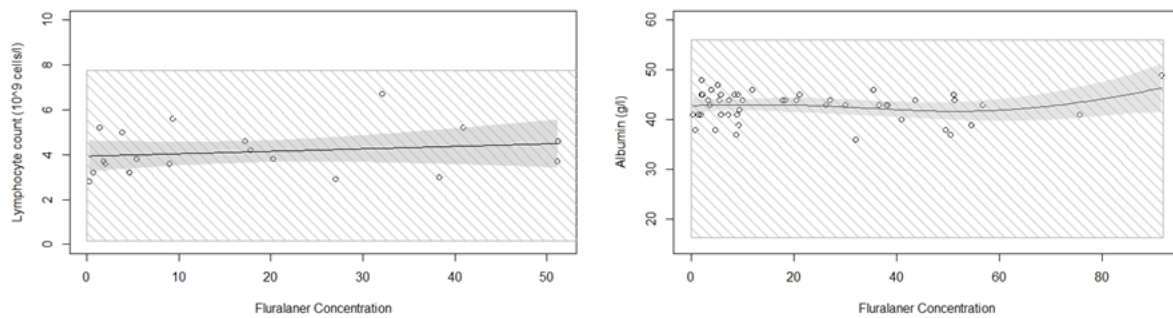


Figure S3.7. Observed values of lymphocyte count and albumin against fluralaner concentration for all koalas (*Phascolarctos cinereus*) (n=5) involved in this study and located at Phillip Island Nature Park, Victoria, Australia. Black line is the predicted trend line, grey polygon represents the standard deviation, and grey hashed polygon represents published reference ranges. (Species360 Zoological Information Management System (ZIMS), 2022, Fabijan et al., 2020, Pye et al., 2012).

APPENDIX B: Chapter 5 supplementary material

Table S5.1. Summary table of environment and climate variables used for randomForest models, including data source and extraction methodology. Variables were included due to their documented influence on sarcoptic mange (caused by *Sarcoptes scabiei*) and bare-nosed wombats (*Vombatus ursinus*).

Predictor variables	Data source	Description
<p>Annual Mean Temperature</p> <p>Temperature Annual Range</p>	<p>Australia, Current Climate (1976-2005), 30 arcsec (~1km)</p> <p>Accessed via EcoCommons 20 July 2025 Vanderwal, Jeremy. (2012). All future climate layers for Australia - 5km resolution. James Cook University.</p> <p>https://data-explorer.app.ecocommons.org.au/?tab=eco-data&facetCollection=Australia+current+and+future+climate+data&facetTimeDomain=Current&facetMonth=Non+monthly+data&pageSize=10&pageStart=0</p>	<p>Bioclimatic variables</p> <p>Annual mean temperature (°C) and temperature annual range (the difference between the warmest and the coldest month) was sourced from Australia, Current Climate (1976-2005) layer from EcoCommons. Extract multi values to points tool in ArcGIS Pro version 3.1.0 (ESRI, 2023) was used to extract each climate variable for each camera site.</p>
<p>Annual Precipitation</p> <p>Seasonal Precipitation (Coefficient of Variation)</p>	<p>Australia, Current Climate (1976-2005), 30 arcsec (~1km)</p> <p>Accessed via EcoCommons 20 July 2025 Vanderwal, Jeremy. (2012). All future climate layers for Australia - 5km resolution. James Cook University.</p> <p>https://data-explorer.app.ecocommons.org.au/?tab=eco-</p>	<p>Bioclimatic variables</p> <p>Annual precipitation (mm) and seasonal precipitation (coefficient of variation) was sourced from Australia, Current Climate (1976-2005) layer from EcoCommons. Seasonal precipitation is a measure of the variability of rainfall over a season and is calculated by dividing the standard deviation of seasonal precipitation by its mean, then multiplying by 100. Extract multi values to points tool in ArcGIS Pro version 3.1.0 (ESRI, 2023) was used to extract each climate variable for each camera site.</p>

	data&facetCollection=Australia+current+and+future+climate+data&facetTimeDomain=Current&facetMonth=November+monthly+data&pageSize=10&pageStart=0	
Land use Distance to farmland	ABARES 2021, Catchment Scale Land Use of Australia – Update December 2020, Australian Bureau of Agricultural and Resource Economics and Sciences, Canberra, February, CC BY 4.0, DOI: 10.25814/aqjw-rq15	<p>Land Use</p> <p>Land use data was sourced from the Australian Bureau of Agricultural and Resource Economics and Sciences. Land use was extracted from camera point data and also percentage of vegetation type in a predicted 5ha home range (5ha buffer polygon around camera points) (Evans, 2008) using ArcGIS Pro version 3.1.0 (ESRI, 2023). We compared the two extracted data and decided to simplify the number of variables, land use point data could capture the variation of land use across the study sites, and therefore point data was used for land use type in this study. Zonal histogram function in ArcGIS Pro version 3.1.0 (ESRI, 2023) was used to extract the number of pixels from each polygon. Extract multi values to points tool was then used to extract the land use at the camera sites and combine with camera site data.</p> <p>Distance to modified pasture</p> <p>To determine the distance to modified pasture, we extracted the grazing modified pasture feature from the land use layer in ArcGIS Pro version 3.1.0 (ESRI, 2023) sourced from Australian Bureau of Agricultural and Resource Economics and Sciences. The spatial analyst distance accumulation tool in ArcGIS Pro version 3.1.0 (ESRI, 2023) was used to determine the straight-line distance from the camera location to the closest point of the grazing modified pasture polygon. Extract values to points tool was then utilised to get the distance values for each camera site and join to camera site data.</p>

<p>Vegetation</p>	<p>National Vegetation Information System (NVIS) Version 7.0 Major Vegetation Groups. https://www.dcceew.gov.au/environment/environment-information-australia/national-vegetation-information-system/data-products</p>	<p>Vegetation</p> <p>Vegetation type was sourced from the national vegetation information system data base. Vegetation type was collapsed from major groups into functional groups for the purposes of this study which were: forests, rainforests, shrublands and heathlands. Vegetation class was extracted from camera point data and also percentage of vegetation type in a predicted 5ha home range (5ha buffer around camera points) (Evans, 2008) using ArcGIS Pro version 3.1.0 (ESRI, 2023). Ie compared the two extracted data and decided to simplify the number of variables, vegetation point data could capture the variation of vegetation across the study sites, and therefore point data was used for vegetation type in this study.</p>
<p>Elevation</p> <p>Topographic roughness</p>	<p>Geoscience Australia (2008) GEODATA 9 second DEM and D8: Digital Elevation Model Version 3 and Flow Direction Grid 2008. Bioregional Assessment Source Dataset. Viewed 25 July 2025, http://data.bioregionalassessments.gov.au/dataset/ebcf6ca2-513a-4ec7-9323-73508c5d7b93</p>	<p>Elevation</p> <p>The Digital Elevation Model sourced from GeoScience Australia was used to generate elevation values. Extract multi values to points tool in ArcGIS Pro version 3.1.0 (ESRI, 2023) was used to get the elevation of each camera site.</p> <p>Terrain roughness</p> <p>The Digital Elevation Model from GeoScience Australia was utilised to calculate terrain roughness (which is the difference between the maximum and the minimum value of a cell and its 8 surrounding cells). The terrain function in the Raster package in RStudio (Hijmans, 2023) was utilised to calculate roughness. The extract multi values to points tool in ArcGIS Pro version 3.1.0 (ESRI, 2023) was then used to extract the roughness values for each camera site.</p>
<p>Distance to freshwater</p>	<p>DEECA 2025, Vicmap Hydro. Department of Energy, Environment and</p>	<p>Distance to freshwater</p> <p>Watercourse polylines and waterpoint data was sourced from Vic Datashare. The watercourse</p>

	<p>Climate Action. HY_WATER_POINT and HY_WATERCOURSE layers. Published 18/07/2025 https://datashare.maps.vic.gov.au/search?q=uuid%3D23a264f2-d5aa-5b73-95a2-812454053147</p>	<p>layer was manually edited to reflect permanent water visually based of world topographic map. Waterpoint data consisted of farm dams and fire waterpoints on public land. The Near tool in ArcGIS Pro version 3.1.0 (ESRI, 2023) (with Geodesic specified) was used simultaneously with both water layers to calculate the straight-line distance between the camera site and the permanent source and the distance to the nearest permanent water source was recorded.</p>
Time since fire	<p>DEECA 2025, Fire History Records of Fires across Victoria. Department of Energy, Environment and Climate Action. FIRE_HISTORY layer Published 15/1/2025 https://datashare.maps.vic.gov.au/search?q=uuid%3Dd817f3be-a7a2-5847-9944-c0f2fdb39444</p>	<p>Fire history To generate time since fire we utilised the Fire history frequency layer sourced from Vic datashare portal. With this layer we pooled entries into 4 categories based on time since fire (including both prescribed fire and wildfire). These categories were years since fire: 1 (1-4 years), 2 (5-9), 3 (10-19), 4 (20+) and 5 (no data or no known recorded fires). We then used spatial join function in ArcGIS Pro version 3.1.0 (ESRI, 2023) to amalgamate this fire information with each camera site.</p>

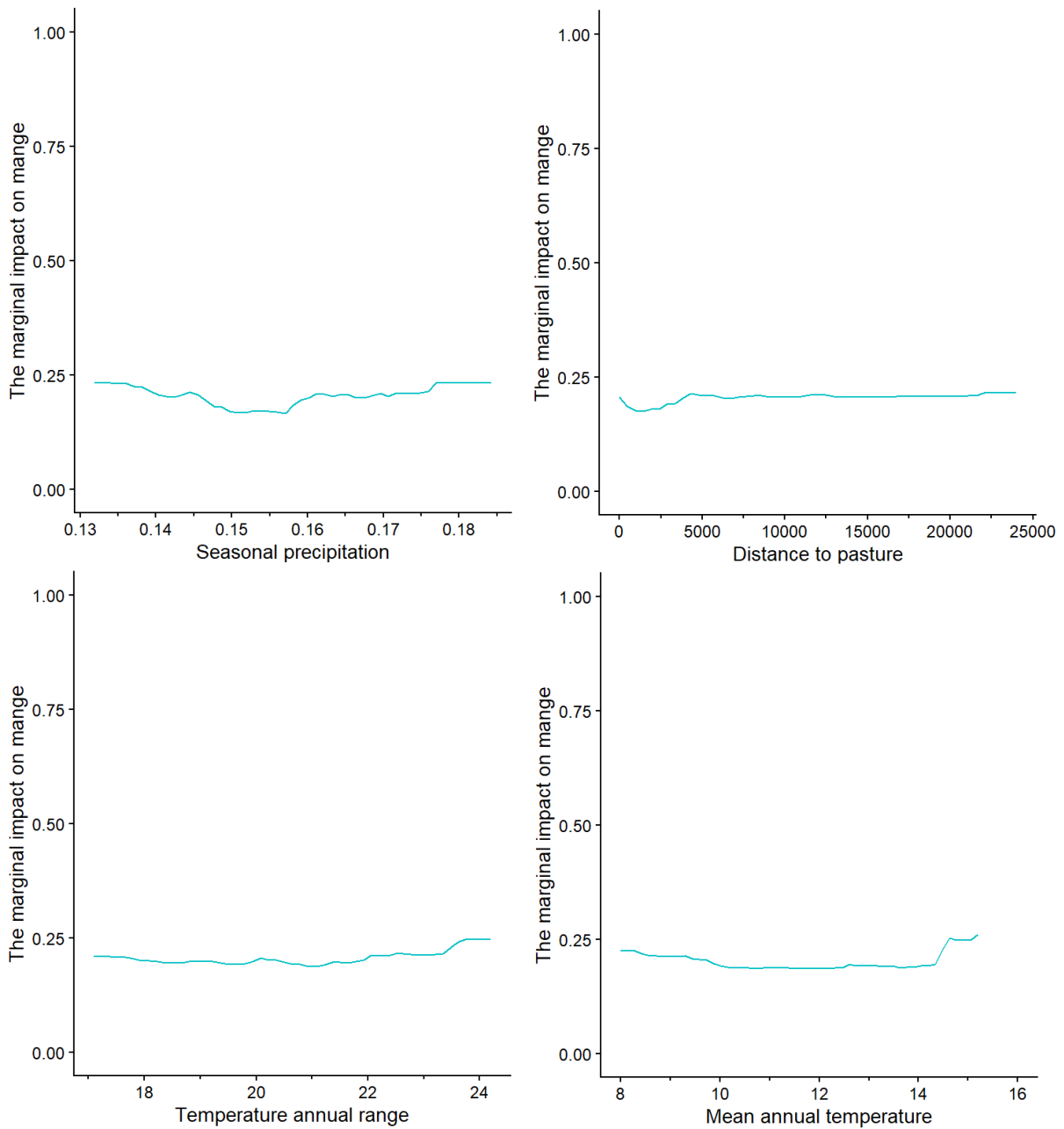


Figure S5.1. Partial dependence plots showing the effects of seasonal precipitation, distance to pasture, temperature annual range and mean annual temperature which had smaller contributions to the variation in mange patterns predicted by the randomForest model.

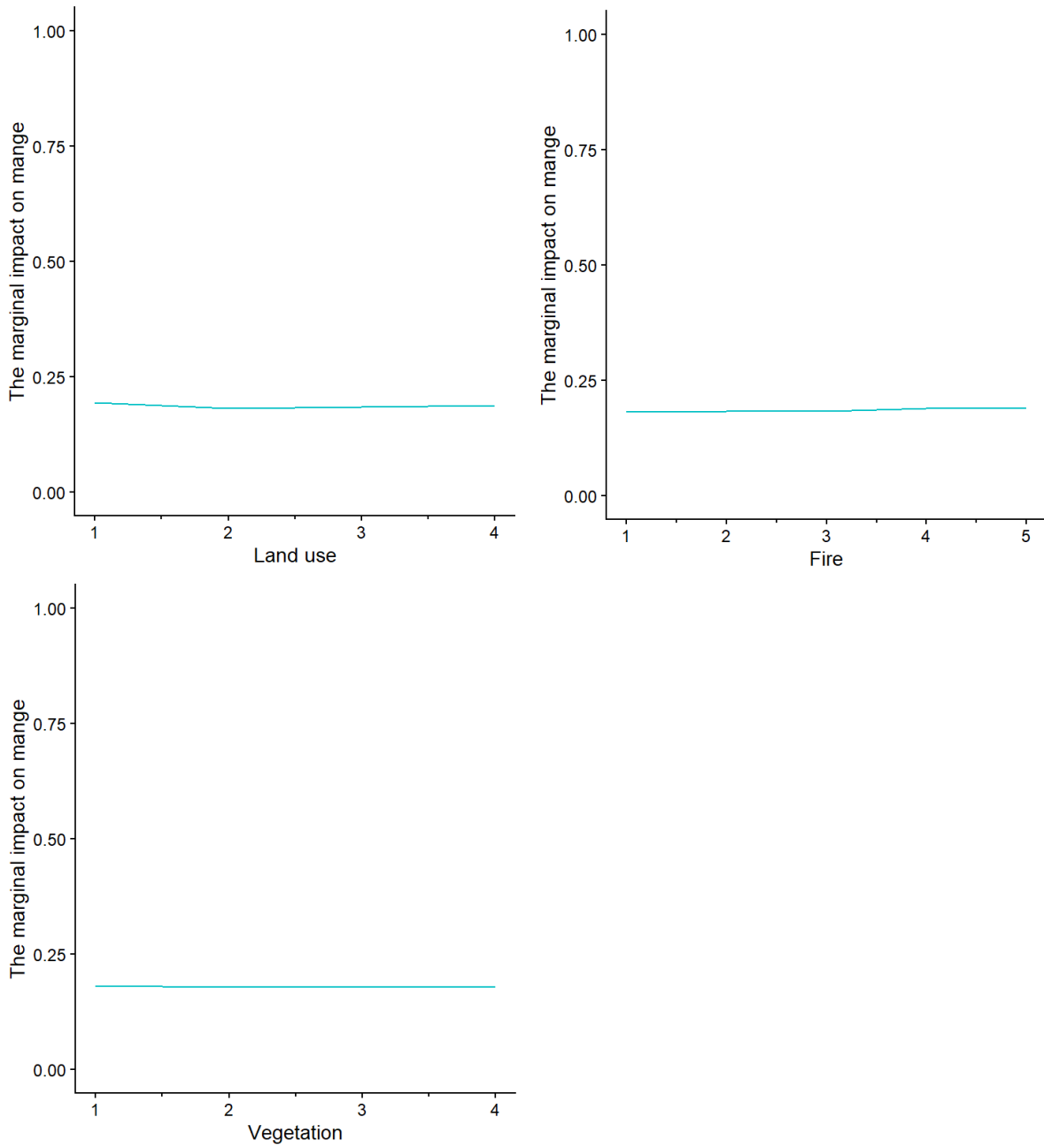


Figure S5.2. Partial dependence plots showing the effects of land use, fire, and vegetation which had smaller contributions to the variation in mangle patterns predicted by the randomForest model.

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