

The effects of the 2019–2020 megafires on koalas and the nutritional quality of their habitat



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A thesis submitted for the degree of Doctor of Philosophy of the
Australian National University

Dedication

I dedicate this thesis to my mother Anne and my late father Murray.
Thank you both for giving me every opportunity in the world to achieve
this.

*“Those who contemplate the beauty of the earth find reserves of
strength that will endure as long as life lasts” – Rachel Carson, Silent
Spring*

Declaration

I, Murraya Lane, declare that this thesis, in fulfillment of the requirements for the award of Doctor of Philosophy, in the Research School of Biology at the Australian National University, is my own work unless otherwise referenced or acknowledged.

This work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution.

This thesis contains four data chapters that have been written as independent research articles, two of which have been published in peer-reviewed journals and another under review. Authorship of all chapters was therefore collaborative, which is reflected in the writing style. The conceptual development, planning and execution of each chapter was my own responsibility as a post-graduate student of the Australian National University. I was guided and mentored by my supervisory panel. I also acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Stipend Scholarship.

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Ethics

This research was approved by the NSW Government Department of Planning, Industry and Environment Animal Ethics Committee (permit number 200421/04) and was conducted in accordance with the Australian Code for the care and use of animals for scientific purposes.

COVID-19 Impact Statement

I began my PhD on 12 February 2020. On 11 March 2020 the World Health Organisation (WHO) declared the novel coronavirus (COVID-19) a worldwide pandemic. The State governments of The Australian Capital Territory (ACT), where the Australian National University (ANU) is located, and New South Wales, where my field sites were located, declared a public health emergency on 16 March 2020. In response to this, ANU campus was closed from March to July 2020 and again from August to November 2021. There were also government-imposed lockdowns and restrictions on travel during these periods. COVID-19 caused unavoidable disruptions to my research and many other Higher Degree by Research (HDR) students. This is reflected in the design and timing of some of my data collection. For example, I could not commence fieldwork sooner post fire, and the lockdowns from August – October 2021 in the ACT meant that one of my seven rounds of leaf collection for a temporal study was delayed.

Publications and presentations

Throughout this degree, I have contributed to the following:

Journal articles

- Lane, MR, Youngentob KN, Clark RG, and Marsh KJ (2024) The nutritional quality of post-fire eucalypt regrowth and its consumption by koalas in the New South Wales Southern Tablelands. *Australian Journal of Zoology* **71**(3). <https://www.publish.csiro.au/ZO/pdf/ZO23024>
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Conference presentations

- Oral presentation at the 2022 Australian Mammal Society Conference, Perth, WA, Australia
- Oral presentation at the 2022 Ecological Society of Australia Conference, Wollongong, NSW, Australia
- Research School of Biology HDR Student Conference 2021
- Research School of Biology HDR Student Conference 2022
- Research School of Biology HDR Student Conference 2023

Invited presentations

- Oral presentation for Mulligans Flat as part of the 'Paperbark Science Series' (2023)
- Oral presentation for 'Friends of the Australian National Botanic Gardens' (2023)
- Oral presentation for 'Cold Country Koalas' by NSW Government Local Land Services (2022)

Media opportunities

- I have featured in several media releases and articles throughout my PhD:

- ABC News - <https://www.abc.net.au/news/2021-10-30/scientists-witness-extraordinary-resilience-of-koalas/100570494>
- Independent photographer, Michael Weinhardt - <https://michaelweinhardtphotography.com/stories/crises>
- National Public Radio (NPR) - <https://www.npr.org/2020/03/14/810152967/australias-fire-ravaged-forests-are-recovering-ecologists-hope-it-lasts>
- The Canberra Times - <https://www.canberratimes.com.au/story/8423706/koalas-show-some-resilience-in-fire-affected-areas-study/>
- Win News Canberra - [Koalas that survived Black Summer may be under pressure again](#)
- Australian Conservation Foundation - https://www.linkedin.com/posts/australian-conservation-foundation_phd-candidate-murraya-lane-has-all-the-activity-7153625306426232833-nGQK
- Featured in a documentary about the plight of koalas that premiered in cinemas in June 2024 and was part of a panel to answer any questions: <http://www.filmprojects.com.au/the-koalas.html>

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Abstract

Bushfires are a major global ecosystem disturbance, particularly in Australia's fire-prone dry sclerophyll *Eucalyptus* forests. Understanding the impacts of fire on wildlife ecology can facilitate the development of management actions that improve conservation outcomes for threatened species. The 2019–2020 megafires in Australia were extensive, burning thousands of hectares of eucalypt forests and killing and injuring many native animal species, including koalas (*Phascolarctos cinereus*), which rely on eucalypt foliage. Eucalypts can resprout after fires, potentially providing food for koalas, but the effects of fire on food quality and folivore ecology remain poorly understood during the post fire recovery period. There is also a lack of information about whether rehabilitated koalas can successfully reintegrate into burnt landscapes. This thesis addresses these key knowledge gaps. The main themes are: 1) koala health in the period following fire, 2) how fire and rehabilitation affect koala movement patterns over time, and 3) how the nutritional composition and palatability of eucalypt browse are impacted by fire in the longer term.

In chapter one, I investigated the effects of fire and rehabilitation on koala health by measuring haematological and biochemical parameters, chlamydial shedding, and body condition scores of rehabilitated and non-rehabilitated koalas in burnt and unburnt landscapes at several timepoints. Most measurements were within normal ranges and similar between koala groups, demonstrating that living in burnt areas and being in care did not exacerbate disease or negatively impact koala health or body condition.

In chapter two, I GPS-tracked 32 koalas for up to nine months in burnt and unburnt landscapes, measuring home ranges, mean nightly distance, farthest distance moved from release site, and total displacement distance (straight-line

distance from their release location to recapture location). Rehabilitated koalas (individuals who were in care after the fires and released back to their original capture location) moved farther from their release site, had larger displacement distances, and larger home ranges than non-rehabilitated koalas, suggesting that rehabilitation, rather than living in a burnt landscape, influenced their movement patterns.

In chapter three, I conducted a feeding study with four captive koalas to observe their consumption of epicormic growth from eight eucalypt species and measured foliar total and available nitrogen and two types of plant secondary metabolites (PSMs) that deter herbivory. Koalas consumed more epicormic foliage from species within the *Symphyomyrtus* subgenus compared to the *Eucalyptus* subgenus, with *Symphyomyrtus* species also having higher concentrations of nitrogen. These results suggest koalas are more likely to browse from *Symphyomyrtus* species after fire and may acquire more protein from these species.

In chapter four, I sampled foliage from two eucalypt subgenera, *Symphyomyrtus* and *Eucalyptus*, in burnt and unburnt woodland over a year to observe changes in foliar chemistry. Epicormic growth initially had higher concentrations of nitrogen, which decreased over time. Concentrations of formylated phloroglucinol compounds (FPCs; compounds that deter herbivory) were also higher in epicormic growth. This demonstrates that koalas may face a trade-off when eating epicormic leaves.

Collectively, my research on koala health, movements and post-fire changes in nutritional composition shows that burnt forests can be suitable habitat and provide adequate food for rehabilitated and non-rehabilitated koalas. These findings are crucial for developing informed conservation strategies to better protect koalas and

other marsupial folivores in fire-prone landscapes. Policies should recognise that burnt habitat can contain valuable food resources in a relatively short timeframe and that, after animals are rehabilitated, they can be returned to their rescue location even if the area was burnt.

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Introduction

Bushfires – a complex disturbance process

Bushfires are a globally relevant and significant disturbance process in many terrestrial ecosystems (Bowman *et al.* 2009; Keeley *et al.* 2011; Levine 1999). They have the capacity to shape biodiversity and influence the structure, patterns and overall composition of landscape vegetation (Bond and Keeley 2005; Bradstock *et al.* 2012; He *et al.* 2019; Levine 1999; Pausas and Keeley 2009). Fires can have both positive and negative effects on species and ecosystems. On the one hand, many areas with regular fire events have high levels of species richness and diversity (He *et al.* 2019; Kelly and Brotons 2017). Conditions following fire can favour many plant species through increased resource availability and removal of biomass and increased light conditions, with some plants even depending on fire for their growth and development to compete with other species in the environment (Bowman and Murphy 2010). On the other hand, fire can have damaging effects on the environment and the plant and animal species living within it, destroying important habitat and causing mortality and injury to wildlife (Dickman 2021; Levine 1999; Martin *et al.* 2016; Ward *et al.* 2020). The degree to which the ecological impacts of fire are beneficial or detrimental can depend on a number of factors, including the size, frequency and severity of fires. A common theme is that animal and plant species are vulnerable to large and frequent fires (Gawryszewski *et al.* 2020; Williams and Bradstock 2008; Yates *et al.* 2008).

Effects of bushfires on animals and their habitats

Fire can have both direct and indirect effects on wildlife (Nieman *et al.* 2022; Sutherland and Dickman 1999). Although fires can cause direct mortality (Doherty *et*

al. 2015; Santos *et al.* 2022; Tomas *et al.* 2021), many individuals that are not killed by the smoke or flames can survive (Hale *et al.* 2022; Jolly *et al.* 2022). Their response to the post-fire landscape can be highly variable and species- or context-specific. Following fire, canopy and ground cover can be reduced, which may make animals more vulnerable to predators (Doherty *et al.* 2022), remove important shelters or nesting sites (Banks *et al.* 2011; Inions *et al.* 1989) and affect some species' ability to thermoregulate (Cristescu *et al.* 2023). If post-fire resources are limited, there may be increased competition for available resources within and between species (Sutherland and Dickman 1999). However, fire can be beneficial to many species. For example, predatory species often move into burnt areas as their prey species have less shelter after a fire event, and therefore hunting success is improved (Doherty *et al.* 2023; Geary *et al.* 2020; McGregor *et al.* 2014). Similarly, bats were drawn to burnt areas due to the increased availability of insects immediately after fire (Braun de Torrez *et al.* 2018), further demonstrating the immediate positive effects of fire for some species. Fire can also impact the chemical composition and biomass of foliage (Thapa *et al.* 2022; Zhang *et al.* 2015), which can affect the quantity and quality of food available to herbivores in either a positive or negative manner. These examples reiterate the complexity of fire and their effects on wildlife.

Fire in the Australian context

The Australian continent, particularly the south-eastern region, is dominated by dry sclerophyll forests that are incredibly fire-prone (Attiwill and Adams 2013; Cheney 1976; Russell-Smith *et al.* 2007), with hundreds of thousands of square kilometres of land impacted by fires annually (Bradstock *et al.* 2012; Craig *et al.*

2002). The conditions in Australia lend themselves to frequent fire events, with low rainfall over much of the continent, and hot, dry summers in many parts of the country (Attiwill and Adams 2013). Fire has been central to indigenous Australian culture, with a long history of using fire as a land management tool (Bowman 2003; Fletcher *et al.* 2021; Whelan *et al.* 2006). Following European settlement, fire regimes changed (Bond and Keeley 2005; Gill 1975), which created a more homogenised landscape that reduced vegetation complexity (Bowman 2003; Enright and Thomas 2008). This likely contributed to declines and contractions of native plant and animal species (Bowman 2003; Gill 1975). Given how fire-prone much of the Australian continent is, many of the native flora and fauna have developed adaptive strategies that allow them to tolerate fire (Gill 1975; He *et al.* 2019; Whelan *et al.* 2006). It is important to distinguish however, that this tolerance is not to fire itself or to all fire regimes (Whelan *et al.* 2006). For example, certain traits may enable survival or reproduction following a fire event, but this does not mean species can withstand all fire events (Gill 1975).

The 2019–2020 megafires

Australia has experienced numerous extreme fire events throughout its history (Hasson *et al.* 2009), however, the 2019–2020 megafires that burned in south-eastern Australia from August 2019 to March 2020 were unprecedented in scale (Collins *et al.* 2021), impacting over 10 million hectares of land (Gallagher *et al.* 2021; Wintle *et al.* 2020). These fires had profound effects on Australia's native fauna and flora (Gallagher *et al.* 2021; Legge *et al.* 2022a; Legge *et al.* 2022b; Ward *et al.* 2020). The habitat of 832 native vertebrate fauna species was affected by fire, with 70 taxa having more than 30% of their habitat burnt (Ward *et al.* 2020). This

habitat included 7.34 million hectares of eucalypt forests and woodlands (Godfree *et al.* 2021).

Responses of eucalypt forests to fire

The forests and woodlands of Australia are dominated by the *Eucalyptus* genus, which comprises over 900 species (Bayly *et al.* 2013; Noble 1989). Many eucalypt forests are both fire-prone and fire-adapted (Gill and Zylstra 2005; Midgley 2013; Pausas *et al.* 2017; Possingham *et al.* 1995). Consequently, many eucalypt species have developed traits that aid survival in response to high frequency fire (Gill 1975; Mount 1964; Mutch 1970). This includes resprouting strategies that allow them to recover rapidly after fire (Burrows 2002; Burrows *et al.* 2010; Pausas and Keeley 2017). A common strategy of many plant species is basal resprouting whereby new shoots grow from the base of the plant (Pausas and Keeley 2017). However, eucalypts are prolific epicormic resprouters (Burrows 2013), with epicormic buds present in the aerial shoots or stems (Pausas and Keeley 2017). Epicormic buds lay dormant beneath the bark and are activated following a major stress event, such as fire (Pausas and Keeley 2017). Epicormic resprouting has several advantages over basal resprouting, including rapid regeneration of leaf area across the tree, which also provides shelter and protection for other tree species, particularly those sprouting basally (Burrows 2013).

Epicormic growth is characterised by leaves that are often different in morphology from mature canopy foliage. Eucalypts have morphologically and anatomically different juvenile and adult leaf phases (England and Attiwill 2006; Johnson 1926; Potts and Jordan 1994). Eucalypts typically begin their life with juvenile leaves, which are often broad, thin, sessile (i.e., attached directly to the main

stem of the plant), glaucous and horizontally oriented (James and Bell 2001). As the tree matures, it undergoes a phase change known as heteroblasty, where the juvenile leaves gradually give way to adult leaves that are normally thicker, narrower, vertically oriented, and waxy or glossy (Hamilton *et al.* 2011; James and Bell 2001). Once the tree has transitioned, it typically retains the adult leaf form for the remainder of its life, unless it is subjected to a stress event that stimulates epicormic regrowth (Springett 1978). This epicormic growth begins in the juvenile form, regardless of whether a tree is young or mature (Nicolini *et al.* 2003). Thus, in eucalypts, the terminology of juvenile and adult phase leaves refers to the leaf growth form rather than a particular leaf age. The differences in eucalypt growth forms between epicormic and adult phase leaves mean that the effects of fire on food quality for herbivores cannot be predicted simply based on leaf age (i.e., young vs. mature leaves).

Arboreal marsupial folivores

Eucalypt forests provide food and shelter to numerous wildlife species, including many arboreal marsupial folivore species (Tyndale-Biscoe 2005). There are four marsupial folivores that are known to feed extensively on eucalypt foliage. They are the koala (*Phascolarctos cinereus*), the greater glider (*Petauroides spp.*), the common ringtail possum (*Pseudocheirus peregrinus*) and the common brushtail possum (*Trichosurus vulpecula*) (Cork and Catling 1996; Cork and Foley 1991). Eucalypt foliage, like that of many other plants, has relatively low concentrations of essential nutrients (e.g., protein), and high concentrations of several herbivore-deterrent plant secondary metabolites (PSMs) (Au *et al.* 2019; Marsh *et al.* 2017; Marsh *et al.* 2019; Moore *et al.* 2005; Moore *et al.* 2004b). PSMs are chemical

compounds that can act as defences against herbivory, causing a range of physiological effects (Dearing *et al.* 2005; Iason 2005; Iason and Villalba 2006; Wiggins *et al.* 2006). They can act directly as toxins, they can reduce the palatability of food, or they can affect the digestibility of nutrients (DeGabriel *et al.* 2009; Iason 2005; Moore *et al.* 2004c).

Nutritional factors, including the concentrations of PSMs, can have a strong effect on the feeding preferences of marsupial folivores (DeGabriel *et al.* 2009; Lawler *et al.* 1998; Moore and Foley 2000; Moore and Foley 2005; Moore *et al.* 2004c; Scrivener *et al.* 2004). There are a number of PSM groups present in eucalypts that can deter herbivory and limit food intake of marsupial folivores, including tannins (Marsh *et al.* 2003), formylated phloroglucinol compounds (FPCs) (Marsh *et al.* 2007; Moore *et al.* 2005; Moore *et al.* 2004b; Wiggins *et al.* 2006) and unsubstituted B-ring flavanones (UBFs) (Beale *et al.* 2022; Marsh *et al.* 2021). Notably, FPCs have only been detected in species from the eucalypt subgenus *Symphyomyrtus* (common name: 'symphyomyrtle') and UBFs have only been detected in the *Eucalyptus* subgenus (common name: 'monocalypt') (Eschler *et al.* 2000; Goodger *et al.* 2016; Heskes *et al.* 2012; Tucker *et al.* 2010). Tolerance to different PSMs differs among the eucalypt folivores and their preference for particular eucalypt species is a reflection of this as well (Moore *et al.* 2004a). Variations in nutritional quality and tree palatability across landscapes likely contribute to the patchy distribution of arboreal marsupial folivores in Australia's eucalypt forests (Moore *et al.* 2004c).

Fire and food for eucalypt folivores

While there has been a substantial amount of research on herbivore deterrent PSMs in adult-phase eucalypt foliage (Marsh *et al.* 2019; Moore *et al.* 2004c), there is limited research in epicormic growth on the concentration of PSMs and nitrogen (a proxy for protein), and how this compares to adult-phase foliage. Additionally, information is limited on how epicormic foliar chemistry changes over time as leaves mature. While marsupial folivores have been documented in areas with post-fire epicormic regrowth (Anderson *et al.* 2019; Lunney *et al.* 2004; Matthews *et al.* 2007), little is known about the extent to which marsupial folivores can consume this type of foliage.

Young adult-phase eucalypt foliage often contains higher concentrations of PSMs (O'Reilly-Wapstra *et al.* 2007) and is also higher in nitrogen (Marsh and Adams 1995; Song and Liu 2019), which may also be true for epicormic growth. Aside from the trees that retain their canopy, epicormic foliage will be the first type of growth available after fire, and potentially the only source of food in some areas. Therefore, understanding if folivores can consume it and if it is nutritionally adequate, is essential for assessing their ability to survive in burnt habitat. Understanding how fire can affect foliar chemistry will provide important information about the nutritional quality of fire-affected landscapes and help us to understand how fire may influence habitat suitability for marsupial folivores.

Koalas and fire

Koalas were one of many species affected by the 2019–2020 megafires, with estimates of more than 60,000 killed or injured (Van Eeden *et al.* 2020). After fire, koalas are at risk of starvation, dehydration, and infections associated with fire-

related injuries and the effects of smoke inhalation (Cristescu and Frere 2020). Members of the public and wildlife rehabilitators rescued many koalas that were either injured or where there were concerns over habitat suitability in the immediate aftermath of the fires (Cope *et al.* 2024; Lunney *et al.* 2022). Koalas dominated triage units, representing 75% of animals that came into care in Victoria (Parrott *et al.* 2021). Many koalas that were rescued had burn injuries and/or were in poor condition (Dunstan *et al.* 2021). While the health and condition of animals is routinely monitored in care, there is often a lack of post-release monitoring, including for koalas (Guy *et al.* 2013; Lunney *et al.* 2004); therefore, information regarding animal health and welfare after they leave care is limited. Post-release monitoring of rehabilitated koalas is crucial for determining whether they survive and persist in burnt landscapes, and the potential alternative welfare outcomes for koalas if released into unburnt habitat.

Relatively few studies have examined the response of koalas to fire. Prior to my research, post-fire studies on koalas primarily focused on how fire impacts koala abundance (Curtin *et al.* 2001; Lunney *et al.* 2007). While these studies provide valuable insights, it is also crucial to understand how fire may indirectly affect koalas through habitat modification, including nutritional changes to eucalypt chemistry, and how these changes may impact koala landscape use and population health over the long term.

Fire has the potential to exacerbate other major threats, such as disease. Chlamydia is a bacterial disease affecting many koalas, with prevalence ranging from 0 to 90% across populations (Polkinghorne *et al.* 2013; Quigley and Timms 2020). It is considered to be a key threat contributing to population declines (Craig *et al.* 2014; Rhodes *et al.* 2011). Chlamydia and other diseases can be exacerbated by

stress (McAlpine *et al.* 2017; Narayan 2017). Therefore, following fire, koalas may be more susceptible to disease that could also impact their ongoing survival in a burnt landscape.

Landscape use by koalas can be influenced by habitat degradation and fragmentation (McAlpine *et al.* 2004; Rus *et al.* 2021), human activities and traffic (Phillips 2016) and stochastic events such as fire and drought (Seabrook *et al.* 2011). Studies have shown that koala movements, home ranges and foraging behaviours are strongly influenced by habitat quality and the availability of resources (Crowther *et al.* 2022; Davies *et al.* 2013). However, surprisingly little is known about the effects of fire on resource availability and habitat quality for koalas. The effects of fire on the movement patterns of koalas after fire and habitat use have occasionally been documented, showing that koalas can maintain home ranges within burnt areas (Matthews *et al.* 2016).

Following the 2019–2020 megafires, a number of studies, including my own, were initiated to address key knowledge gaps that were needed to deliver more effective conservation strategies and to improve policy and management guidelines for fire response. These studies were undertaken to determine how koalas utilise burnt landscapes months after fire and how fire influences food quality and availability. Given their specialised feeding behaviour (Moore and Foley 2000) and the strong influence of the nutritional landscape on koala occurrence and abundance (Au 2018; Au *et al.* 2019), post-fire nutritional changes to koala habitat could have major impacts on their survival and conservation. This knowledge will also aid management of koalas in care after fire, as it can help determine if and when koalas can be released into burnt landscapes.

Aims of this research

The overall aim of this research was to investigate the effects of wildfire on an arboreal marsupial folivore, the koala, and their habitat, specifically focusing on how fire influences their use of a fire-affected landscape and how it may impact eucalypt foliage, their primary food source. This research provides valuable insights that could improve the management and rehabilitation practices of koalas and other marsupial folivores that are affected by fire, aiding their conservation. Ultimately, observing how fire and rehabilitation affect koala movements following a major wildfire event, and measuring the nutritional quality of these landscapes, will demonstrate whether koalas can live in burnt areas.

Thesis outline

My thesis contains a series of data chapters that explore components of the indirect effects of fire on koalas. These include the impacts on movement, feeding behaviour, browse quality, and how rehabilitation and human intervention affect behaviour and health. Finally, this thesis provides valuable information on an understudied population of koalas in the Southern Tablelands of New South Wales, Australia.

Does the health of koalas differ between a burnt and unburnt environment?

In my first chapter, I examine the impacts of both fire and rehabilitation on koala health by measuring key parameters in both rehabilitated and non-rehabilitated individuals. Given the severity of the 2019–2020 megafires and the poor condition of many koalas that came into care, monitoring their condition and monitoring their reintegration into a burnt landscape after rehabilitation provides valuable information

that can improve the management and rehabilitation practices of wildlife, by determining the viability of releasing koalas into burnt landscapes.

How do koalas move within burnt areas relative to unburnt areas after fire?

My second chapter looks at the effects of fire on koala home ranges and movements, observing individuals living in adjacent burnt and unburnt landscapes. I also investigated how rehabilitation affects movements and home ranges, given that many individuals that were rescued after the fires spent months in care and were released into a physically changed landscape with non-rehabilitated individuals. In this research, I was able to use an experiment design with treatment and control groups to better determine the effects of disturbance (fire and rehabilitation) on movements. Many other movement or tracking studies like this are often opportunistic and are limited in their ability to make these kinds of comparisons. Understanding how koalas move within fire-impacted landscapes provides important information about how animals respond to and recover after a major fire event. In conjunction with the research in my first chapter, this data can better inform the management and care of koalas after fire.

Can koalas feed on epicormic growth?

Epicormic growth is often readily available after fire and may be an important or only source of browse for koalas in post-fire landscapes; however, whether marsupial folivores can feed on this type of regrowth is relatively unknown. For my third chapter, I conducted a feeding trial to observe whether captive koalas would feed on the epicormic growth of different eucalypt species and quantified chemical constituents in the foliage that are known to impact koala feeding. This information

has the capacity to improve the care and rehabilitation of koalas by helping to determine the optimal timing for their release into burnt habitat. It can also help to predict how koalas will fare in burnt landscapes if they are not injured by fire or brought into care.

How does the nutritional quality of eucalypt foliage change over time in a burnt and unburnt landscape?

In my final chapter, I determined whether foliar chemistry changed over time in epicormic growth and adult-phase foliage, as changes to the nutritional landscape across time could have important consequences for marsupial folivores. Studies that observe changes across different spatial and temporal scales, using repeated measures, provide a more robust understanding of ecological processes (Estes *et al.* 2018). For this research, I measured the foliar chemistry of epicormic growth in a burnt landscape and adult-phase foliage in an unburnt landscape for one year, to look at how composition differed between foliage types and across time. The information from this study helps us understand longer-term impacts of fire on landscapes and wildlife.

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Chapter 1: Health assessments of koalas after wildfire: A temporal comparison of rehabilitated and non-rehabilitated individuals

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1.1 Abstract

Many koalas (*Phascolarctos cinereus*) required rehabilitation after the 2019–2020 Australian megafires. Little is known about how the post-release health of rehabilitated koalas compares to non-rehabilitated koalas. We evaluated health parameters in rehabilitated koalas and non-rehabilitated koalas in burnt and unburnt habitat in southern New South Wales, Australia. Health checks were undertaken within six weeks of fire (rehabilitated group), 5–9 months post-fire and 12–16 months post-fire. Body condition improved over time in rehabilitated koalas, with similar condition between all groups at 12–16 months. Rehabilitated koalas therefore gained body condition at similar rates to koalas that remained and survived in the wild. The prevalence of *Chlamydia pecorum* was also similar between groups and timepoints, suggesting wildfire and rehabilitation did not exacerbate disease in this population. While there was some variation in measured serum biochemistry and haematology parameters between groups and timepoints, most were within normal reference ranges. Our findings show that koalas were generally healthy at the time of release and when recaptured nine months later. Landscapes in the Monaro region exhibiting a mosaic of burn severity can support koalas, and rehabilitated koala health is not compromised by returning them to burnt habitats 4–6 months post-fire.

Keywords: marsupial folivore; bushfires; health parameters; rehabilitation; body condition

1.2 Introduction

Fire is a natural and often essential part of the Australian landscape, with the dry sclerophyll forests in the south-east region considered to be one of the most fire-

prone regions in the world (Attiwill and Adams 2013; Penman *et al.* 2007; Russell-Smith *et al.* 2007). Australia has experienced many wildfires throughout history; however, the 2019–2020 bushfire season was unprecedented in both severity and scale (Wintle *et al.* 2020). Between August 2019 and March 2020, 12.6 million hectares burnt on the eastern side of Australia across the states of Victoria, New South Wales (NSW), Queensland and South Australia (State of NSW and Department of Planning Industry and Environment 2020; Wintle *et al.* 2020). The effects on native wildlife were significant, with losses estimated in the billions (Van Eeden *et al.* 2020).

The koala (*Phascolarctos cinereus*) accounted for about 75% of the wildlife brought into emergency triage units in Victoria (Parrott *et al.* 2021). Hundreds of koalas were also rescued in NSW (Lunney *et al.* 2022) and South Australia (Dunstan *et al.* 2021), with rescue efforts continuing for weeks to months after the fires. There is no uniform approach to releasing fire-affected koalas after rehabilitation; some are returned to their rescue locations in burnt habitats while others are translocated to nearby unburnt habitats (Dunstan *et al.* 2021; Lunney *et al.* 2022; Parrott *et al.* 2021). Both approaches have risks that have not been adequately quantified. For example, translocated koalas often move farther from their release sites relative to koalas that remain in situ, which can place them at greater risk of predation and vehicle strikes (Menkhorst *et al.* 2019; Whisson *et al.* 2012). In contrast, burnt habitats may be lower quality with less food available and increased exposure to predators (Lunney *et al.* 2007; Zylstra 2019).

Koalas are dependent on *Eucalyptus* trees for both food and shelter (Crowther *et al.* 2014; Martin and Handasyde 1999), and these resources are often depleted or patchily distributed in burnt areas (Matthews *et al.* 2016). Many eucalypt species can

regenerate from epicormic buds on branches and stems relatively quickly after a fire (Burrows 2002; Burrows 2008; Clarke *et al.* 2013b; Waters *et al.* 2010; Williams *et al.* 2012). However, it is unclear how these changes affect habitat quality for koalas, and hence whether fire influences the longer-term health of individuals and populations living in burnt habitats. For example, in some unburnt areas, diseases such as chlamydiosis and, potentially, koala retrovirus (KoRV) can have substantial impacts on animal health, breeding success and population density (McCallum *et al.* 2018). Chlamydial infertility in particular is considered to play a major role in the decline in some koala populations (Polkinghorne *et al.* 2013; Quigley and Timms 2020). Environmental stressors, such as bushfires, have been hypothesised to further exacerbate disease, as well as to influence susceptibility to infections in wildlife in general (Albery *et al.* 2021). Given the extent of the 2019–2020 megafires (e.g., more than 3.5 million hectares (or 25%) of suitable koala habitat burnt in NSW State of NSW and Department of Planning Industry and Environment 2020) and that fires are predicted to become more common and extreme with climate change (Lucas *et al.* 2007), there is a critical need to understand whether there are longer-term health risks for rehabilitated koalas released into burnt habitats and whether the health of koalas in burnt habitats differs from those in unburnt habitats.

During and following the 2019–2020 megafires, community members and volunteers rescued 33 koalas from fire-impacted areas in the NSW Snowy Monaro Shire (Lunney *et al.* 2022), of which 31 had initial health data available for this study. Koalas that recovered after being in care were released at their rescue location into a burnt habitat. Ten (32%) of these koalas were also fitted with GPS tracking collars as part of a post-release monitoring program, along with 25 non-rehabilitated koalas from the same vicinity. The non-rehabilitated koalas occupied a mosaic of burnt and

unburnt habitats, providing a unique opportunity to compare the health of rehabilitated koalas before and after release with their non-rehabilitated counterparts. When possible, we collected data on body condition, chlamydial shedding and blood biochemistry and haematology at three timepoints for rehabilitated koalas: the time of rescue (January to March 2020), the time of release (June to December 2020) and the time of collar removal (February to June 2021). We collected the same data for non-rehabilitated koalas during the latter two time periods.

The comparative health data collected in this study provide a novel opportunity to address a key gap in our understanding of whether rehabilitated koalas should be returned to burnt habitats after their recovery. Furthermore, the study provides important baseline information about the general health and background levels of disease in a significant but understudied koala population, as well as improving the knowledge base about disease prevalence and severity across the distribution of the koala (Grogan *et al.* 2018; McCallum *et al.* 2018). Many koala populations across south-eastern Australia are in decline due to a combination of habitat destruction, fragmentation, wildfire, vehicle strikes, dog attacks and disease (Gonzalez-Astudillo *et al.* 2017; Lunney *et al.* 2007; McAlpine *et al.* 2015; McCallum *et al.* 2018). Accordingly, koalas are federally listed as ‘Endangered’ in Queensland, NSW, and the Australian Capital Territory (Department of Agriculture 2022). These data will also provide important information about the management implications of rescuing and rehabilitating koalas after fire.

We expected that rehabilitated koalas would be in poorer body condition when they were first rescued but would recover to a similar or possibly better condition at their time of release compared to non-rehabilitated koalas living in a fire-impacted

landscape. We also anticipated that koalas in a burnt habitat (rehabilitated or non-rehabilitated) would be in poorer body condition than koalas in an unburnt habitat if food quantity or quality were limiting. If fire or rehabilitation affected koala condition, we also expected to see indicators of poorer health in blood biochemistry and haematology parameters. Finally, we expected that there may be differences in the prevalence or the severity of symptoms of chlamydial disease between koalas in burnt and unburnt habitats.

1.3 Materials and Methods

1.3.1 Study Area

The Snowy Monaro Shire in NSW, Australia, incorporates the sub-alpine region that extends from the Australian Capital Territory in the north to the Victorian border in the south, west to the Kosciuszko Ranges and east to the Kybeyan range. Within the Snowy Monaro Shire, there are three main bioregions, including the South Eastern Highlands (59%), Australian Alps (23%) and the South East Corner Region (18%) (Snowy Monaro Regional Council 2020). Koalas are known to occur in the Macanally–Numeralla Ranges, east of the Monaro tableland, within the South Eastern Highlands bioregion (State Government of NSW and Department of Planning and Environment 2014). These areas comprise north–south ridges with steep sloping terrain and an elevational range of 800–1233 m. The main vegetation class in the area is Southern Tablelands dry sclerophyll forest, which is dominated by red stringybark (*Eucalyptus macrorhyncha*) and scribbly gum (*E. rossii*) (Zylstra 2019). Brittle gum (*E. mannifera*) and broad-leaved peppermint (*E. dives*) are also abundant in this area (Allen 2015). Ribbon gum (*E. viminalis*) and candlebark (*E. rubida*) are present in creek lines, and snow gums (*E. pauciflora*) are found on

slopes at higher elevations (Leavesley and Rhind 2008; Office of Environment and Heritage NSW National Parks and Wildlife Services 2012). Understory vegetation is dominated by *Acacia spp.* (Martin *et al.* 2020).

1.3.2 Koala Groups

Following bushfires in late January 2020, we obtained health data from 31 koalas (15 males, 16 females) that were rescued from Peak View (36.07° S, 149.38° E), Numeralla (36.17° S, 149.33° E) and Countegany (36.18° S, 149.45° E) in the Snowy Monaro Shire (Figure 1). After veterinary triage (described in more detail in the “data collection” section) and initial care at the Australian National University, the koalas were transported to licensed local wildlife carers, where their care continued until release. Twelve of these koalas were also selected for post-release GPS monitoring (the “rehabilitated” group; nine males and three females). Release of these koalas began in June 2020, with all koalas being released at their rescue locations by December 2020.

Between May and September 2020, additional non-rehabilitated koalas were located and captured in Peak View (n = 9) and Numeralla (n = 16). Koalas were located either by daylight ground-based searches, or by a drone fitted with a thermal imaging camera (Witt *et al.* 2020). They were captured using the noose and flag method described by Madani *et al.* (2020) and, after being examined by an experienced wildlife veterinarian (see “data collection” section) and fitted with a LiteTrack 60 VHF/GPS tracking collar (Lotek, Havelock, New Zealand), were released on the same day. The koalas captured at Numeralla were found in unburnt habitat, approximately 4 km from the nearest fire ground (the “unburnt non-rehabilitated” group; 5 males and 11 females), while those captured in Peak View

were in a habitat within the burn scar (the “burnt non-rehabilitated” group; 4 males and 5 females) (Figure 1). All koalas that retained their collar were recaptured eight to nine months later for collar removal and a final veterinary check (see “data collection” section). Supplementary material 1 contains a full list of koalas assessed at each point in the study.

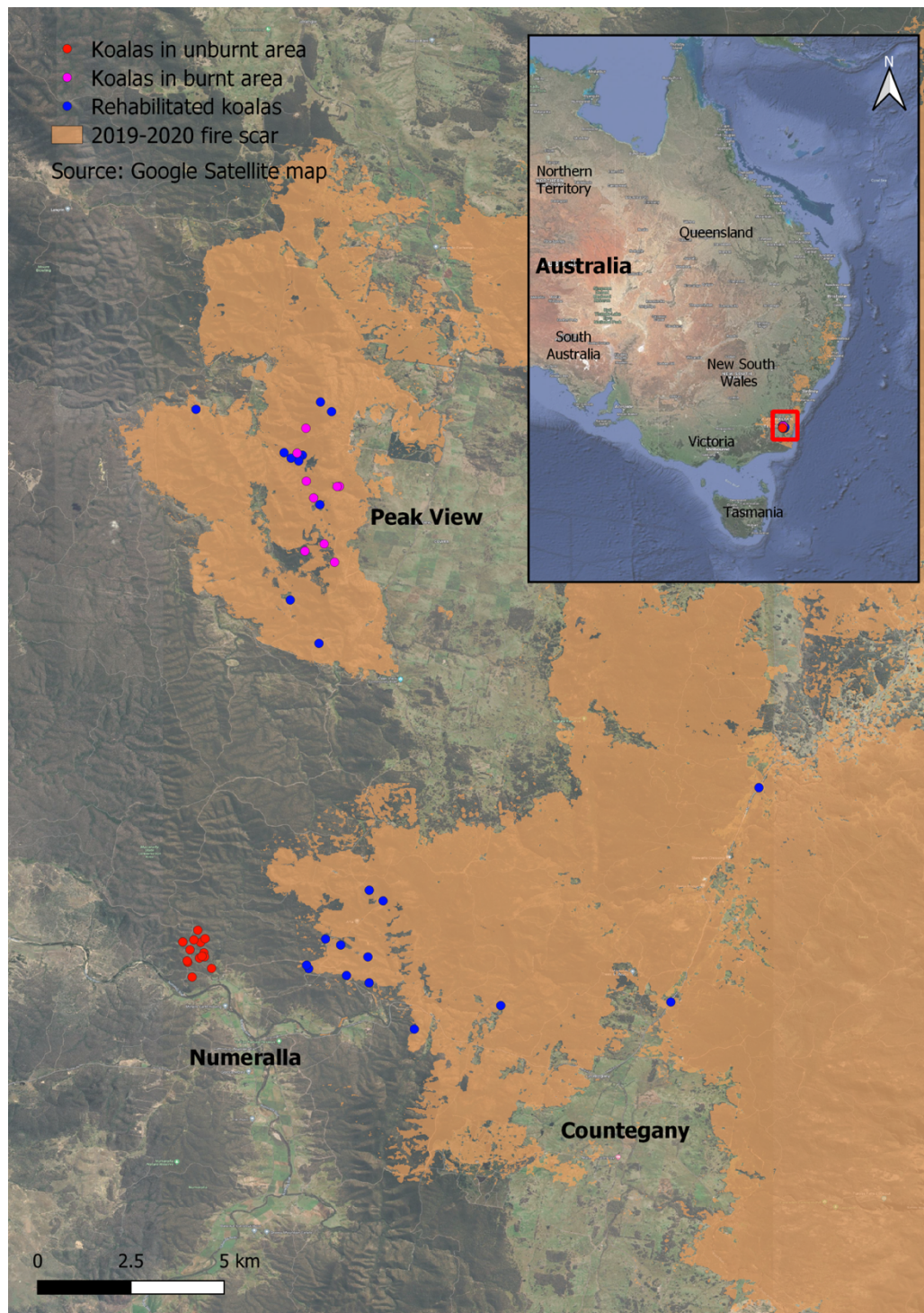


Figure 1. Capture locations of all rescued koalas (blue) as well as non-rehabilitated koalas captured in burnt habitat (pink) or unburnt habitat (red) against the 2019–2020 fire scar (orange) (State Government of NSW and Department of Planning and Environment 2020) (map data ©Google). The inset shows the location of the study site in south-eastern Australia.

1.3.3 Data Collection

Health data were collected from each koala at up to three different timepoints depending on their group (Table 1). These timepoints were:

- Rescue: an initial health check within 24 h of rescue for the rehabilitated group only.
- Pre-release: a pre-release check (5–9 months after rescue) for rehabilitated koalas that were selected for post-release monitoring, and a pre-collaring check for burnt non-rehabilitated and unburnt non-rehabilitated koalas captured for the monitoring study.
- Recapture: a final check up to nine months after the pre-release check for all monitored koalas that retained their tracking collars.

Table 1: Number of koalas given health checks from each group. The groups are rehabilitated koalas (note that only a subset of individuals was tracked post-release and therefore received a pre-release and recapture check), non-rehabilitated koalas (individuals caught, collared and released on the same day) in burnt habitat and non-rehabilitated koalas in unburnt habitat (see Supplementary material 1 for a full list of the individual koalas used in this study).

Health Check (Timeframe)	Rehabilitated	Non-rehabilitated in the	
		burnt habitat	unburnt habitat
Rescue (23 January 2020–13 March 2020)	31	NA	NA
Pre-release (25 May 2020–18 December 2020)	12	9	16
Recapture (5 February 2021–16 May 2021)	4	8	6

Health checks were conducted by wildlife veterinarians while koalas were under heavy sedation or general anaesthesia (see appendix (A1) for Koala Health Hub's koala health protocol for sampling of koalas following capture and restraint). Premedication with Alfaxalone (Jurox 10 mg/mL; up to 3 mg/kg intramuscular) was followed by maintenance on oxygen delivered by a face mask and/or isoflurane (VCA 1 mL/mL at 1–3% to effect) with an oxygen flow of 2 L/min. Koalas were maintained in lateral recumbency, with spontaneous breathing and relaxed muscle tone for the physical examination and data collection. Anaesthesia was monitored with heart rate, respiratory rate and temperature taken at regular intervals and depth assessed via palpebral and withdrawal reflexes. Koalas were checked for the presence of burns or any other clinically important findings, and a variety of general parameters were recorded, including sex, presence of pouch young (for females), age (from tooth wear score) and weight. Tooth wear score was determined by assessing the wear of the fourth upper premolars according to Martin (1981). Veterinarians also assessed physical condition, examining abdominal fill status and aural health. Body condition was assessed by one of two wildlife veterinarians using a standardised technique of palpation of the muscles on either side of the protruding ridge of bone on the scapula (spine of the scapula), and was scored out of 5 (Dunstan *et al.* 2021; Legione *et al.* 2017; Patterson *et al.* 2015; Sarker *et al.* 2020).

Blood was collected from the cephalic vein into serum and EDTA tubes for haematology, biochemistry and genetic analysis, and blood smears were made in the field. During cold conditions, blood draw was assisted by wrapping the forelimb in a warm towel. Ocular swabs were collected by gently and carefully rolling the swab (premoistened with sterile saline) across conjunctiva on both eyes, while urogenital swabs were collected from the urogenital sinus (females) or penile urethra (males).

In the first health check only (rescue or pre-release check depending on koala group), an ear tissue biopsy was collected in 80% ethanol, a microchip (Trovan, UK) placed subcutaneously within the intrascapular space and an individually numbered plastic ear tag (3.5 cm × 1 cm) (Leader Products, Shanghai, China) placed into the right pinna of females and left pinna for males, using an aseptic technique. At the pre-release check, koalas were also fitted with a LiteTrack 60 VHF/GPS tracking collar (Lotek, Havelock, New Zealand) that weighed 80 g around the neck, with at least two finger widths of space between the neck and collar. The tracking collars were programmed to collect five GPS points overnight (1900 h to 0700 h) at three-hourly intervals. Each collar had a unique frequency that was tested prior to releasing the koala. An elastic weak link was incorporated into each collar to ensure release in the event of snagging.

1.3.4 Sample Processing

After collection, blood and swab samples were transported to the Australian National University. Upon arrival, 0.5 mL blood in EDTA was pipetted into a new vial for haematological analysis. Serum tubes were centrifuged at 6500× g for 10 min and the serum was transferred to a new vial for biochemical analysis. Blood and serum samples were refrigerated at 4 °C for up to 48 h and swabs were stored at ambient temperature for up to five days, pending analysis at the Sydney School of Veterinary Science, University of Sydney. Haematological parameters measured included PCV (packed cell volume), TPP (total protein), haemoglobin, erythrocytes, MCV (mean corpuscular volume), MCHC (mean corpuscular haemoglobin concentrations), MCH (mean corpuscular haemoglobin), platelet count, leukocytes, neutrophils, lymphocytes, neutrophil/lymphocyte ratio, monocytes, eosinophils and

basophils. Haematocrit and total plasma protein were determined by capillary tube centrifugation and refractometer, respectively. White cell count was determined using a Sysmex haematological analyser (XN-1000RF Vet, Roche Diagnostics, Basel, Switzerland) and differential counts obtained by light microscopy.

Biochemical parameters measured included urea, creatinine, AST (aspartate aminotransferase), ALP (alkaline phosphatase), GGT (gamma-glutamyl transferase), bilirubin, phosphate and ALT (alanine transaminase). Serum biochemical analysis was conducted on a Konelab Prime biochemistry analyser (30i, Thermo Fisher Scientific Oy, Vantaa, Finland).

Chlamydial detection was conducted using an established qPCR (Hulse *et al.* 2018) for chlamydial 23S, ompB and koala beta-actin as a sample quality control, following DNA extraction on a magnetic bead-based 96-well extraction system (Kingfisher Flex, Thermo Fisher Scientific) with positive and negative controls.

1.3.5 Genetic Sample Extraction and Sequencing

As a part of the koala whole genome survey, genomic analysis was conducted on 20 koalas from the Snowy Monaro Shire (Hogg *et al.* 2023). DNA was extracted from 200 μ L of whole blood from koalas in Peak View (non-rehabilitated in the burnt: n = 5, rehabilitated: n = 6) and Numeralla (non-rehabilitated in the unburnt: n = 9) using the standard MagAttract HMW DNA kit (QIAGEN, Hilden, Germany) protocol with elution in 60 μ L buffer AE. The quantity of DNA was determined by Qubit 2.0 (Thermo Fisher Scientific) and the quality was assessed through 0.8% agarose TBE gel stained with SYBR Safe (Life Technologies, Thermo Fisher Scientific), where 2 μ L of DNA was stained with 4 μ L of 10% loading dye (Bioline, London, UK). DNA was separated alongside a 1-kb size standard (Bioline) for 30 min at 90 V, bands

were visualised using a Gel Doc XR + (Bio-Rad, Hercules, CA, USA) under ultraviolet light and images were analysed with ImageLab (Bio-Rad). DNA samples underwent additional QC, Illumina DNA PCR-free library preparation (Illumina, San Diego, CA, USA) and whole genome sequencing (WGS) at the Ramaciotti Centre for Genomics (UNSW). Samples were sequenced as 150 bp paired ends on a Novaseq S4 flowcell obtaining ~30× coverage per sample. Resequencing data from the 20 koalas were aligned with the reference genome (GCA_002099425.1_phaCin_unsw_v4.1_genomic.fasta (Johnson *et al.* 2018)) and variants were called using the DRAGEN Germline platform v3.8.4 (Illumina) (Miller *et al.* 2015). Joint genotyping across all samples was performed using DRAGEN JointGenotyping v3.8.4.

1.3.6 Population Genetic Analysis

Variants were restricted to biallelic SNPs using GATK v4.2.0.0 (McKenna *et al.* 2010), any sites with missing data were removed using vcftools v0.1.14 (Danecek *et al.* 2011). SNPs were further filtered on a minor allele frequency (MAF) of 0.08 to ensure an SNP was seen in at least 2 individuals ($3/2N$). Linked SNPs were removed with PLINK v1.90 (Purcell *et al.* 2007) using an R^2 of 0.4, a window size of 50 SNPs and a step of five SNPs. Using R v4.1.1 (R Core Team 2021), the vcf file was then transformed to a genind object using the adegenet package v2.4.1 (Jombart 2008) and a PCoA analysis was conducted. Using the set of SNPs filtered only on missing data, we estimated the runs of homozygosity using the `homozyg` function in PLINK. We used a sliding window of 50 SNPs with regions considered as homozygous if they were at least 100 kb and 100 SNPs. We allowed for one heterozygous SNP per window and five SNPs per ROH. In order to call a run of

homozygosity (ROH), we required at least one SNP per 50 kb and the maximum gap between two SNPs was 200 kb and at least 5% of windows were required to contain a homozygous SNP to be considered an ROH. Heterozygosity and F_{is} were calculated using the `populations` command in `Stacks v2.2` (Catchen *et al.* 2013) using biallelic sites only with no additional filtering as to not bias heterozygosity estimates.

1.3.7 Data Analysis

Four of the biochemical and haematological parameters (urea, AST, ALT and MCV) were log transformed prior to statistical analysis as they were not normally distributed. We used the `lme4` package (version 1.1.31) (Bates *et al.* 2015) in R (v3.6.3) (R Core Team 2021) to test whether body condition and each of the biochemical and haematological parameters differed between koala groups and/or health checks. For each model, the health parameter was the dependent variable and koala group, health check and the interaction between the two were the fixed effects. We also included the individual koala identifier as a random effect because some koalas received multiple health checks. If effects were significant ($p < 0.05$), we used the `emmeans` package (v1.6.3) (Lenth 2021) to conduct pairwise t-tests to determine which treatments differed from others. We used the same packages to test for differences in each health parameter between koalas that tested either positive or negative to Chlamydia. We did not test for differences in infection rates with ocular Chlamydia due to the small number of positive cases. Urogenital Chlamydia status was a fixed effect and individual koala was a random effect. No haematological data were available for any rehabilitated koalas for the recapture check due to unexpected degradation of the blood samples during transportation.

This means that haematological comparisons are only between rescue and pre-release health checks for rehabilitated koalas.

We investigated differences between rehabilitated koalas that lived or died by conducting logistic regressions of survival against individual health parameters. It was only feasible to test one health covariate at a time because of missing values in these covariates and the small number of individuals that died ($n = 7$).

1.4 Results

1.4.1 Survival Rates

Of the 56 koalas that received a health check, 10 koalas died or were euthanized. This included 7 of the 31 koalas that were rescued post-fire, with a 78% survival rate in care for the rehabilitated koalas. Post-mortems on five of these koalas showed evidence of starvation, poor body condition and a large tapeworm burden in one individual. The other two koalas were euthanized due to multiple contributing factors that made them unreleasable, such as large cysts in the reproductive tract and crepitus in the joints.

After release, two of the twelve rehabilitated koalas that were monitored with GPS collars died during the study (i.e., 17%). One sustained a bite injury two months after release, possibly from another male, and was recaptured and treated but later died. On post-mortem, a sinus was found at the left ventral thigh extending to the greater trochanter of the femur, with necrosis of the bone present. The other koala died seven months post-release, but the cause of death is unknown. No collared koalas in either the burnt or unburnt non-rehabilitated groups died during the study. However, an emaciated koala of advanced age with poor dental condition (worn

down teeth due to age) was captured in the unburnt landscape during the pre-release checks and was euthanised due to welfare concerns.

Two of the GPS collars failed after deployment, with one koala located again and the collar removed. Another 13 koalas dropped their collars before the completion of the study due to weak links breaking or the collar slipping over the head.

1.4.2 Burn Injuries

Only three out of thirty-one rescued koalas had superficial burn injuries, although there were also changes in the skin compatible with healing soft tissue injuries on the extremities of two of the rescued koalas that died. One of the burn injuries in the surviving koalas required regular bandaging and prevented that individual from climbing for a few weeks. The others were treated for burns but did not require bandaging. All three koalas made a full recovery. Interestingly, two non-rehabilitated koalas captured ~4 months post-fire for the monitoring study had evidence of healed burns on their footpads (patches of pale pink skin) and scars without fur around their ear margins, demonstrating that these koalas were physically impacted by the fires but survived and stayed within the burnt area.

1.4.3 Body Condition Score

At the initial health check, many of the rehabilitated koalas had a very low body condition score (BCS), with the average being 1.3 ± 0.14 (SE) out of 5. BCS differed between health checks ($F_{2,79} = 26.81$, $p < 0.001$), particularly between the rescue and pre-release ($p < 0.001$) and rescue and recapture health checks ($p < 0.001$) for

rehabilitated koalas (Figure 2). At the pre-release and recapture health checks, koalas from all groups were in similar body condition ($p > 0.05$; Figure 2).

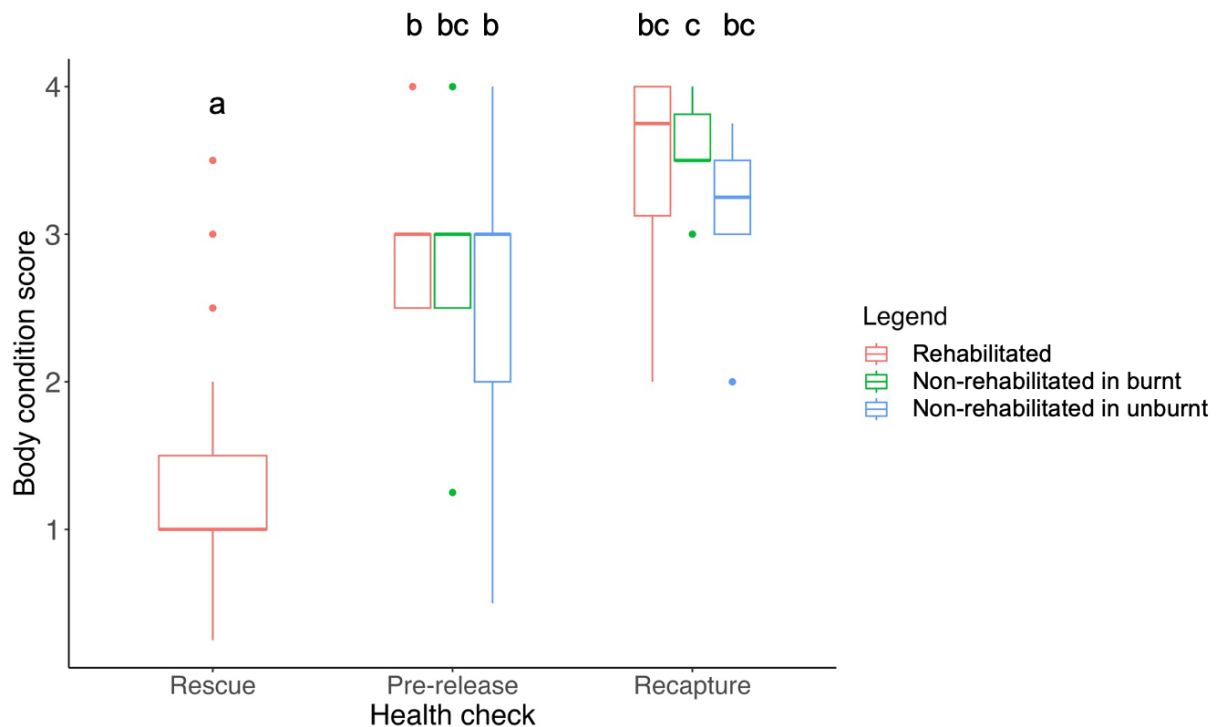


Figure 2. Spread of body condition scores at each health check for koalas within each group. Significance at the level of $p < 0.05$ is denoted with different lettering. Note the long whisker for the non-rehabilitated koalas in the unburnt at the pre-release check is due to one individual of advanced age who was in very poor condition and was subsequently euthanized on welfare grounds. The points outside the boxplots show outliers (individuals who had higher or lower BCS that fell outside the range).

1.4.4 Overall Health and Chlamydia Status

Of the 24 adult female koalas examined in this study, 13 had pouch and/or back young present between January and September 2020. This equated to 44% of

female rehabilitated individuals, 40% of female burnt non-rehabilitated koalas and 36% of female unburnt non-rehabilitated koalas. Two rehabilitated koalas and one unburnt non-rehabilitated koalas lost their pouch young during the study, while all others reared them to independence.

Only 3 of the 56 individual koalas examined tested positive for ocular *Chlamydia pecorum* at any of the three health checks (Table 2) and all three were asymptomatic juvenile individuals who were rescued from the fire grounds. Ocular *Chlamydia pecorum* was not detected in the seven koalas that presented with eye abnormalities, including discharge, corneal ulceration and opacity. In contrast, urogenital shedding of *C. pecorum* was detected in 31 of 56 koalas (55%; Table 2). This included the mothers of two of the juveniles with ocular chlamydial shedding. None of the koalas involved in the post-release monitoring program were treated for chlamydiosis even if they tested positive. Koalas in which urogenital chlamydial shedding was detected at their first health check were also positive at other health checks. Likewise, koalas that were negative tested negative throughout the study. Signs of disease typically associated with urogenital *C. pecorum* were also very low, with 48/56 koalas at their first health check having a rump score of 0/7, which indicates normal fur, normal cloaca and no evidence of wet bottom (Koala Health Hub The University of Sydney). A total of seven koalas scored 1/7 (slight discoloration and mild urine leakage). The highest rump score was 3/7 for one female koala in the unburnt area, which indicates discoloration of the tail area fur and stronger 'wet bottom' smell. For all koalas that received a health check at recapture, eight scored 0/7, nine scored 1/7 and one scored 1.5/7.

Table 2. Number of koalas shedding *Chlamydia pecorum* in each group

	Number of Individuals with Ocular Chlamydia Only	Number of Individuals with Urogenital Chlamydia Only	Number of Individuals with both Ocular and Urogenital Chlamydia
Rehabilitated koalas (<i>n</i> = 31)	2	17	1
Non-rehabilitated in the burnt habitat (<i>n</i> = 9)	0	4	0
Non-rehabilitated in the unburnt habitat (<i>n</i> = 16)	0	10	0

1.4.5 Blood Biochemistry

There were no differences detected between serum creatinine (Figure 3a) and ALP (Figure 3b) concentrations for different koala groups or health checks ($p > 0.05$ for all tests). While we observed some differences in blood biochemistry results between koala groups and/or health checks for other parameters (described below), most individuals were within the published reference ranges (Canfield *et al.* 1989; Dunstan *et al.* 2021) (Figure 3).

For phosphate, there was an interaction between group and health check ($F_{2, 19.73} = 4.86$, $p = 0.019$), likely because phosphate concentrations decreased between the pre-release and recapture checks in rehabilitated koalas but increased in the non-rehabilitated koalas in burnt habitats (Figure 3c). A post-hoc analysis showed that, at the pre-release check, phosphate levels were higher (and above the reference range for many individuals) in rehabilitated koalas compared to koalas in the burnt ($p < 0.001$) and unburnt ($p = 0.003$) non-rehabilitated groups (Figure 3c). There tended to be a difference for group x health interaction for GGT ($F_{2, 27.26} = 3.24$, $p = 0.054$; Figure 3d), with mean values lower at pre-release compared to

recapture in rehabilitated koalas but not in other groups. Furthermore, GGT concentrations were lower for koalas in the rehabilitated group at the pre-release check relative to all other checks for all koala groups ($p < 0.05$ for all pairwise comparisons) but were within the reference range (Figure 3d).

There were differences in the concentrations of both ALT ($F_2, 36.88 = 7.15, p = 0.002$; Figure 3e) and AST between health checks ($F_2, 18.46 = 8.65, p = 0.002$; Figure 3f). ALT concentrations were lower at the final recapture check compared to the pre-release check in both non-rehabilitated koalas in the burnt ($p = 0.008$) and unburnt landscape ($p = 0.025$; Figure 3e). For AST, concentrations were higher in rehabilitated koalas at the rescue check compared to pre-release ($p = 0.003$; Figure 3f).

There was a difference in urea concentrations between koala groups ($F_2, 55.15 = 4.13, p = 0.021$), with non-rehabilitated koalas in the unburnt landscape having lower concentrations of urea at the pre-release check compared to non-rehabilitated koalas in the burnt landscape ($p = 0.005$) and rehabilitated koalas ($p = 0.016$; Figure 3g).

There were also differences in bilirubin concentrations between koala groups ($F_2, 61.05 = 3.54, p = 0.035$) and health checks ($F_2, 36.41 = 3.36, p = 0.046$; Figure 3h). Bilirubin levels were higher in non-rehabilitated koalas in the burnt landscape at the pre-release check compared to both rehabilitated koalas ($p = 0.02$) and non-rehabilitated koalas in the unburnt landscape ($p = 0.007$; Figure 3h). Bilirubin concentrations were also higher in rehabilitated koalas at rescue compared to pre-release ($p = 0.002$; Figure 3h).

For most biochemical parameters, values were similar regardless of whether koalas tested positive or negative to urogenital *C. pecorum*. The exceptions were for

ALP ($F_1, 70.38 = 11.85, p < 0.001$) and phosphate ($F_1, 64.711 = 6.36, p = 0.014$), with concentrations for both constituents lower in koalas that tested positive to urogenital chlamydia.

We found a negative association between survival and the log of ALT, suggesting that koalas with high ALT were less likely to survive. Although statistically significant at the $p < 0.05$ level, this finding is considered tentative due to the small sample size (only five koalas where ALT was measured died).

Legend

- ▢ Rehabilitated
- ▢ Non-rehabilitated in burnt
- ▢ Non-rehabilitated in unburnt

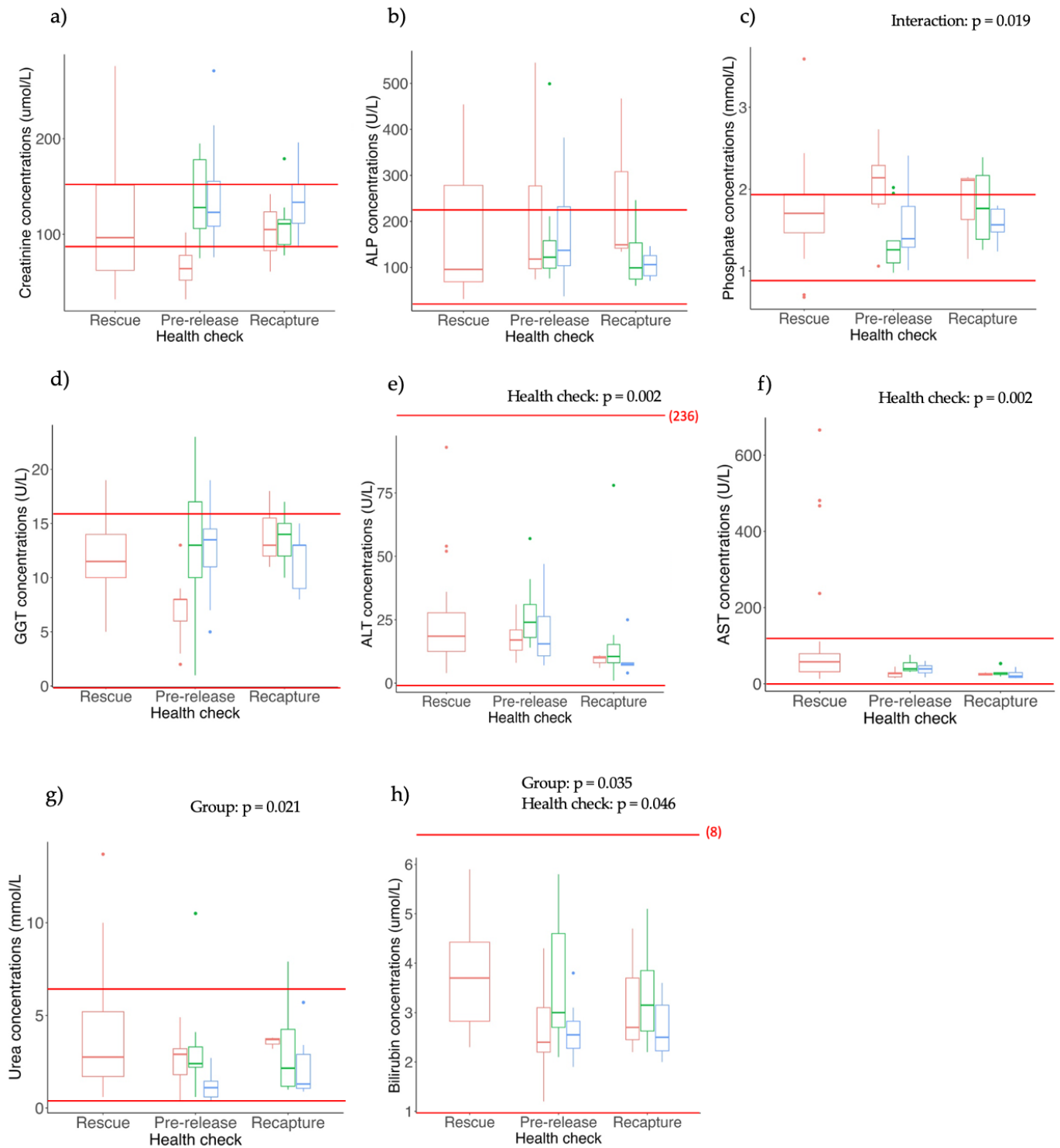


Figure 3. Spread of data within groups and health checks for each biochemical parameter. Each biochemical parameter is represented by a letter from a–h. Red lines indicate the reference range. p-values are provided where differences between koala groups, health checks or the interaction were less than 0.05. Refer to the text for the pairwise comparisons. The points outside the boxplots show outliers (individuals who had higher or lower values for each parameter that fell outside the range).

1.4.6 Haematology

Similar to the blood biochemistry, the haematological results for most individuals were within the published reference ranges (Canfield *et al.* 1989; Dunstan *et al.* 2021) (Figure 4). There were no differences between the koala groups or the health checks for PCV, TPP, haemoglobin, MCHC, platelet count, leukocytes, monocytes, eosinophils or basophils ($p > 0.05$ for all tests; Figure 4a–h). A figure for basophils has not been included as most values were 0.

Concentrations of nucleated red blood cells (NRBCs) differed between health checks ($F_{2, 27.35} = 5.79$, $p = 0.008$; Figure 4i) and were higher at the pre-release check for non-rehabilitated koalas in the burnt landscape compared to non-rehabilitated koalas in the unburnt landscape ($p = 0.039$). NRBCs were also higher at the recapture check compared to the pre-release check in non-rehabilitated koalas in the unburnt landscape ($p = 0.048$).

There were differences between koala groups for MCV ($F_{2, 59.53} = 3.54$, $p = 0.035$; Figure 4j), MCH ($F_{2, 59.70} = 6.003$, $p = 0.004$; Figure 4k) and neutrophils ($F_{2, 48.48} = 4.65$, $p = 0.014$; Figure 4l), and there was also a trend for erythrocytes to differ between groups ($F_{2, 59.25} = 3.006$, $p = 0.057$; Figure 4m). MCV was higher at

the recapture health check compared to the pre-release check for non-rehabilitated koalas in the burnt landscape ($p = 0.045$) and higher at recapture in non-rehabilitated koalas in the burnt landscape compared to non-rehabilitated koalas in the unburnt landscape ($p = 0.011$; Figure 4j). MCH was higher at both the pre-release and recapture checks in non-rehabilitated koalas in the burnt landscape compared to non-rehabilitated in the unburnt landscape ($p = 0.011$ and $p = 0.002$, respectively; Figure 4k). At the pre-release check, neutrophils were above the reference range in almost half of the non-rehabilitated koalas in the burnt landscape and were higher on average than both non-rehabilitated koalas in the unburnt landscape ($p = 0.01$) and rehabilitated koalas ($p = 0.003$; Figure 4l). However, at recapture, neutrophils for non-rehabilitated koalas in the burnt landscape were lower than at pre-release ($p = 0.036$) and most were within the reference range (Figure 4l).

There were differences in lymphocytes for both koala groups ($F_2, 55.43 = 4.96$, $p = 0.01$) and health checks ($F_2, 24.91 = 13.46$, $p < 0.001$; Figure 4n). At pre-release, lymphocytes in non-rehabilitated koalas in the burnt landscape were lower than both unburnt non-rehabilitated koalas ($p = 0.011$) and rehabilitated koalas ($p = 0.008$) during this check. At recapture, lymphocytes were also lower in burnt non-rehabilitated koalas compared to unburnt non-rehabilitated koalas ($p = 0.05$). Finally, in rehabilitated koalas, lymphocyte values were higher at pre-release compared to their first health check at rescue ($p < 0.001$). There were no differences detected in either koala group or health check for the neutrophil/lymphocyte ratio.

There were no differences observed between any of the haematological parameters and Chlamydia status, or between koalas that lived or died ($p > 0.05$ for all tests).

Legend

- ▢ Rehabilitated
- ▢ Non-rehabilitated in burnt
- ▢ Non-rehabilitated in unburnt

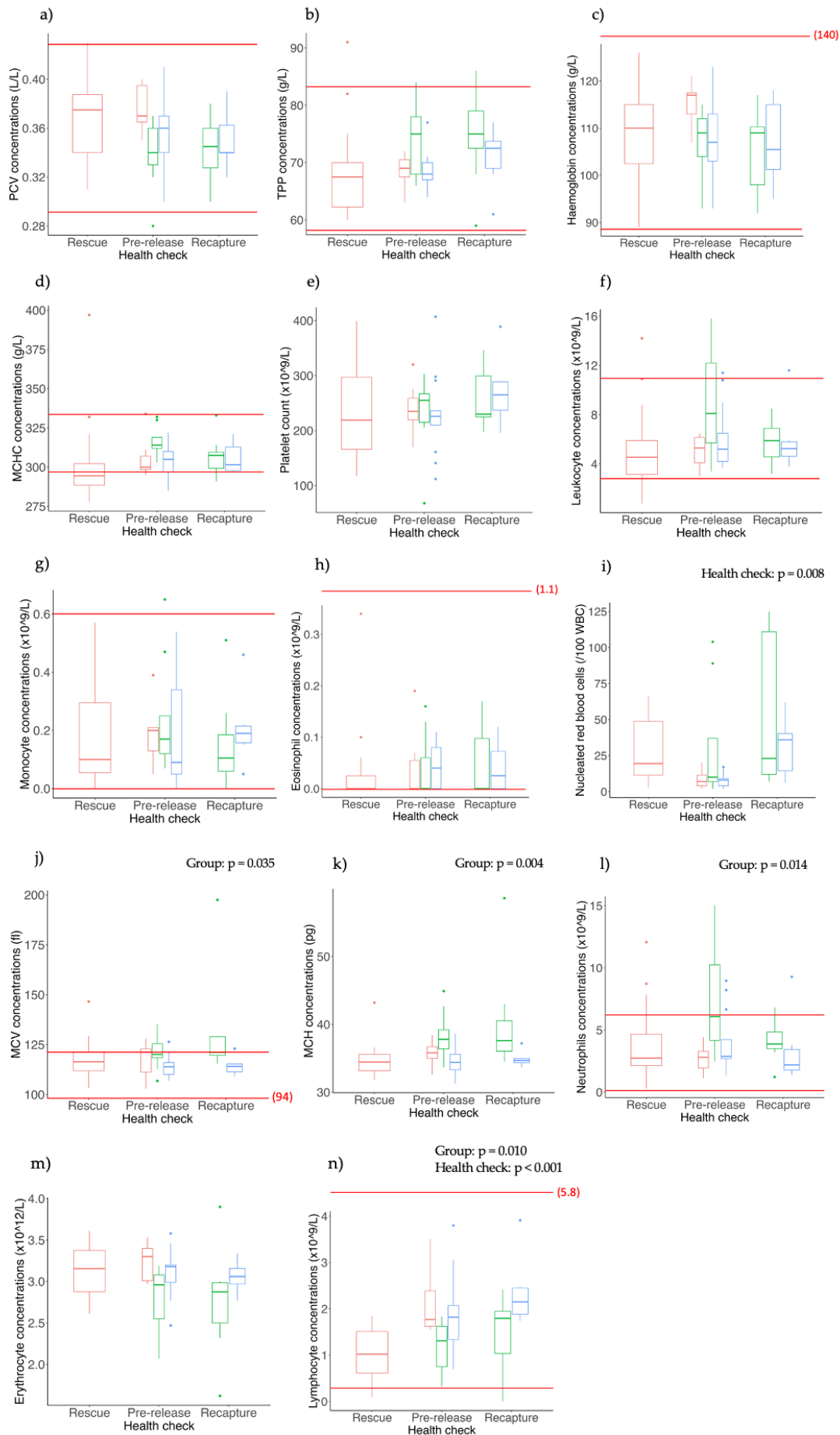


Figure 4. Spread of data within groups and health checks for each haematological parameter. Each haematological parameter is represented by a letter from a–n. Red lines indicate reference range. p-values (<0.05) are provided where differences between koala groups, health checks or the interaction were significant. Refer to the text for the pairwise comparisons. The points outside the boxplots show outliers (individuals who had higher or lower values for each parameter that fell outside the range).

1.4.7 Genetic Analysis

All 20 samples passed quality control and were sequenced with an average 36.4X coverage (range 29.2–49.3) with a total of 18,953,300,000 paired end reads. After filtering on missingness and MAF, there were 9,988,662 SNPs, and after removing linked SNPs there were 948,924 SNPs for downstream analysis.

PCoA showed minimal population structuring among individuals from Peak View and Numeralla, with the majority of individuals clustering together (Figure 5). Observed heterozygosity was similar between the two groups (Numeralla 0.172 ± 0.053 , Peak View 0.174 ± 0.053), as was expected heterozygosity (Numeralla: 0.157 ± 0.035 , Peak View: 0.160 ± 0.035). Fis for both populations was close to zero (Numeralla: -0.005 ± 0.047 , Peak View: -0.0091 ± 0.047). The majority of the ROH were small with the average length of ROH at 196 kbp across all individuals, with the longest ROH identified as 2.79 Mbp (Figure 6).

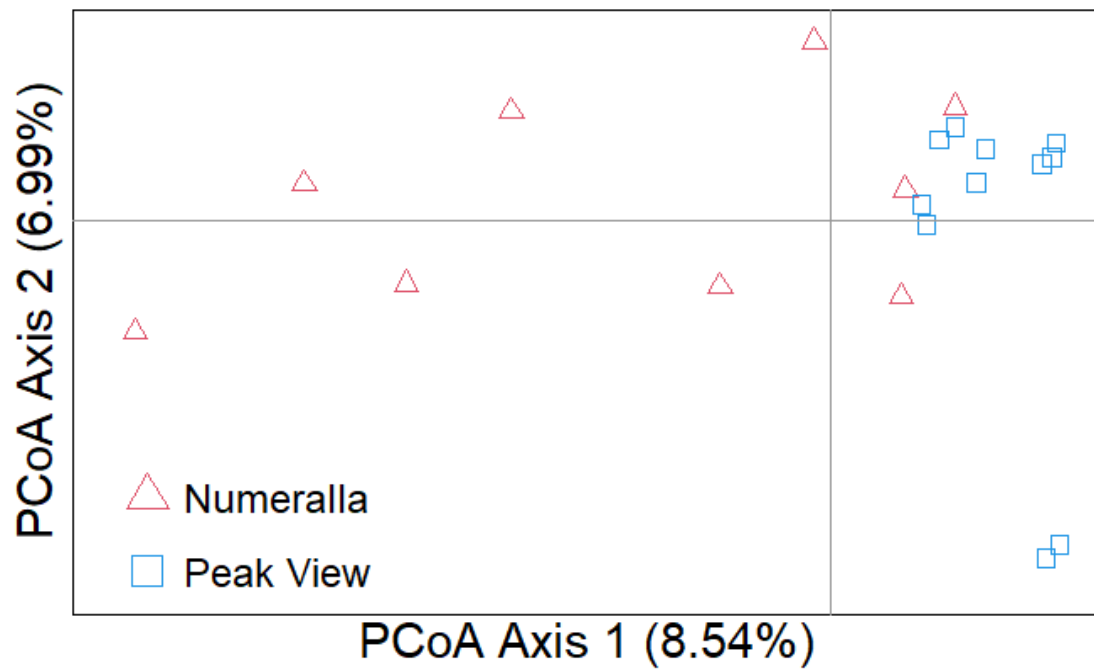


Figure 5. PCoA plot based on 948,924 biallelic SNPs with individuals from two koala groups in the Snowy Monaro region (Numeralla and Peak View, NSW). The values in the brackets are the percentage of variation in the dataset explained by each axis.

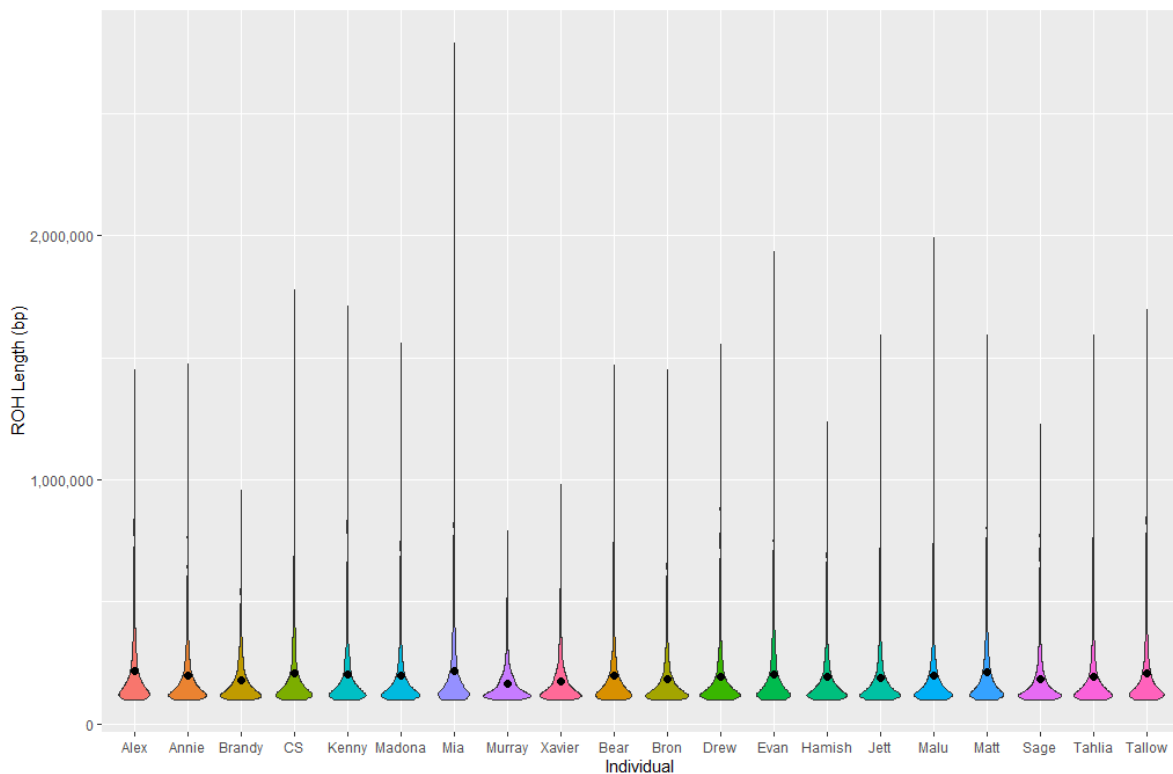


Figure 6. Distribution of runs of homozygosity (ROH) for each individual.

1.5 Discussion

This is the first research on the impacts of bushfires and rehabilitation and release into bushfire-affected areas on the overall health, chlamydial shedding and biochemical and haematological parameters of koalas. This previous knowledge gap hampered decisions about whether to return koalas rescued from fire-affected areas to burnt habitats or whether to translocate them to unburnt habitats. Our results showed that body condition improved in rehabilitated koalas over time, and they were in similar body condition to koalas living in fire-affected and unburnt landscapes at their time of release. Unexpectedly, there was no difference in body condition between koalas in burnt and unburnt habitat, suggesting there was a sufficient quantity and quality of food available to sustain their body conditions in burnt areas. However, more studies are needed to determine whether this pattern is consistent across other koala populations post-fire. While there were detectable differences in some of the measured biochemical and haematological parameters between koala groups and/or health checks, there was no evidence that fire and rehabilitation had any consistent biologically significant effects on the general health of koalas or on the signs or prevalence of disease. Thus, our study suggests that, within the study timeframe post-release, the health of rehabilitated and non-rehabilitated koalas in burnt habitats was not compromised compared to koalas in unburnt habitats.

1.5.1 Body Condition and Overall Health

As expected, koalas rescued from fire grounds were in very poor body condition, with an initial mean score of 1.3 out of 5, where 1–2 is considered emaciated or poor condition, 3 is healthy/adequate and 4–5 is very good/excellent (Dunstan *et al.* 2021; Koala Health Hub The University of Sydney ; Legione *et al.*

2017; Patterson *et al.* 2015; Sarker *et al.* 2020). After their time in care, the BCS of koalas in the rehabilitated group had increased and was similar to non-rehabilitated koalas in the burnt and unburnt group at both the pre-release and final recapture health checks. Koalas in care and after release therefore did not gain or lose body condition at a substantially different rate than koalas that remained and survived in the wild. This means that within four months of fire there were sufficient resources in burnt habitats to support healthy koalas. It also may be worthwhile considering other measures of health prior to release, as Leigh *et al.* (2023) found that both BCS and climbing ability were important determinants of acute survival following release from rehabilitation, with koalas with stronger climbing abilities and higher body condition scores more likely to survive. This suggests that release should be considered if suitable habitat is present, and koalas are in good physical condition.

Landscapes typically burn with a mosaic of fire severity due to variation in weather and topography, and the fire in the study region was no exception. Thus, fire severity ranged from low (only understorey burnt) to extreme (full canopy consumption) in patches across the burnt area inhabited by koalas in our study. We do not know whether the koalas in our study relied on trees with intact canopies or whether they extensively utilised the widespread epicormic regrowth. It is likely that they used both given that rehabilitated koalas were willing to eat epicormic regrowth from some eucalypt species alongside mature foliage while in care (Lane *et al.* 2024). Nevertheless, future studies should attempt to better understand the value of epicormic growth as food for koalas and how it may shape the way that koalas use fire-impacted landscapes and their health.

Drought conditions are known to negatively impact koala populations, affecting mortality rates, breeding success and dispersal of individuals (Melzer *et al.* 2000;

Seabrook *et al.* 2011). It is possible that the initial poor condition and some of the wide variation in values recorded in the biochemical and haematological parameters in the rescued koalas could be due to the extreme drought conditions and heat that were prominent in the lead up to the fire in addition to fire effects. For example, several rehabilitation groups reported that drought conditions had greater impacts on the koalas that came into care during 2019–2020 than the fires (Lunney *et al.* 2022). Droughts and heatwaves can trigger leaf fall and foliage damage, causing a reduction in the availability of sufficient food, ultimately leading to malnutrition, dehydration and possible death in koalas (Degabriele 1981; Gordon *et al.* 1988). A reduction in available browse also creates other indirect consequences for koalas, including increased competition, lower breeding success and higher susceptibility to disease (Cristescu *et al.* 2023). These drought events can also compound the effects of fire and other habitat-altering events (e.g., logging; Lunney *et al.* 2014), making koalas weaker and more susceptible when there is a major bushfire event. It is important to consider that with a changing climate (Lucas *et al.* 2007), more koalas may be displaced or disrupted by both droughts and fire. Understanding how their physiology is affected will be important for future rehabilitation efforts and knowing when to release them.

1.5.2 Burn Injuries

The number of fire-related injuries observed in koalas rescued from the Monaro region was relatively low (only three of thirty-one) compared to rescue efforts in other areas. For example, Parrott *et al.* (2021) reported that 20% of koalas rescued in Victoria during the megafires were euthanised due to fire-related injuries. Likewise, 67.4% of koalas that came into triage units on Kangaroo Island, South Australia,

were suffering from burn injuries, and 45.6% died or needed to be euthanised (Dunstan *et al.* 2021). The lower numbers in our study may have been due to a combination of relatively low encounter rates between searchers and koalas due to low densities (Allen 2015), higher mortality rates from intense fires or difficulties with access to remote areas and restricted fireground access. Despite this, the results from all three studies demonstrate that many more koalas would likely have perished without the necessary care provided by veterinary teams and wildlife carers post-fire. Our findings provide additional data to show that koalas that survive the initial fire event and are later released can live and maintain health in a burnt landscape.

1.5.3 *Chlamydia Status*

In this study, shedding of *C. pecorum*, an intracellular bacterium that is widespread throughout most koala populations (Hulse *et al.* 2020; Polkinghorne *et al.* 2013), was detected in 59% of koalas. Interestingly, very few of the Monaro koalas exhibited clinical signs of disease during any health check, with only one koala in the rehabilitation group euthanised and two requiring antibiotic treatment due to the presence of mild paraovarian cysts. Furthermore, 44% of the adult females who were infected with *C. pecorum* in our study had pouch or back young. These findings place the Monaro koalas in line with other populations that appear to have a low prevalence of clinical disease. *C. pecorum* can cause a range of diseases, with the most common being conjunctivitis and urogenital disease, the latter of which can lead to infertility in females (Cockram and Jackson 1981; Martin and Handasyde 1999; Melzer *et al.* 2000; Quigley and Timms 2020; Wan *et al.* 2011). Koalas from the Monaro region showed higher rates of chlamydial shedding and urogenital disease relative to ocular sites, which is consistent with other

populations in both Queensland (Nyari *et al.* 2017; White and Timms 1994) and Victoria (Patterson *et al.* 2015). *C. pecorum* has also been detected in most koala populations, with the prevalence and severity of *C. pecorum* varying between populations (Hulse *et al.* 2020; Polkinghorne *et al.* 2013), infecting individuals without causing outward and obvious symptoms or disease (Santamaria and Schlagloth 2016). For example, in a koala population in south-eastern Queensland, 71% of koalas tested positive for *C. pecorum*; however, only 9% of individuals showed overt clinical disease (Weigler *et al.* 1988). Furthermore, 82% of females had either back young or pouch young, despite 67% being infected (Weigler *et al.* 1988). In another Queensland population, of the 67% of males that tested positive to urogenital Chlamydia, only one-third showed clinical symptoms (Hulse *et al.* 2020). A low prevalence of clinical disease has also been observed in South Australian populations including in the Mount Lofty Ranges and at Cleland Wildlife Sanctuary (Polkinghorne *et al.* 2013).

Rates of chlamydial shedding were similar between rehabilitated koalas and non-rehabilitated koalas in burnt and unburnt habitats. Furthermore, we did not observe any changes either in the presence of chlamydial infection or the clinical signs of disease in individuals across the course of the study, even though environmental stressors such as bushfire have the potential to exacerbate clinical disease (Albery *et al.* 2021). These results further support the idea that neither fire nor rehabilitation have major effects on chlamydial status in koalas in the Snowy Monaro region. It also supports the idea that there were limited negative health impacts of releasing rehabilitated koalas into burnt habitats in the Monaro region, even with untreated chlamydial infections. It is important to note, however, that *C. pecorum* is primarily sexually transmitted in koalas (Polkinghorne *et al.* 2013;

Quigley and Timms 2020), although it can also be transmitted from mother to young during pap feeding (Nyari *et al.* 2017; Polkinghorne *et al.* 2013). Sexual transmission of Chlamydia may potentially be lower in burnt landscapes, as there may be fewer individuals to interact with due to lower densities.

1.5.4 Genetics

Using a whole genome sequencing (WGS) approach, we assessed the genetic health of koalas from the Monaro region of NSW and report the first genetic analysis of this population of koalas. We show that this population of koalas displays diversity comparable to other populations of koalas (Kjeldsen *et al.* 2019; Kjeldsen *et al.* 2016). An analysis of ROH is able to give insights into inbreeding history of an individual, with long ROH segments reflecting recent inbreeding events and shorter ROH segments reflecting historical inbreeding that has been broken by recombination (Curik *et al.* 2014). The majority of the ROH were <0.4 MBp in length and only eight ROH segments were larger than 1.6 MBp, indicating some recent inbreeding in those individuals. On average, the lengths of ROH in this koala population were lower than other wildlife species including killer whales and rhinoceros (Foote *et al.* 2021; Liu *et al.* 2021). Population structuring indicates higher variation amongst individuals from Numeralla with some mixing between the two sites, which is to be expected as the sites are only 15 km apart. The WGS data here provide a baseline with which to compare the genetic health of Monaro koalas in the future and also add to the growing body of genomic data becoming available for koalas.

1.5.5 Biochemical and Haematological Parameters

Although we detected some differences in haematological and biochemical parameters between koala groups, health checks, Chlamydia status and age classes, values were predominantly within the published reference ranges (Canfield *et al.* 1989; Dunstan *et al.* 2021). While this suggests that the differences noted are unlikely to be biologically meaningful, perhaps reflecting normal variation between and within individuals, it should also be considered that wildlife reference ranges are generated on small sample sizes and either for captive koalas of known health status or free-ranging koalas, the health and stress status of which can only be partially assessed, and so may be broader than true ranges.

Most of the differences observed between biochemical and haematological parameters cannot be easily explained, but some results provide possible insight into the effects of fire and rehabilitation on koala health. The first is for urea. Blood urea and creatinine levels assist with assessment of renal function (Braun *et al.* 2003; Finco 1997; Yakubu and Musa 2012). Both rehabilitated koalas and non-rehabilitated koalas in the burnt landscape had higher concentrations of urea 4–6 months after fire at the pre-release check compared to non-rehabilitated koalas in the unburnt area, but the difference disappeared by the final recapture check 8–9 months later. There could be several reasons for this. Firstly, koalas living in the burnt area may be more dehydrated due to ingestion of leaves with a lower moisture content compared to those in the unburnt area. However, preliminary data suggest that post-fire epicormic regrowth on eucalypts contains more moisture than mature leaves in the canopy (Natural Resources Commission 2022). Furthermore, since creatinine, which can be used with urea to assess dehydration, was not elevated in burnt non-rehabilitated koalas at the pre-release health check and values were mostly within

the reference range for both urea and creatinine, the results may not indicate dehydration. An alternative may be related to urea as an indicator of protein status and thus nutrition (Robert and Schwanz 2013). For example, low creatinine and urea concentrations observed in tammar wallabies (*Notamacropus eugenii*) were linked to nutritional stress (Robert and Schwanz 2013). Similarly, red-necked pademelons (*Thylogale thetis*) had higher plasma urea levels with a higher consumption of nitrogen in the diet (Dellow and Hume 1982). The epicormic regrowth from some eucalypt species can contain relatively high concentrations of nitrogen (a proxy for protein) (Lane *et al.* 2024). It is plausible that koalas in care or living in burnt habitat may have been eating diets higher in protein prior to their pre-release check and that this may have contributed to the difference in urea status. Future studies could investigate links between dietary protein intake and levels of urea in koalas.

A number of enzymes found predominantly in the liver can be indicators of hepatocellular damage and disease (Kaplan 1972; Sherman 1991; Stojević *et al.* 2005; Whitehead *et al.* 1999; Whitfield 2001). These include AST, ALT, ALP and GGT. These enzymes can be non-specific and so should be observed in conjunction with one another and with clinical signs (Bennett *et al.* 2008; Center 2007). Elevated levels of AST and GGT have been associated with lymphosarcoma and liver disease in koalas (Canfield *et al.* 1989; Spencer and Canfield 1996), as well as diabetes mellitus (Hemsley *et al.* 1998) and oxalate nephrosis, a prevalent disease in South Australian koalas (Speight *et al.* 2014). A study on chlamydiosis in koalas also found elevated AST and ALT levels; however, this was during treatment with subcutaneous injections and the results could potentially have been explained by localised myopathy (Griffith 2010). We found no differences in AST and ALT levels between Monaro koalas that tested either negative or positive for *C. pecorum*. Furthermore,

mean values for all liver enzymes were within the reference ranges for both negative and positive cases of Chlamydia. This may not be surprising given that most infections were subclinical.

Elevated AST can also be attributed to haemolysis and liver and muscle damage (Kaneko *et al.* 2008; Letendre *et al.* 2018). It is likely that the elevated levels in rehabilitated koalas at their rescue health check were associated with impacts of poor nutrition and stress metabolism on hepatocyte integrity, rather than primary liver disease. Elevated AST has been associated with stress in a variety of marsupials, including capture stress in western ringtail possums (*Pseudocheirus occidentalis*) (Clarke *et al.* 2013a), trauma from fighting in hairy-nosed wombats (*Lasiiorhinus latifrons*) (Gaughwin and Judson 1980) and oxalate nephrosis (Speight *et al.* 2014). Bilirubin, a bile pigment formed when haemoglobin breaks down and important in liver metabolism (Bosma 2003; Stocker *et al.* 1987; Wolf 1999), was higher in rehabilitated koalas when they came into care compared to when they were released. In horses, which are hindgut fermenters like koalas, elevated bilirubin can be associated with fasting (Gronwall and Mia 1972). It is possible that this could also have been an indication that rescued koalas did not have access to sufficient food in the wild.

Leukocytes (white blood cell count (WBC)) form a major part of the immune system (Janeway Jr *et al.* 2001), and include lymphocytes, neutrophils, monocytes, eosinophils and basophils (Janeway Jr *et al.* 2001). While differences were detected in lymphocyte and neutrophil concentrations, most values were within reference ranges, and the higher values observed in some non-rehabilitated koalas in the burnt area at pre-release were within the reference range at recapture. The neutrophil/lymphocyte ratio (N:L) is sometimes used to assess inflammatory

response (Faria *et al.* 2016; Forget *et al.* 2017), with an elevated neutrophil/lymphocyte ratio associated with stress and illness in laboratory animals and domestic pets (Hickman 2017; Neumann 2021; Swan and Hickman 2014). In this study, we found no differences between koala groups or health checks for the N:L ratio. Although linked to stress and disease, Canfield *et al.* (1989) found that neutrophil and lymphocyte proportions and ratios can be highly variable in healthy koalas.

Variation was also observed in nucleated red blood cells (NRBCs), with differences detected between health checks, though the reason for this is unclear. NRBCs are immature red blood cells (Constantino and Cogionis 2000) and their presence in the bloodstream can be associated with bone marrow damage and a number of diseases in people (Constantino and Cogionis 2000; Schaefer and Rowan 2000). In koalas, however, NRBCs are relatively common and are not always indicative of disease (Bolliger and Backhouse 1960; Canfield *et al.* 1989; Hajduk *et al.* 1992; Spencer and Canfield 1995). Reference ranges for NRBCs have been reported to be between 0 and 8–10 for koalas (Spencer and Canfield 1994; Spencer and Canfield 1995). Higher NRBCs have been linked with disease in koalas, for example, Fabijan *et al.* (2020) found that koalas with neoplasia had significantly higher counts of NRBCs and koalas with ocular Chlamydia also had higher NRBC counts compared to those who had urinary tract and reproductive disease, though the mechanism for this is unclear. Higher counts of NRBCs have also been documented in koalas with anaemia (Spencer and Canfield 1995). The values observed in the koalas from the Southern Tablelands are much higher and more variable than reports from other studies. While this could suggest underlying health issues, their body condition and other results suggest that they are otherwise in a

good health condition. While the presence of NRBCs in these koalas could suggest regenerative anaemia, young koalas can also have higher counts of NRBCs.

1.6 Conclusions

This study provides important baseline health data for the Snowy Monaro population of koalas. Koalas in the Monaro region of NSW appear to be in good health, with low prevalence of clinical Chlamydia and relatively high proportions of adult females with joeys. The study population had adequate to excellent body condition by the completion of the project, even though 19 of the 32 tracked koalas were living in burnt habitats. From a management perspective and given the health status of koalas in this region, it is important to try and conserve this seemingly healthy population and the valuable habitat that can support them.

This is the first study to demonstrate that the general health indicators of koalas living in burnt habitat between 4 and 16 months after fire were similar to koalas living in unburnt habitat during the same time period. The health of koalas released into burnt habitats after rehabilitation mirrored that of koalas that remained in situ after fire in both burnt and unburnt habitats. These findings suggest that, in the Monaro, uninjured koalas do not need to be removed from burnt habitats and rehabilitated koalas can be released into fire-impacted areas, provided there is access to patches of trees with canopy cover or epicormic growth. However, more research is needed in this area to determine at what point there is too little food, and the other implications of rehabilitation, such as movements and habitat use. Many of the koalas in this study were living within the fire scar and utilising burnt areas. In the future, it would also be important to look at the home ranges of koalas and the nutritional composition of the landscape to better understand how koalas are using

fire-impacted areas. Future research could also explore whether the findings from this study are consistent across other regions and koala populations. Retaining uninjured koalas in the landscape alleviates resources needed to maintain animals in care and enables them to recover in their natural environment, which may be less stressful overall. Inevitably, there will be more bushfires in the Australian landscape, and the implications of this study have direct applications to management decisions and rehabilitation efforts for koala populations post-fire.

Data Availability Statement

All raw fastq sequences, aligned bam files and metadata for koala genomes from the Koala Genome Survey are available at https://awgg-lab.github.io/australasiangenomes/species/Phascolarctos_cinereus.html (accessed on 4 July 2020). Raw data used for analyses of health parameters, body condition and Chlamydia status is online at the Australian National University Data Commons Library:

<https://datacommons.anu.edu.au/DataCommons/rest/display/anudc:6328?layout=def%3adisplay>

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1.7 Appendix

A1: sampling of koalas following capture and restraint from the Koala Health Hub:

<https://drive.google.com/file/d/1MyOOQwXsIfAZAVwipCDv2NwYuMXTy9XU/view>

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1.9 Supplementary material

Supplementary material 1: List of all koalas including group, health check, sex, tooth wear class, presence of pouch young and outcome.

Koala name	Group	Health checks				Sex	Tooth wear class	Presence of back(B)/pouch (P) young	Outcome
		Rescue	Pre-release	Recapture					
Sterling	Rehabilitated	Yes				F	4		Died
Miles	Rehabilitated	Yes				M	4A	NA	Died
Allison	Rehabilitated	Yes				F	4	B	Euthanised
AJ	Rehabilitated	Yes				F	3	B	Euthanised
Billie	Rehabilitated	Yes				F	3		Euthanised
Isabella	Rehabilitated	Yes				F	2		Euthanised
Malu	Rehabilitated	Yes				M	5	NA	Euthanised
Ash	Rehabilitated	Yes				F	3	P	Released
Mallee	Rehabilitated	Yes				F	2	B	Released
Kim	Rehabilitated	Yes				F	3	P + B	Released
Hayley	Rehabilitated	Yes				F	1	NA	Released
Imogen	Rehabilitated	Yes				F	1	NA	Released
Kath	Rehabilitated	Yes				F	1	NA	Released
Camo	Rehabilitated	Yes				F	1	NA	Released
Montanna	Rehabilitated	Yes				F	2		Released
Gabou	Rehabilitated	Yes				M	5	NA	Released
Craig	Rehabilitated	Yes				M	4	NA	Released

Koala name	Group	Health checks				Sex	Tooth wear class	Presence of back(B)/pouch (P) young	Outcome
		Rescue	Pre-release	Recapture					
Beck	Rehabilitated	Yes				M	4	NA	Released
William	Rehabilitated	Yes				M	1	NA	Released
Tahlia	Rehabilitated	Yes	Yes			F	2	P	Released and GPS-tracked
Amelia	Rehabilitated	Yes	Yes			F	2	NA	Released and GPS-tracked
Jessie	Rehabilitated	Yes	Yes			F	3	P + B	Released and GPS-tracked
Tallow	Rehabilitated	Yes	Yes	Yes		M	2	NA	Released and GPS-tracked
Bear	Rehabilitated	Yes	Yes	Yes		M	3.5	NA	Released and GPS-tracked
Paul	Rehabilitated	Yes	Yes			M	3	NA	Released and GPS-tracked
Rick	Rehabilitated	Yes	Yes	Yes		M	5	NA	Released and GPS-tracked
Ian	Rehabilitated	Yes	Yes			M	4	NA	Released and GPS-tracked
Jarrah	Rehabilitated	Yes	Yes			M	2	NA	Released and GPS-tracked
Matthew	Rehabilitated	Yes	Yes			M	2.5	NA	Released and GPS-tracked
Mark	Rehabilitated	Yes	Yes			M	2	NA	Released and GPS-tracked
Hamish	Rehabilitated	Yes	Yes	Yes		M	2	NA	Released and GPS-tracked
Claire	Non-rehabilitated in burnt		Yes	Yes		F	4	P	GPS-tracked
Romane	Non-rehabilitated in burnt		Yes	Yes		F	4		GPS-tracked
Bronwen	Non-rehabilitated in burnt		Yes	Yes		F	3	P	GPS-tracked
Sage	Non-rehabilitated in burnt		Yes	Yes		F	4B		GPS-tracked

Koala name	Group	Health checks			Sex	Tooth wear class	Presence of back(B)/pouch (P) young	Outcome
		Rescue	Pre-release	Recapture				
Peggy	Non-rehabilitated in burnt		Yes	Yes	F	2		GPS-tracked
Evan	Non-rehabilitated in burnt		Yes	Yes	M	4B	NA	GPS-tracked
Drew	Non-rehabilitated in burnt		Yes	Yes	M	5	NA	GPS-tracked
Jett	Non-rehabilitated in burnt		Yes		M	1	NA	GPS-tracked
Owen	Non-rehabilitated in burnt		Yes	Yes	M	2	NA	GPS-tracked
Koala	Non-rehabilitated in unburnt		Yes		F	5	N	Euthanised
Rosalie	Non-rehabilitated in unburnt		Yes	Yes	F	3		GPS-tracked
Tamara	Non-rehabilitated in unburnt		Yes		F	4	P	GPS-tracked
Grace	Non-rehabilitated in unburnt		Yes		F	5	P	GPS-tracked
Annie	Non-rehabilitated in unburnt		Yes		F	5		GPS-tracked
Madonna	Non-rehabilitated in unburnt		Yes	Yes	F	4C	P	GPS-tracked
Mia	Non-rehabilitated in unburnt		Yes		F	1	NA	GPS-tracked
Zinger	Non-rehabilitated in unburnt		Yes		F	1	NA	GPS-tracked
Jaime	Non-rehabilitated in unburnt		Yes		F	2	P	GPS-tracked
Brandy	Non-rehabilitated in unburnt		Yes		F	2	NA	GPS-tracked
CS	Non-rehabilitated in unburnt		Yes	Yes	F	2		GPS-tracked
Xavier	Non-rehabilitated in unburnt		Yes	Yes	M	4A	NA	GPS-tracked
Rod	Non-rehabilitated in unburnt		Yes		M	5	NA	GPS-tracked

Koala name	Group	Health checks				Sex	Tooth wear class	Presence of back(B)/pouch (P) young	Outcome
		Rescue	Pre-release	Recapture					
Murray	Non-rehabilitated in unburnt		Yes	Yes	M	4B	NA		GPS-tracked
Kenny	Non-rehabilitated in unburnt		Yes	Yes	M	5	NA		GPS-tracked
Alex	Non-rehabilitated in unburnt		Yes		M	2	NA		GPS-tracked

Chapter 2: Home ranges and movements of an arboreal folivore after wildfire: comparing rehabilitated and non-rehabilitated animals in burnt and unburnt woodlands

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2.1 Abstract

Wildfires can have complex effects on wildlife populations. Understanding how post-fire conditions affect the movement ecology of threatened species can assist in better conservation and management, including informing the release of rescued and rehabilitated animals. The 2019–2020 megafires in Australia resulted in thousands of animals coming into care due to injury or concerns over habitat degradation. This included hundreds of koalas (*Phascolarctos cinereus*), for which relatively little was known about how fire affected habitat suitability, or when rehabilitated animals could be returned to burnt areas. We compared the movements of 32 koalas across three experimental groups – non-rehabilitated koalas in burnt habitat, non-rehabilitated koalas in nearby unburnt habitat, and rehabilitated koalas returned to their rescue location in burnt habitat in New South Wales, Australia. We GPS-tracked koalas for up to nine months and compared home ranges, mean nightly distance moved, farthest distance moved from release site and total displacement distance between treatment groups. We found no differences in koala movements and home range size between non-rehabilitated koalas in burnt and unburnt habitat. However, rehabilitated koalas moved farther from their release site, had larger displacement distances, and larger home ranges than non-rehabilitated individuals. Regardless of their experimental group, we also found that males moved farther than females each night. Additionally, our resource selection analysis showed that, koalas preferred low and moderately burnt habitats over all other fire severity classes. Experimental frameworks that incorporate “treatment” and “control” groups can help isolate disturbance effects on animal movements. Encouragingly, despite catastrophic wildfires, burnt woodlands provided adequate resources for koalas to persist and recover. Furthermore, rehabilitated koalas re-integrated into the burnt landscape

despite moving farther from their release sites than non-rehabilitated individuals. Studies like this improve our understanding of the ecological impacts of fire on species and their habitats, and will be instrumental in informing wildlife management and conservation efforts as wildfires increase in frequency and severity worldwide in response to climate change.

Keywords: *Wildlife rehabilitation, habitat, koala, landscape disturbance, endangered species, conservation, radio-tracking*

2.2 Introduction

Animals move through their environment to find and defend resources, disperse, breed, avoid predation, interact with conspecifics, and seek shelter (Doherty *et al.* 2019; Nimmo *et al.* 2019). These essential movements normally occur within a defined area, which is often described as a home range (Burt 1943; Cooper Jr 1978; Dingle 2014). Several intrinsic and extrinsic factors can influence home range size, including body size (Lindstedt *et al.* 1986; McNab 1963), age (Badyaev *et al.* 1996; Saïd *et al.* 2009), sex (Cederlund and Sand 1994; Gardiner *et al.* 2019), food availability and quality (Hanya *et al.* 2006; McGregor *et al.* 2023; Saïd *et al.* 2009; Schradin and Pillay 2006), climate (Morellet *et al.* 2013; Rivrud *et al.* 2010), and landscape fragmentation (Gardiner *et al.* 2019). Understanding the factors that affect home range size can provide important information about the space and resources needed by species and populations, explain the drivers of movement patterns (Spencer 2012), and assist with conservation and management planning.

Landscape disturbance from natural and anthropogenic sources (e.g., fire, floods, land clearing, logging, etc) can influence the structure of faunal and floral

communities and can fragment landscapes by creating physical barriers to movement (Driscoll *et al.* 2021; Silva *et al.* 2020; Youngentob *et al.* 2013). These can directly and indirectly affect how animals utilise and move within their environment (Berry *et al.* 2016; Nimmo *et al.* 2019; Silva *et al.* 2020; Whelan *et al.* 2002). Fire is a common stochastic disturbance process in many terrestrial environments and can result in short-term effects on animal movement patterns due to the immediate emigration of individuals out of a landscape that are trying to escape fire fronts, and cause longer-term shifts in movement patterns within burnt areas due to changes in habitat suitability and altered competition and predation dynamics (Engstrom 2010; Nimmo *et al.* 2019; Whelan *et al.* 2002). Changes in habitat suitability after fire can include the destruction or modified pattern of food and shelter availability. This can place substantial physiological stress on animals, which can negatively impact reproductive success, thermoregulation, and susceptibility to other threats such as predation and disease (Cristescu *et al.* 2023; Davies *et al.* 2013b; Degabriele 1981; Doherty *et al.* 2015; Hradsky 2020; Melzer *et al.* 2000; Narayan 2019). The ability to move and find resources in a burnt landscape can therefore affect the survival and recovery of individuals (Joly *et al.* 2012; Nimmo *et al.* 2014; Nimmo *et al.* 2019; Palm *et al.* 2022; Watson *et al.* 2012b).

Past research into the effects of fire on home ranges shows considerable variation between mammal species. Some species show no change in home range size following wildfire (Cook 2010; MacGregor *et al.* 2013; Thompson *et al.* 1989; Vernes and Pope 2001), while others expand their home ranges following fire (Green 2005; Herzog *et al.* 2014) or reduce them (Pasch and Koprowski 2011). Increases in home range sizes have been attributed to easier movement through landscapes to find resources in the absence of understorey vegetation (Herzog *et al.* 2014) or the

need to move farther to find food if its availability is reduced (MacGregor *et al.* 2013; Sutherland and Dickman 1999). Where home range sizes were similar after fire despite a measurable change in resource availability, this has been attributed to individuals accessing one resource in burnt areas and another in adjacent unburnt areas (MacGregor *et al.* 2013). The different responses reinforce the complex effects of fire on animal movements and home ranges and highlight the need to improve our understanding of the mechanisms that underly the varied patterns in wildlife population responses to fire.

The temperate forests of the south-east region of Australia are dominated by tree species of the *Eucalyptus* genus and are incredibly fire-prone (Attiwill and Adams 2013; Bradstock *et al.* 2012; Lucas *et al.* 2007; Russell-Smith *et al.* 2007). This region is also subjected to lower rainfall and hot dry summers, which are significant contributors to major wildfires (Attiwill and Adams 2013). From August 2019 to March 2020, extensive bushfires burned over 12 million hectares of eastern Australia (Wintle *et al.* 2020). These fires were unprecedented in both scale and severity (Dickman *et al.* 2022; Legge *et al.* 2022; Wintle *et al.* 2020). Throughout this time, there were extensive wildlife rescue and rehabilitation efforts (Dunstan *et al.* 2021; Parrott *et al.* 2021). One species that received particular attention was the koala. During the 2019–2020 fires, an estimated 60,000 koalas were killed or injured (Van Eeden *et al.* 2020), and over 900 taken into wildlife care triage units (Cope *et al.* 2024; Lunney *et al.* 2022; Parrott *et al.* 2021).

Fire affects the distribution and abundance of koalas (Cristescu *et al.* 2023; Melzer *et al.* 2000), and could also influence short-term behavioural shifts in landscape use due to changes in population density, food quality and availability. Koalas, like many other animal species, form distinct home ranges, with males and

females tending to overlap (Davies *et al.* 2013a; Ellis *et al.* 2002; Hindell and Lee 1988; White 1999). Koala home range sizes can be influenced by a number of factors, such as the sex and age of the animal, population density, and the availability and quality of feed trees (Logan and Sanson 2002; Martin 1999; Radford Miller 2012; Ramsay 1999). Very few studies have investigated the effects of fire and rehabilitation on movements and home range sizes of the koala (but for exceptions see Lunney *et al.* 2004; Matthews *et al.* 2016). Evaluating home range size after fire can provide insight into disturbance related changes in habitat quality, population health, and resilience, which can help to improve landscape and wildlife management (Ward *et al.* 2022; Ward *et al.* 2020).

Understanding how rehabilitation in captivity may affect the longer-term recovery of individuals after fire is also important for decision-making around this type of intervention. Rehabilitation success is often determined by recovery and survival of animals in care to the point of release (Lunney *et al.* 2004); however, a better metric is survival post-release, which is not always monitored or recorded (Hernandez 2019; Nichols and Armstrong 2012). Some studies have found that, after release, rehabilitated animals move farther than non-rehabilitated individuals, which may be due to several factors, including being displaced or even losing the skills to compete and survive following time in care (Gaydos *et al.* 2013; Goldenberg *et al.* 2022; Tolhurst *et al.* 2016). Increased movements and dispersal can be energetically costly (Bonte *et al.* 2012; Roff 1977), lead to increased exposure to predators (Yoder *et al.* 2004) and mortality (Goldenberg *et al.* 2022; Linnell *et al.* 1997; Roff 1977). Post-release effects such as these could be exacerbated when the release environment has been affected by disturbance, such as fire (Cope *et al.* 2024). For example, there could be greater conflict with non-rehabilitated individuals due to

increased competition for limited food resources. Since fire severity and frequency in many parts of the world are predicted to increase with climate change (Lucas *et al.* 2007), we need to improve our understanding of post-release issues to effectively manage the reintegration of rehabilitated individuals into surviving populations of threatened species.

This study addresses identified knowledge gaps in managing wildlife living in landscapes impacted by fire, including the success of post-rehabilitation release (Cope *et al.* 2024). The specific aim was to determine whether fire and/or rehabilitation affect the home range sizes and movements of koalas. We addressed this aim within an experimental framework, which is commonly lacking in these types of studies due to the stochastic nature of fire and wildlife rehabilitation (Cope *et al.* 2024). We monitored koalas fitted with GPS collars across three treatment groups: 1) non-rehabilitated koalas in burnt habitat, 2) non-rehabilitated koalas in unburnt habitat, and 3) rehabilitated koalas released into their original burnt habitat. Each koala was monitored for up to nine months.. Since severe fire can influence the availability of foliage biomass (Jhariya and Raj 2014; Shaffer *et al.* 2018), we expected that food resources would be diminished for koalas in the burnt landscape, resulting in them moving farther each day to obtain adequate nutrition and have larger home ranges compared to individuals in unburnt habitat. Because rehabilitated koalas had to reintegrate into the population in burnt habitat, where competition for resources may be increased, we also expected that they would have larger home ranges and movements compared to non-rehabilitated koalas.

2.3 Materials and methods

2.3.1 Study sites

The study was located in the New South Wales (NSW) Snowy Monaro Shire, a local government area in Australia spanning just over 15,000 km² and located approximately 130 km south of the Australian Capital Territory (Gould *et al.* 2019). Much of the koala tracking work was located on the Macanally-Numeralla ranges. The area is on steep terrain, dominated by north-to-south facing ridges (Office of Environment and Heritage NSW National Parks and Wildlife Services 2012). The elevation ranges from 800-1233 m above sea level (Leavesley and Rhind 2008) and the underlying geology is comprised of Palaeozoic Era sediments, with fluvial sandstones and lacustrine sediments amongst granite plutons and alkali basalts (Martin *et al.* 2020). Overstorey vegetation is dominated by red stringybark (*Eucalyptus macrorhyncha*) and scribbly gum (*Eucalyptus rossii*), with ribbon gum (*E. viminalis*) and candlebark (*E. rubida*) more prominent in creek lines, and snow gums (*E. pauciflora*) at higher elevations (Office of Environment and Heritage NSW National Parks and Wildlife Services 2012). Brittle gum (*Eucalyptus mannifera*), broad-leaved peppermint (*Eucalyptus dives*), and apple box (*Eucalyptus bridgesiana*) were also common in some areas (Adams 2023).

The Monaro Tableland, which encompasses the study area, is within a rain shadow zone (Keith and Bedward 1999; Office of Environment and Heritage NSW National Parks and Wildlife Services 2012). Prior to the fires in 2019, annual rainfall in the nearest city, Cooma, was 318.6 mm, which is well below the annual average of 528.1 mm (Bureau of Meteorology 2020). Mean daily minimum and maximum temperatures in the Snowy Monaro Shire at these sites average 9°C to 27°C in summer, and -3°C to 13°C in winter (Martin *et al.* 2020).

The “burnt” site was burnt by wildfire in January 2020, with large areas of moderate to complete canopy loss (figure 1). The “unburnt” site was located approximately 15 km to the south and was not directly impacted by fire. Both sites were connected by continuous forest cover, and had similar tree species composition.

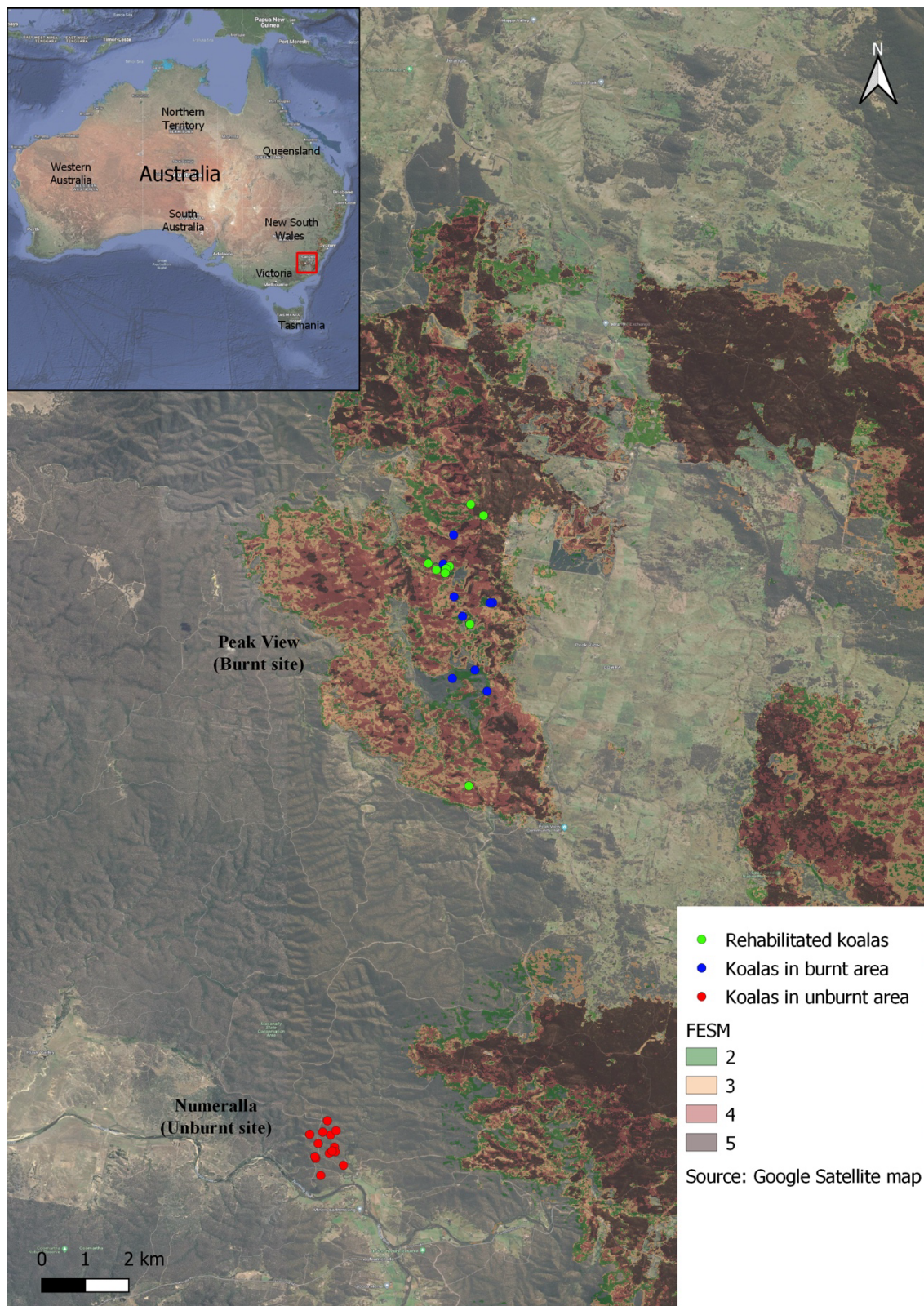


Figure 1. Initial capture locations of all GPS-tracked rehabilitated koalas (green), and non-rehabilitated koalas in burnt habitat (blue) and unburnt habitat (red) (map data ©Google). Fire extent and severity mapping (FESM) (State Government of NSW and

Department of Planning and Environment 2020) is also shown, with 2 representing the lowest severity (burnt understorey with unburnt canopy), 3 representing moderate (partial canopy scorch), 4 representing high (completed canopy scorch, +/- partial canopy consumption) and 5 representing extreme (complete canopy consumption). The inset shows the location of the study area within south-eastern Australia.

2.3.2 Koala groups

We used three treatment groups of koalas: 1) non-rehabilitated koalas in burnt habitat, 2) non-rehabilitated koalas in unburnt habitat, and 3) rehabilitated koalas released into the burnt habitat where they were originally rescued. These were the same groups of koalas that were outlined in chapter one. The number of koalas in each group was a combined function of the available numbers of individuals and logistical considerations regarding tracking larger numbers of animals in multiple areas. The rehabilitated koalas comprised ten individuals that had been rescued from burnt habitat in the study area between early January 2020 and early March 2020 due to poor body condition and bushfire-related injuries. Rehabilitated koalas were released between June 2020 and November 2020. All rehabilitated koalas apart from a young, orphaned male were released at their capture location. The young male was housed and released with an adult female and her joey ~4.6 km from his capture location. Release timing depended on the overall health of each koala (subject to a health check – see “Health checks, collar fitting and release”) and evidence that there was food available in the area from which they were rescued (trees with intact canopy or producing new growth).

Non-rehabilitated koalas in burnt (n = 9) and unburnt landscapes (n = 13) were located between May 2020 and October 2020 using a combination of daytime ground-based searches and night-time thermal drone searches (Witt *et al.* 2020). These koalas were captured using the noose and flag method (Madani *et al.* 2020), placed into canvas bags and transported by car (usually less than 1 km) for a health check by a wildlife veterinarian.

2.3.3 Health checks, collar fitting and release

Koalas in all three groups were given a health assessment under sedation by an experienced koala veterinarian immediately prior to being fitted with a GPS tracking collar and released (Lane *et al.* 2023). The koalas ranged in age from <2 years to >10 years old (based on tooth wear; Martin 1981) and included 15 males and 17 females (Supplementary material 1).

While sedated, we fitted koalas with a Lotek LiteTrack 60 collar (Lotek Havelock North, New Zealand) with VHF and GPS capabilities. These collars weighed approximately 80g each and had a “weak link” made of elastic that was designed to break under strain. Koalas were placed in a pet carrier covered with a towel in a quiet room to recover from sedation. All of the non-rehabilitated koalas from the burnt and unburnt landscapes were released on the same day of their capture, at their capture location.

2.3.4 Koala tracking and monitoring

Given that koalas are nocturnal, collars collected five GPS points per 24-hour period at three-hourly intervals between 1900 h and 0700 h for the duration of the study. This data was stored on the collars and could be downloaded remotely using a

paired PinPoint Commander (Lotek, Havelock North, New Zealand). If there was no movement detected for 24 hours, a mortality signal would activate.

We visually checked koalas daily for the first two weeks following the collar fitting, twice per week for the following two weeks, and then weekly when possible for the remainder of the study. Koalas were located using their unique VHF signal. Once a collared koala was located, we observed them with binoculars to check for any signs of injury, illness or decline of health. Occasionally, there were times when a koala could not be visually accessed at a scheduled point in time due to, for example, inaccessible roads or the koala had moved to a property that we did not have permission to access. When this occurred, we remotely downloaded their GPS data to check that their daily movements were normal (i.e., that they were moving around at night).

2.3.5 Collar removal

We removed collars from koalas after approximately nine months, unless the collar had already fallen off due to breakage of the weak link or slipping over the koala's head. Koalas were captured using the same noose and flag method, and received another veterinary health check under sedation (Lane *et al.* 2023). Once recovered, koalas were released back into the same tree from which they were captured.

2.3.6 Home range calculations

In the context of this study, home ranges were defined as the area used by koalas (50% and 95% kernel utilisation distributions) across the entire period over which they were tracked.

Before calculating home ranges, we cleaned the GPS data to remove points with poor spatial accuracy. I investigated the horizontal dilution of precision (HDOP) as a potential method to clean the data but found that the data still contained points that were clearly inaccurate and needed to use an alternative method that worked better. We removed any points where the recorded altitude fell outside the range available at the sites because preliminary inspection revealed that these locations were invariably inaccurate. We then calculated the straight-line distance and difference in altitude between each GPS fix. If either difference was unlikely given the site and terrain, we removed the point. We were able to determine which point to remove by comparing it to the other GPS fixes obtained that night, as it was almost always an obvious outlier (i.e., a large distance away from other points or at a much higher altitude etc.). Finally, if the distance moved between consecutive points was greater than 200m, we visually checked the location on a map relative to the previous and next point. In this case, we removed any points where a koala appeared to have moved to another location and then returned to the first at the following fix, because this scenario was unlikely. After this process, we further thinned the data to two GPS points per koala per night (22:00 and 07:00), to get the best spread and timing where koalas were most likely to have moved.

We calculated 50% and 95% kernel utilisation distributions (KUDs) for each koala using a bivariate normal kernel method in the *adehabitatHR* package (v0.4.19) (Calenge 2006) in R Statistical Software (v3.6.3) (R Core Team 2020). This widely-used method (Bouyoucos *et al.* 2020; Bryant *et al.* 2024; Nordberg *et al.* 2021; Worku *et al.* 2021) defines home range as the minimum area in which an animal has some specified probability of being located. The h value (smoothing parameter), which controls the “width” or size of the kernel function around each point was set to

the “reference” bandwidth defined as “*href*” (Calenge and Fortmann-Roe 2011). We generated asymptotes from the *hrBootstrap* function in the *adehabitatHR* package to determine which koalas did not have a home range. These were individuals that were still moving significant distances, which suggest they had not ‘settled’ or were not in a defined ‘range’. These koalas were not used in statistical comparisons of home ranges. Using the output from the analysis, we generated maps of home ranges in QGIS v.3.22.8 (QGIS Development Team 2021).

2.3.7 Calculation of movements

We calculated the nightly movement distance for each koala by adding the straight-line distances between each sequential GPS point between 1900 h and 0700 h the following morning. We used these values to calculate the average nightly distance moved by koalas throughout the study, as well as the average nightly distance moved during different seasons. We calculated the displacement distance for each koala by measuring the straight-line distance between the point at which koalas were released after being fitted with a tracking collar and the location at which they either lost their collar or were recaptured. We calculated the greatest distance moved by measuring the farthest distance a koala was recorded from its initial capture location.

2.3.8 Resource use analysis

We used resource selection analysis to investigate whether koalas use habitat in different fire severity classes in proportion to its availability within their home ranges.

Using the *raster* package (Hijmans 2024) in R and the NSW fire extent and severity mapping spatial dataset (State Government of NSW and Department of Planning and Environment 2020) we calculated the proportional availability of each fire severity class (as described by the State Government of NSW and Department of Planning and Environment 2020) within the home range of each koala that spent time in burnt habitat ($n = 18$). We also extracted the fire severity class at every GPS point used to generate home ranges, and calculated the proportion that fell within each of the five fire severity classes for each koala. We used the *compana* function in the *adehabitatHS* package (Calenge and Basille 2024) to perform a compositional analysis of habitat use (Aebischer *et al.* 1993), comparing proportional fire severity class use by each koala relative to the proportional availability of each fire severity class within their home range.

2.3.9 Statistical analyses

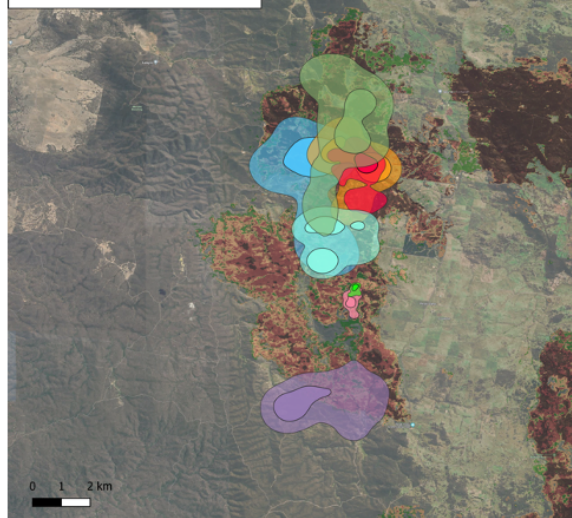
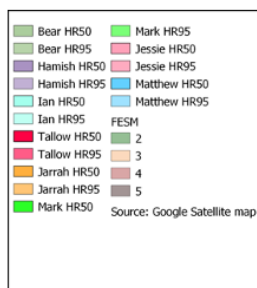
All analyses were performed using R Statistical Software (v3.6.3) (R Core Team 2020). We investigated whether home range size and movement data (displacement distance, greatest distance moved and mean nightly distance) differed between koala groups (rehabilitated, or non-rehabilitated in burnt or unburnt habitat), sexes or their interaction using ANOVAs. We also used an ANOVA to investigate whether mean nightly distance differed seasonally between male and female koalas. For greatest distance moved and displacement distance, all koalas tracked less than 50 days were excluded from analyses to avoid potential correlations between distance moved and the length of time tracked. We also excluded two female koalas from the non-rehabilitated unburnt group ('Rosalie' and 'Brandy') from all analyses because they moved from the unburnt habitat into burnt habitat during the study. Thus, their

movements may have been influenced by characteristics of both unburnt and burnt habitat, and it was not appropriate to consider them within the unburnt group for comparative purposes. Despite this, their data are still interesting and could be informative, so we have included them descriptively in the results. Displacement distance, greatest distance moved, and 50% and 95% KUDs were all logged prior to analysis to achieve normal distribution. If results of the ANOVA were statistically significant ($p < 0.05$), we used the *emmeans* package (v.1.6.3) (Lenth 2021) to conduct pairwise t-tests to determine which treatments differed from the others ($p < 0.05$).

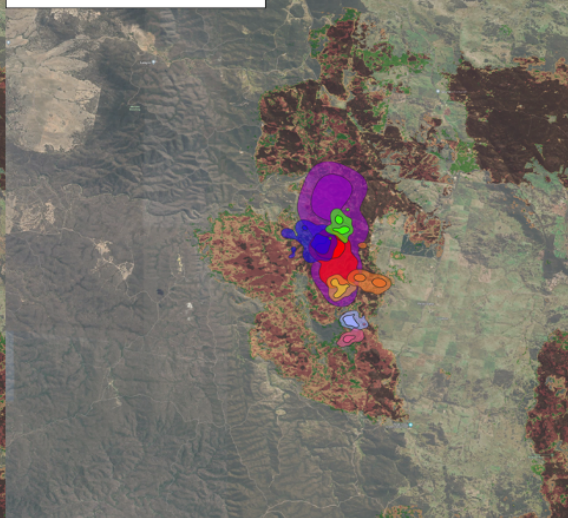
2.4 Results

Thirty-two koalas across the three groups were followed for between 9 and 256 days each (Supplementary material 1). Both rehabilitated and non-rehabilitated koalas from the burnt landscape used burnt habitat extensively (figure 2). The average nightly distance moved by each koala ranged from 74 to 448 m (Supplementary material 1), although 14 koalas moved more than 1 km in a night at least once during the study. There was no difference in the mean nightly distances moved by koalas between the three groups ($F_{2, 26} = 1.35$, $p = 0.28$; figure 3a). However, male koalas moved farther each night than females ($F_{1, 26} = 24.35$, $p < 0.001$), especially during spring ($F_{1, 24} = 33.03$, $p < 0.001$) and summer ($F_{1, 17} = 34.21$, $p < 0.001$; figure 4).

a.



b.



c.

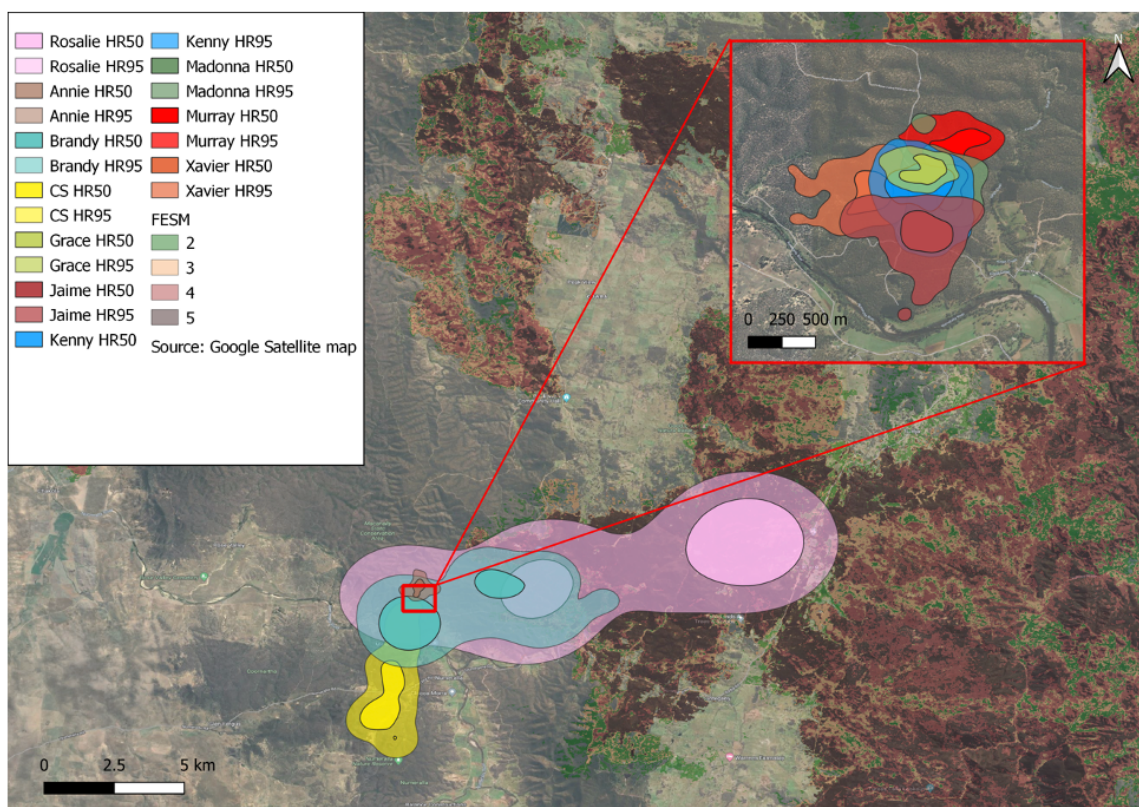
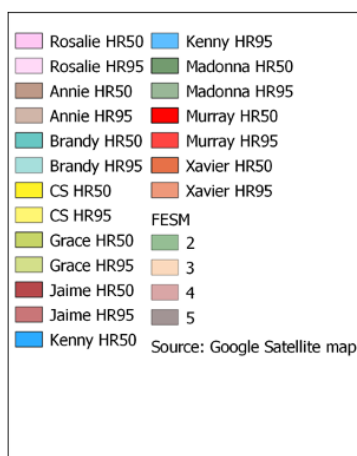


Figure 2. Home ranges at KUD 50% (darker colours) and 95% (lighter colours) for rehabilitated koalas (a), non-rehabilitated koalas in burnt habitat (b) and non-rehabilitated koalas in unburnt habitat (c) (map data ©Google). Part c also shows two koalas that moved from unburnt into burnt habitat (Brandy and Rosalie). The inset on figure 2c shows the home ranges of six koalas in the unburnt area with comparatively smaller home ranges. Note that the scale on figure 2c differs from that of 2a and b.

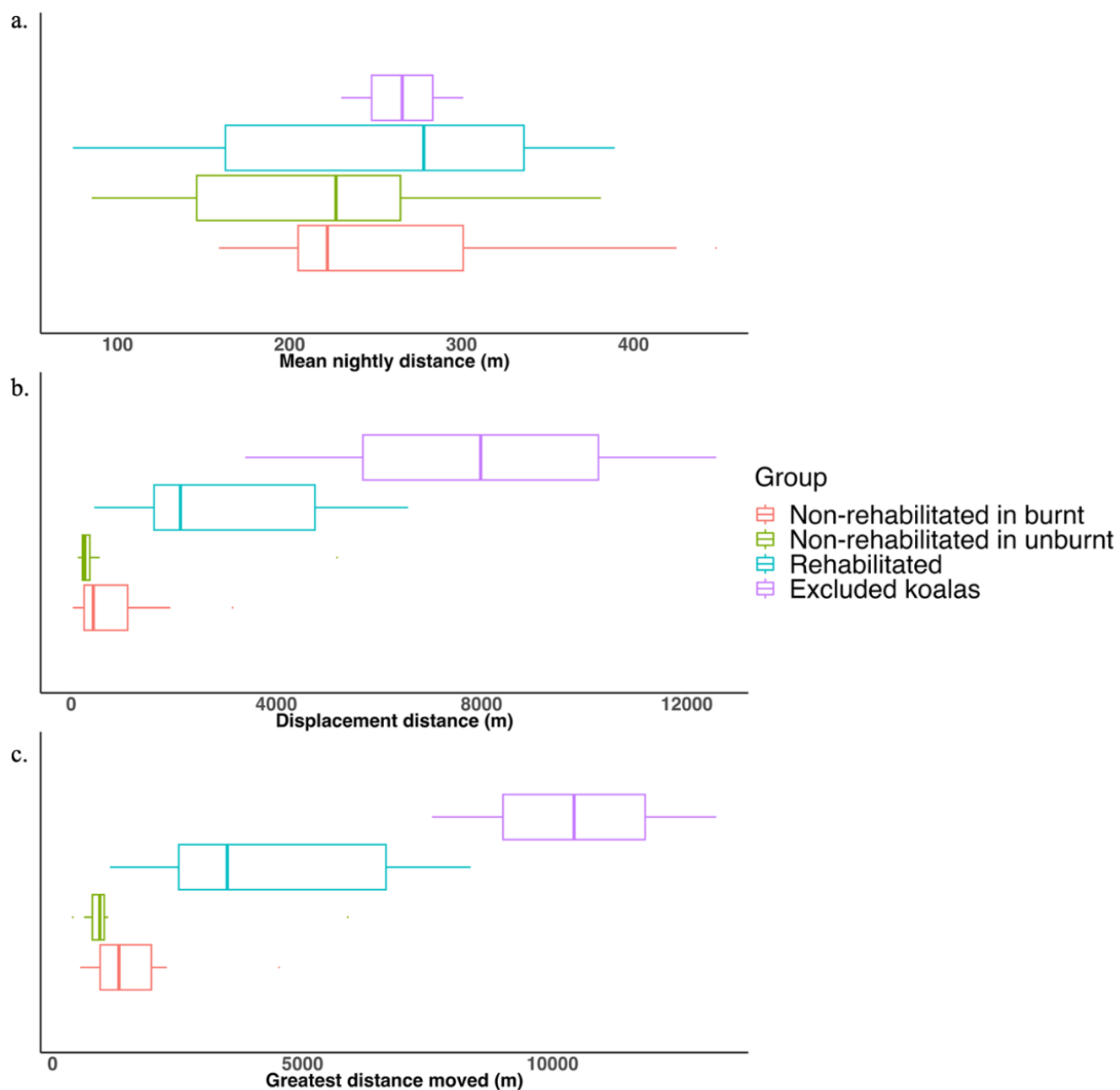


Figure 3. Mean nightly distance moved by all koalas ($n = 30$) (a), displacement distance for koalas tracked >50 days ($n = 23$) (b), and greatest distance moved from release point for koalas tracked >50 days (c) in each study group. Excluded non-rehabilitated koalas ($n = 2$) moved from unburnt habitat into burnt habitat, and therefore did not fit the criteria for any group. Note that the lines within each bar are the median value.

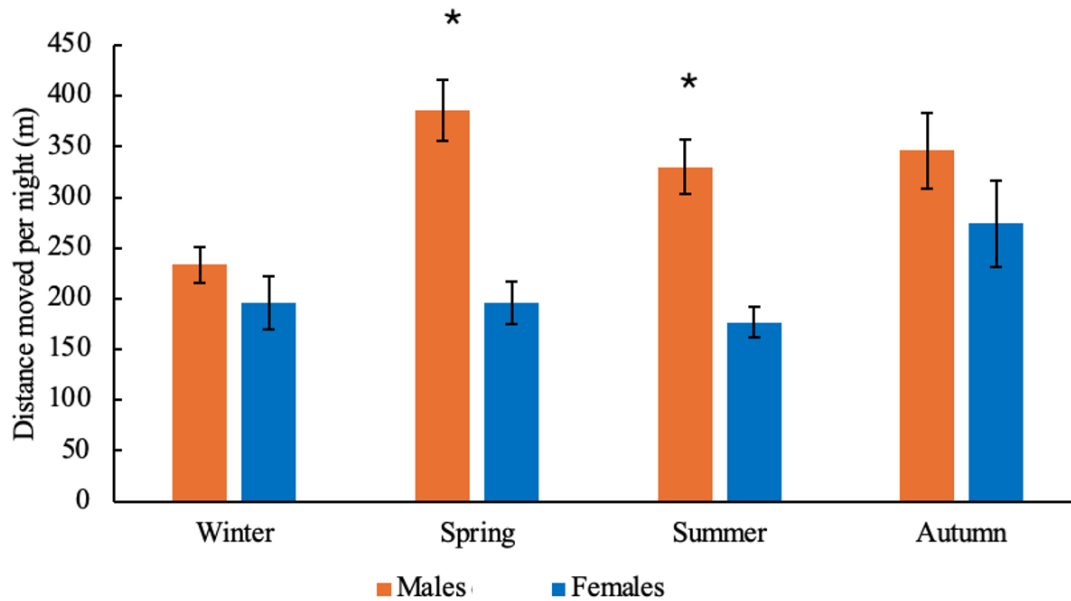


Figure 4. Mean $\pm$ SE nightly distances moved by koalas during different seasons. Seasons with an asterisk above them indicate that the distances moved are significantly different ($p < 0.05$) between males and females.

The maximum distance from the release location varied between koalas. (405 m to 8,362 m for koalas included in statistical analyses; Supplementary material 1). Similarly, the displacement distance between the first and last locations recorded for each included koala varied, ranging from 32 m to 6,567 m. For koalas tracked more than 50 days, both displacement distance, and greatest distance moved differed between koala groups ($F_{2,19} = 4.93$, $p = 0.019$; $F_{2,19} = 5.94$, $p = 0.010$, respectively; figure 3b and 3c). On average, rehabilitated koalas had a displacement distance over three times that of non-rehabilitated koalas in both the burnt ($t(19) = 2.345$, $p = 0.03$) and unburnt landscapes ($t(19) = 2.615$, $p = 0.017$; figure 3b). We found a similar pattern for the greatest distance moved. The greatest distance moved was 2.5 times higher in rehabilitated koalas compared to non-rehabilitated in the burnt area ($t(19) = 2.240$, $p = 0.037$) and was three times higher compared to non-

rehabilitated in the unburnt ($t(19) = 3.032, p = 0.007$; figure 3c). Displacement distance and greatest distance moved did not differ between males and females ($F_{1, 19} = 0.295, p = 0.594$; $F_{2, 19} = 0.392, p = 0.539$, respectively).

We determined home-range sizes for 26 koalas (8 rehabilitated koalas, 8 non-rehabilitated koalas in the burnt landscape, 8 non-rehabilitated koalas in the unburnt landscape and for the two koalas that moved from unburnt area into burnt (Supplementary material 1). For the six koalas that did not reach an asymptote generated using the *hrBootstrap* package, five were tracked for less than 50 days (one rehabilitated, one non-rehabilitated in the burnt, and three non-rehabilitated in the unburnt). The sixth koala was tracked for 120 days (a rehabilitated female), but she moved consistently in one direction. Home range size for the other 24 koalas in the treatment groups (11 females and 13 males) varied from 4.8 to 279.8 hectares at 50% KUD and 18.5 to 1,543.1 hectares at 95% KUD (figure 2; Supplementary material 1). At 95% KUD, mean home range size was different between koala groups ($F_{2, 20} = 3.639, p = 0.045$), with post-hoc analyses showing larger home range sizes for rehabilitated koalas compared to non-rehabilitated individuals in the unburnt landscape ($t(20) = 2.291, p = 0.033$; Table 1). 50% KUD, koalas in the rehabilitated group tended to have larger home ranges than koalas in the non-rehabilitated groups ($F_{2, 20} = 3.041, p = 0.070$; Table 1). On average, home range sizes for males and females were not different at both 50% KUD ($F_{1, 20} = 0.121, p = 0.732$) and 95% KUD ($F_{1, 20} = 0.114, p = 0.739$), with large variation between individuals of both sexes. There was no group by sex interaction observed at either 50% or 95% KUD.

There was substantial overlap in the home ranges of tracked koalas at the 95% KUD, particularly in the unburnt area, but less so at the 50% KUD. For koalas living in the burnt landscape ($n=16$), the core home range (50% KUD) of seven koalas did

not overlap with any other tracked individuals. However, for the remaining nine koalas, overlap occurred with at least one individual and up to a maximum of three individuals. For koalas in the unburnt landscape, only one individual had a core home range that did not overlap with any other koalas. There was more extensive overlap for the remaining nine koalas in the unburnt landscape, with overlap observed with at least one other individual, and maximum overlap occurring with eight other tracked individuals.

Table 1: Mean $\pm$ SE home range size at 50% and 95% kernel utilisation distribution (KUD) for koala groups and excluded non-rehabilitated koalas that used both habitat types.

Group	50% KUD (ha)	95% KUD (ha)
Rehabilitated koalas (n=8)	151.5 $\pm$ 39.8	773.8 $\pm$ 206.5
Non-rehabilitated in burnt habitat (n=8)	58.0 $\pm$ 28.0	250.8 $\pm$ 123.3
Non-rehabilitated in unburnt habitat (n=8)	45.1 $\pm$ 33.6	194.7 $\pm$ 142.7
Excluded koalas (n=2)	1178.1 $\pm$ 598.8	5880.4 $\pm$ 2741.7

Relative to their availability, koalas showed a preference for low and moderately burnt habitat over all other severity classes, including unburnt ($\lambda = 0.228$, $p = 0.002$) (table 2). They also used habitat in the high fire severity class more often relative to its availability than the extreme or unburnt classes (table 2). Fire severity classes were ranked in the following order of preference: low severity (class 2), moderate severity (3), high severity (4), extreme severity (5) and unburnt (0).

Table 2: Relative preference for fire severity categories by koalas considering availability. A description of the categories is provided as per State Government of NSW and Department of Planning and Environment 2020. The mean availability is the percentage of home ranges that comprised each severity class, averaged across the home ranges of all koalas that spent some time in burnt habitat and had a home range (n = 18). Symbols indicate whether koalas demonstrated a slight (+) or strong preference (+++) for, or avoidance (- or ---) of different fire severities.

	Severity class	Description	Mean [range] availability (%)	0	2	3	4	5
0	Unburnt	0% canopy and understorey burnt	17.7 [0.0 – 66.1]	0	---	---	---	-
2	Low	>10% burnt understorey >90% green canopy	16.1 [4.6 – 32.]		0	+	+++	+++
3	Moderate	20-90% canopy scorch	24.1 [5.7 – 43.6]			0	+++	+++
4	High	>90% canopy scorched <50% canopy biomass consumed	22.8 [8.0 – 40.5]				0	+++
5	Extreme	>50% canopy biomass consumed	19.2 [0.4 – 60.6]					0

2.5 Discussion

Understanding how environmental disturbances such as wildfire affect animal movements and home ranges is important for assessing and guiding the conservation and management of endangered species. In our study, the use of “control” groups within an experimental framework was particularly valuable because it allowed us to compare movement distances and home range sizes of rehabilitated and non-rehabilitated koalas in burnt and unburnt eucalypt woodland to understand how both bushfire and rehabilitation influenced movement behaviour. Our key findings were that: 1) contrary to expectations, the movements and home ranges of non-rehabilitated koalas were similar in burnt and unburnt habitat, and 2) as anticipated, displacement distances (distance between release and recapture points), the farthest distance moved from the release point, and home ranges were largest on average in rehabilitated koalas compared to their non-rehabilitated counterparts. Interestingly, our compositional analysis of habitat use showed that koalas preferred low to moderate burn severity, relative to all other burn severity categories. These findings have important implications for our current understanding of the value of burnt habitat for koalas and other marsupial folivores, and for refining intervention and release guidelines for koalas that are rescued from fire-affected areas.

2.5.1 Effects of fire on home ranges and movement

Our predictions of larger home ranges and longer movements in burnt habitat were based on the idea that fire reduces habitat quality (e.g. less food available; Masters 1993) and competitive pressure from conspecifics due to fire-related mortality (Crownier and Barrett 1979), forcing individuals to travel greater distances

to find adequate food and/or allowing them to expand into unoccupied habitat immediately after a fire event. Unquestionably, leaf biomass, and therefore food availability, is substantially reduced in eucalypt forests after high severity fire (Qin *et al.* 2022). Likewise, moderate to severe wildfire can substantially reduce koala abundance in burnt compared to unburnt areas (Cristescu *et al.* 2023; Law *et al.* 2022; Phillips *et al.* 2021). Despite these changes, movement distances and home range sizes of non-rehabilitated koalas in burnt habitat were similar to those in unburnt habitat within our study timeframe, which spanned five to sixteen months post fire.

By the time our study commenced, food availability may not have been limiting for the remaining animals. Eucalypts are well-adapted to fire, and many species produce new growth from epicormic buds within days or weeks (Burrows 2002; Pausas and Keeley 2017). In addition, a reduction in the availability of canopy foliage may be offset by an increase in the nutritional quality of epicormic leaves in a relatively short time after fire. This is further supported by our compositional analysis of habitat use, which demonstrated that koalas showed a preference for low to high burn severity over unburnt habitat, which could be linked to the epicormic foliage in these areas being more nutritious. Recent studies have shown that epicormic leaves are higher in nutritional quality in some eucalypt species than the adult-phase leaves that are present in unburnt areas (Lane *et al.* 2024). This may have contributed to our findings, since many trees in the burnt landscape had at least some epicormic growth at the time we initiated our study.

It was interesting to note that two koalas in our study who were initially captured as part of the group in unburnt habitat moved into burnt areas. Although they were

excluded from statistical analyses, this behaviour is worth discussing. At a minimum, the movement from unburnt to burnt habitat reinforces the idea that burnt landscapes can provide habitat for koalas. Additionally, the compositional analysis showed that koalas preferred low, moderate and highly burnt areas over extremely burnt areas and even unburnt areas, demonstrating that burnt areas can support koalas, and may even be favoured if the canopy has not been completely consumed. Other studies have observed similar results, with some grazing and browsing mammals attracted to burnt areas for the new growth of plants recovering from fire (Allred *et al.* 2011; Archibald *et al.* 2005; Klop *et al.* 2007; Moe *et al.* 1990). The movement of individuals from unburnt to burnt areas should also be considered in the context of post-fire recovery, whereby populations in unburnt areas can drive the recolonisation of burnt habitat (Shaw *et al.* 2021; Watson *et al.* 2012a). It would be valuable to design future studies to investigate the emigration of koalas from unburnt to burnt habitat to better understand how this may contribute to population recovery after wildfire. Additionally, to better improve our understanding of koala movements after fire, individuals should be tracked pre- and post-fire to better determine what movements are 'normal' and what movements may be driven by fire and living in a burnt landscape.

2.5.2 Effects of rehabilitation on home ranges and movement

Rescuing and rehabilitating wildlife can be stressful for animals (Vogelnest 2008), and the risks to animal welfare need to be weighed against the potential benefits for both the individual and the population (Cope *et al.* 2022). Some species have a low survival rate after release following rehabilitation (Beringer *et al.* 2004), while other species reintegrate back into the wild with minimal expense to fitness

(Myers and Young 2018). The effects of rehabilitation and release can be complex, with various factors contributing to outcomes (Leigh *et al.* 2023; Molony *et al.* 2007). We found that rehabilitated koalas had larger home ranges and larger displacement distances from their release site than non-rehabilitated koalas in both the burnt and unburnt landscape. While several studies have looked at koala movements following release from rehabilitation (e.g., Ellis *et al.* 1990; Goldingay and Dobner 2013; Leigh *et al.* 2023; Matthews *et al.* 2016), only Matthews *et al.* (2016) also compared their findings to non-rehabilitated koalas in the same area. In contrast to us, they found that rehabilitated koalas in Port Stephens had similar home range sizes to non-rehabilitated koalas. There are a number of differences between this study and ours. For example, in Matthews *et al.* (2016) study in Port Stephens, much of the land where the research was conducted was forest fragments surrounded by agricultural land and urban housing blocks, so koalas may not have been able to extend their home ranges. Additionally, it is not specified how long koalas were in care for, which could affect their behaviour after release. Future research is needed to better understand which factors drive these differences and could provide insights into when koalas will reintegrate most easily into their habitat after rehabilitation.

The larger movements of rehabilitated koalas are unlikely to be due to their release into burnt habitat *per se*, because their movements differed from non-rehabilitated koalas in the same area. Previous studies have found that rehabilitated koalas that are released away from their capture location often disperse to other areas (Beaman *et al.* 2023; Leigh *et al.* 2023). However, this does not explain our findings either because koalas in all groups were released at the location from which they were captured. Instead, there are other possible explanations, such as larger movements to refamiliarise themselves with the landscape, or competitive exclusion

by established, non-rehabilitated koalas. For example, rehabilitated red foxes (*Vulpes vulpes*) had larger home ranges and travelled farther from their release site than wild individuals, which was attributed to captivity causing territorial displacement (Tolhurst *et al.* 2016). To address this, future research could look at interactions among rehabilitated and non-rehabilitated koalas in the same landscape to better understand their social dynamics, particularly when male-female and male-male interactions are likely to be high, such as during the breeding season (Watchorn and Whisson 2019). This could guide more effective management of rescued koalas and aid rehabilitation and release practices.

Despite the greater distance moved and larger area used by rehabilitated individuals, measured health parameters were similar between koalas in all groups throughout the study (Lane *et al.* 2023). Furthermore, there was no change in the chlamydia status of individuals (a disease which can be triggered by stress; Weigler *et al.* 1988), and koalas in all groups improved in body condition over the study timeframe and had high rates of survival (Lane *et al.* 2023). This suggests that any costs associated with moving away from release locations did not have substantial longer-term (i.e. at nine months post-release) effects on the health of individuals. In combination with our findings, there is strong evidence from multiple studies that rehabilitated koalas are capable of integrating back into the landscape, including when it is burnt, and they can successfully form home ranges, although they may initially need to move farther from their release site to re-establish their territory (Goldingay and Dobner 2013; Leigh *et al.* 2023; Matthews *et al.* 2016).

Based on our findings, koalas in fire-affected landscapes that are injured or in poor body condition should be taken into care, but otherwise, uninjured koalas should be allowed to remain in burnt landscapes, as long as some browse is

available. For animals that are taken into care, our study demonstrates that rehabilitated individuals can be safely released at their rescue location rather than moving them to areas that are less disturbed, a practice that is sometimes undertaken (Leigh *et al.* 2023). Our findings should give wildlife carers confidence that rehabilitated koalas can be successfully released into burnt habitat, and to incorporate this practice into release policy. More research like this is needed across the range of the koala, since tree species composition can have substantial effects on food quality for koalas before and after fire (Lane *et al.* 2024). In addition, climatic conditions can vary considerably across the koala's large distribution and reduced canopy cover after fire may impact thermoregulation more substantially in some regions than others. During hot weather, koalas are known to select cooler microclimates (Briscoe *et al.* 2014; Crowther *et al.* 2014), and if the tree canopy is lost or reduced after fire, koalas have less access to shade. The ability to develop region specific guidelines would assist policy-makers, landscape managers and the wildlife care sector to more effectively conserve koalas and their habitat in the face of increasingly severe and frequent fires from anthropogenic climate change (Rhodes *et al.* 2006).

2.5.3 Challenges of interpreting animal movements

Studying animal movements and interpreting the outcomes of home range analyses can be challenging, as many animal species do not use their home range, or move, in a uniform way (Harris *et al.* 1990). It is often assumed that animal movement data captured at a particular time adequately reflects normal behaviour, however it fails to recognise dispersal and shifts in habitat use due to changes in the landscape and environmental conditions (Powell and Boitani 2012). Core home

range measurements (i.e., 50% KUD in our study) focus on where animals spend the majority of their time, and they remove longer and more directional movements that are considered 95% KUD. We observed that many of the rehabilitated koalas gradually moved away from their release sites until they remained in one area more consistently. These gradual directional movements were captured in the 95% KUD, whereas the 50% KUD was more concentrated around the area where koalas completed the study (see figure 2).

Similar to some of the koalas in our study, Ream *et al.* (1985) observed a lone female wolf dispersing a large distance and then settling into a well-defined home range, and intensively using two core areas. Spencer (2012) suggested that the reason for this type of behaviour may simply be exploration that resulted in finding a good place to stay and settle down. While our KUD analyses represent the area used by koalas over the study period, the movements of some koalas (e.g. moving from unburnt to burnt habitat or dispersing after release from rehabilitation) may not be typical of home range use and extent at other times. Researchers should be mindful of these issues when interpreting the output from home range analyses (Fieberg and Börger 2012; Laver and Kelly 2008; Worton 1987). In addition, end users, such as policy makers and conservation practitioners, need to use caution when translating this type of output into management recommendations. For example, there may be limited value in using “home range” size from rehabilitated koalas to understand the typical amount of area needed to support koalas in the Monaro region.

Our study, like many tracking studies, was restricted in scope by the logistical limitations of tracking many different individual animals across rugged terrain (Saunders *et al.* 2022). However, a larger number in each group would have

provided more statistical power, particularly given the large variation between koalas in core home range sizes in our study area (5 ha to 280 ha). The “trend” observed in 50% KUD between rehabilitated and non-rehabilitated koalas ($p = 0.07$) may have been significant with additional data. Notably, the variation that we observed between individual koala home ranges is not unusual and similar variability has been reported in other koala tracking studies (Ashman *et al.* 2020; Ellis *et al.* 2002; Ellis *et al.* 2009; Law *et al.* 2024; Martin 1999; Matthews *et al.* 2016). This demonstrates that, in any study, there is a need to consider how large variations in movement behaviour between animals might influence the capacity to address project aims when selecting the number of individuals to monitor.

2.5.4 Other findings

Other studies have found that movement parameters differ between males and females (De Oliveira *et al.* 2013; Matthews *et al.* 2016; Ryan *et al.* 2013). We found that male koalas moved farther each night on average than females, particularly during spring and summer. This is likely because spring and summer are the breeding season for koalas (Law *et al.* 2018). Male koalas also often have larger home ranges than females (Ellis *et al.* 2009; Lassau *et al.* 2008; Matthews *et al.* 2016; White 1999) and tend to disperse farther (Davies *et al.* 2013a). In our study, home range sizes were similar between the sexes, and the longest movements or dispersals away from the release site were undertaken by females. This demonstrates that variability in movements is not explained by sex alone, but also by differences between individuals.

The home ranges observed in this population were generally larger than other studied koala populations. Other studies in NSW have reported home range sizes

ranging from 4.9-58.9 ha using the fixed kernel method (FK) at 95% (Goldingay and Dobner 2013; Kavanagh *et al.* 2007; Lassau *et al.* 2008; Matthews *et al.* 2016), which is considerably smaller than the 95% KUD reported for the non-rehabilitated koalas in our study (250.8 hectares in the burnt and 194.7 hectares in the unburnt). Some studies in Queensland, however, have reported larger home ranges, including up to 135 ha in Blair Athol (Ellis *et al.* 2002). It is important to acknowledge that home range size estimates will vary depending on the method or technique used. Kernel methods are used to calculate utilisation distributions (i.e. distribution of an animal's position) by smoothing locational data and creating a density estimate (Van Winkle 1975; Worton 1989), and are thought to be more accurate measures compared to minimum convex polygons (MCPs) and the harmonic mean (Downs and Horner 2008; Worton 1987; Worton 1995). MCPs are thought to either under- or over-estimate home range size, depending on sample size (Downs and Horner 2008) and over-estimates have also been documented for the harmonic mean (Naef-Daenzer 1993). For example, Goldingay and Dobner (2013) found larger home ranges when using MCPs (e.g., average of 37.4 ha compared to the 8 ha at FK 95%). Despite this, it is likely that the larger home ranges and movement distances in our study are not just an artefact of using different home range methodologies, but demonstrate that some koalas in the NSW Monaro use larger areas than koalas that have been studied elsewhere.

There are a number of reasons why home range sizes differ between populations in other mammal species. These include differences in the availability of resources (Schradin *et al.* 2010), disturbance (O'Donnell and delBarco-Trillo 2020), environmental conditions (such as rainfall; Davies *et al.* 2013a) and population densities (Glessner and Britt 2005; Schoepf *et al.* 2015), with populations at lower

density having larger home ranges than areas with higher densities (Bengsen *et al.* 2016). Several of these factors likely contribute to the larger home ranges of koalas recorded in our study. For example, the Snowy Monaro region has a relatively low density koala population compared to some other areas, with Cristescu *et al.* (2023) estimating 0.032 koalas per hectare in burnt habitat at Peak View, NSW and 0.041 koalas per hectare in unburnt habitat in Numeralla, NSW. Higher density populations in other parts of NSW are typically around 0.3 koalas per hectare (Law *et al.* 2021), and can be much higher in other states (e.g., 10.1-18.4 koalas per hectare recorded in Cape Otway, Victoria between 2011 and 2013; Whisson *et al.* 2016). The quality of food resources may be lower or more dispersed in our study area compared to other areas (State of NSW and Office of Environment and Heritage 2011). However, this region is one of the few areas in Australia where koala populations appear to be stable rather than declining (Allen 2010). Our study provides the first information about the movements and home ranges of this understudied population of koalas, which will assist with the development of local management plans.

2.6 Conclusions

Studies such as this one that document the movement ecology of species after fire play an important role in understanding the ecological implications of wildfire, and can help guide conservation efforts by evaluating how burnt landscapes are used. Incorporating an experimental design with “treatment” and “control” groups in the same study can help identify disturbance effects on animal movements and landscape use with more certainty than comparing data collected from different areas, with different populations and/or at different times. Our findings fill important knowledge gaps about post-rehabilitation release success and the value of burnt

habitat to rehabilitated and non-rehabilitated animals, and can assist with decisions about the rescue and release of wildlife in burnt landscapes.

Encouragingly, despite the catastrophic wildfires of 2019–2020, our study found that burnt woodlands in the Monaro region of the NSW Southern Tablelands provided adequate resources for koalas to persist and recover, which is further supported by the high body condition of all koalas at the end of the study (Lane *et al.* 2023). In addition, apart from habitat that burnt in the extreme category, koalas spent proportionally more time in burnt than unburnt areas within their home ranges. The relatively mild conditions and higher than average rainfall from multiple years of La Niña after the fires also may have contributed positively to landscape and wildlife recovery. With wildfires predicted to increase in frequency and severity worldwide in response to climate change (Jones *et al.* 2020), the success of wildlife management and conservation efforts will likely depend on improving our understanding of the ecological impacts of fire on species and their habitats (Santos *et al.* 2022).

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2.8 Supplementary material

Supplementary material 1: Movement summaries for GPS-tracked koalas, including koala group, sex and age. Nightly distances are calculated by adding the straight line distances between each GPS point recorded between 7 pm and 7 am each night.

Greatest distance is the farthest distance that each koala was recorded from their initial capture/release location. Displacement is the straight line distance between the first and last capture locations for each koala.

Koala	Group ^a	Sex ^b	Age (approx.) ^c	Days tracked	Home range (50%) (ha)	Home range (95%) (ha)	Mean nightly distance (m)	Greatest distance moved (m)	Displacement distance (m)
Tallow	R	M	5-6	225	51.62	418.07	310	3495	2130
Bear	R	M	3-4	252	267.5	1489.34	345	8362	5737
Hamish	R	M	2-3	252	176.85	1044.05	300	7645	6567
Matthew	R	M	~3	178	276.55	1543.09	389	5690	3767
Tahlia	R	F	~2	120	NA	NA	210	3170	2084
Jessie	R	F	3.5-5.5	94	12.6	65.59	108	1149	1149
Jarrah	R	M	1-2	47	235.77	884.89	256	3689	2141
Ian	R	M	5.5-6.5	63	186.27	724.7	346	1878	449
Mark	R	M	1-2	44	4.79	20.74	147	382	125
Amelia	R	F	1-2	30	NA	NA	74	282	182
Romane	B	F	~6	256	45.91	219.83	301	1873	285
Claire	B	F	~6	256	20.78	88.41	221	1251	32
Evan	B	M	6.5-7.5	228	8.51	48.08	273	993	824
Sage	B	F	6.5-7.5	228	18.19	60.75	205	555	295
Drew	B	M	9-10	231	24.65	80.08	448	831	153

Koala	Group ^a	Sex ^b	Age (approx.) ^c	Days tracked	Home range (50%) (ha)	Home range (95%) (ha)	Mean nightly distance (m)	Greatest distance moved (m)	Displacement distance (m)
Bronwen	B	F	3.5-5.5	224	25.18	116.51	159	1404	568
Owen	B	M	~2.5	225	247.81	1085.68	425	4531	3147
Peggy	B	F	~2.5	183	72.98	307.10	222	2285	1933
Jett	B	M	1-2	38	NA	NA	191	677	172
Kenny	U	M	~10	228	16.16	64.61	345	908	555
Madonna	U	F	7.5-9	245	7.4	32.11	173	981	244
Xavier	U	M	5.5-6.5	224	9.46	49.07	381	1112	146
Murray	U	M	10-14	227	8.03	29.47	262	631	244
CS	U	F	~3	227	279.84	1191.05	171	5904	5184
Jaime	U	F	2-3.5	247	11.69	62.32	227	1007	301
Grace	U	F	9-10	195	6.01	15.83	112	405	127
Annie	U	F	9-10	60	22.24	113.26	267	850	289
Zinger	U	F	1-2	12	NA	NA	121	221	140
Mia	U	F	1-2	9	NA	NA	85	159	147
Alex	U	M	1.5-3	12	NA	NA	243	360	178
Rosalie	E	F	3.5-5.5	253	1776.82	8622.17	230	13273	12573
Brandy	E	F	~2	109	579.3	3138.68	301	7590	3393

^a R = rehabilitated, B = non-rehabilitated in burnt habitat, U = non-rehabilitated in unburnt habitat, E = excluded (i.e. moved from unburnt to burnt habitat)

^b F = female, M = male

^c Age as determined by tooth wear score

Chapter 3: The nutritional quality of post-fire eucalypt regrowth and its consumption by koalas in the New South Wales Southern Tablelands

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3.1 Abstract

Most forests and woodlands of south-eastern Australia are fire prone. These landscapes are typically dominated by tree species within the *Eucalyptus* genus, and many of these are proficient epicormic resprouters after fire. Several marsupial folivores rely on *Eucalyptus* foliage, including the koala. Little is known about the nutritional composition of epicormic leaves and whether folivores can utilise them. Following the 2019–2020 megafires, we offered koalas epicormic leaves from eight eucalypt species that occur in the New South Wales Southern Tablelands and measured concentrations of foliar chemical constituents known to influence koala feeding (total and available nitrogen, formylated phloroglucinol compounds (FPCs), and unsubstituted B-ring flavanones (UBFs)). Captive koalas ate epicormic leaves from the four eucalypt species belonging to the symphyomyrtle subgenus but ate relatively little from the monocalypt species. Available nitrogen was higher in symphyomyrtle epicormic regrowth compared to monocalypts and differed between species. Concentrations of FPCs and UBFs also differed between eucalypt species. Our results suggest that post-fire epicormic regrowth from symphyomyrtle species may be more readily browsed than monocalypts by koalas in the NSW Southern Tablelands. Therefore, some burnt areas are likely to be better quality habitat for koalas than others.

Keywords: *Marsupial folivores, bushfire, browse quality, epicormic leaves, plant secondary metabolites, dry matter intake, nitrogen, herbivory*

3.2 Introduction

Fires can cause major disturbance to ecosystems (Vernes *et al.* 2001) and are a common occurrence in the Australian landscape. The dry sclerophyll forests in south-eastern Australia are considered to be one of the most fire-prone areas in the world (Attiwill and Adams 2013; Melzer *et al.* 2000; Penman *et al.* 2007; Russell-Smith *et al.* 2007). Several arboreal marsupial species utilise the eucalypt forests of south-eastern Australia and feed on the foliage of a variety of eucalypt species. These include: the common brushtail possum (*Trichosurus vulpecula*), the common ringtail possum (*Pseudocheirus peregrinus*), the southern greater glider (*Petauroides volans*) and the koala (*Phascolarctos cinereus*) (Moore *et al.* 2004a). Their distribution throughout eucalypt forests and woodlands is patchy, and this is thought to be, in part, due to nutritional factors (Moore *et al.* 2004c).

Eucalypt foliage, like other plant material, can vary considerably in the types and/or concentrations of plant nutrients and herbivore-deterrent plant secondary metabolites (PSMs) both between and within species (Lawler *et al.* 1998; Lawler *et al.* 2000; Moore *et al.* 2004a; Wallis *et al.* 2010). This creates a nutritionally complex landscape that can influence diet selection and folivore distribution (Au *et al.* 2019; Jensen *et al.* 2014). There has been much research on how chemical constituents in the mature foliage of eucalypts influence feeding by browsing marsupials (Beale *et al.* 2022; Marsh *et al.* 2003a; Marsh *et al.* 2007; Moore *et al.* 2005), but little is known about the chemical composition of eucalypt post-fire regrowth and whether it is utilised by arboreal folivores after fire.

Resprouting after fire is a key functional trait and strategy for many plant species (Clarke *et al.* 2013; He 2014; Pausas *et al.* 2016), and, as a group, eucalypts are considered to be the most proficient and successful epicormic

resprouters (Bradstock *et al.* 2012; Burrows 2002; Burrows 2008b; Clarke *et al.* 2013; Waters *et al.* 2010). In many eucalypt species, after fire, numerous buds can develop from meristems across the entire bark at different depths (Bradstock *et al.* 2012; Burrows 2013; Burrows 2008a; Waters *et al.* 2010), enabling them to produce large numbers of shoots on both the bole and branches of the tree (Clarke *et al.* 2013). Epicormic resprouters are also able to help maintain tree growth and structure following fire; therefore, the production of epicormic regrowth enables a relatively rapid recovery of forests and woodlands from fire (Pausas and Keeley 2017).

The 2019–2020 Australian megafires were unprecedented in duration, severity and scale, with over 12 million ha burnt between August 2019 and March 2020 (Wintle *et al.* 2020). Although fires can have benefits to biodiversity (Bowman and Murphy 2010; Gill 1994), the intensity and severity of the 2019–2020 bushfires caused large areas of habitat to be significantly altered, including more than a quarter of the remaining, highly suitable koala habitat (or 3.5 million ha) in New South Wales (NSW) (State of NSW and Department of Planning Industry and Environment 2020). In many areas, there were few unburnt refuges for wildlife (State of NSW and Department of Planning Industry and Environment 2020), increasing the need to understand how post-fire conditions affect wildlife and their habitat.

After severe fire, epicormic leaves are normally the first type of growth available (Pausas and Keeley 2017), and yet there have been no studies on the nutritional composition of epicormic leaves for koalas and how this may differ within and between species and from mature foliage. Some studies have observed koalas occupying burnt areas and feeding in trees with epicormic growth (Lunney *et al.* 2004; Matthews *et al.* 2007). However, little is known about the extent to which they feed on them or whether the epicormic leaves from all eucalypt species are equally

palatable. This information is critical to understanding how koalas may utilise a burnt landscape, whether koalas recovering in care can be supported by epicormic leaves and when a landscape may be suitable for koalas to be released.

The aim of this study was to determine whether captive koalas would consume epicormic leaves from eight common eucalypt species in the NSW Southern Tablelands (*Eucalyptus rossii*, *E. mannifera*, *E. dives*, *E. macrorhyncha*, *E. radiata*, *E. viminalis*, *E. rubida* and *E. bridgesiana*). We also compared the nutritional composition of epicormic foliage with mature foliage from the same species collected as part of an earlier study on the nutritional quality of foliage for koalas (Au 2018). A number of foliar constituents are known to influence the feeding choices of eucalypt folivores and the digestibility of essential nutrients (Wallis *et al.* 2010). These include specific PSMs: formylated phloroglucinol compounds (FPCs) in eucalypt species from the *Symphyomyrtus* subgenus (common name symphyomyrtle), unsubstituted B-ring flavanones (UBFs) in species from the *Eucalyptus* subgenus (common name monocalypt), and tannins (Marsh *et al.* 2019; Marsh *et al.* 2003b; Moore *et al.* 2005). Foliar nitrogen (N) (as a proxy for protein) is considered a key limiting nutrient for many eucalypt folivores, and marsupial browsers often select food that is higher in digestible protein and lower in PSMs (Au 2018; Moore and Foley 2005; Stalenberg *et al.* 2014). Understanding the nutritional quality of epicormic regrowth and whether koalas can consume the regrowth has important implications for determining whether fire-affected landscapes can aid in supporting koala populations. This information can also be used to guide decision-making about when it is safe, from a nutritional perspective, to release koalas that were rescued from wildfires.

3.3 Materials and methods

3.3.1 Study animals and housing

Four adult male koalas were kept in individual outdoor roofed enclosures (approximately 2.5 x 2.5 x 3 m, (w x d x h)) containing multiple vertical and angled trunks and branches with forks. The koalas had been rescued from the vicinity of Peak View, NSW, Australia (36.07°S, 149.38°E) after a severe fire burnt through the area in January 2020. Koalas are aged based on the wear of the fourth upper premolars according to Martin (1981) on a scale of 1 (minimal wear; youngest animals) to 7 (root wear; oldest animals). The tooth wear of individual animals was between 2 and 4, and weights ranged from 4.9 to 7.8 kg. While in care, the koalas were offered a variety of eucalypt species found in their natural habitat. Fresh leaves were supplied daily by placing large stems into polythene tubes that were filled with water and secured to branches. Drinking water was available *ad libitum*.

3.3.2 Study design

The study was designed so that eight eucalypt species (table 1) were offered separately in a series of experiments that were undertaken between 4th April and 12th June 2020. Four of the species belong to the symphyomyrtle subgenus and the other four to the monocalypt subgenus (table 1). The selected species are used to varying degrees by koalas in the Central and Southern Tablelands koala management area (KMA) for feeding, shelter and/or social purposes (State of NSW and Office of Environment and Heritage 2018) (table 1).

Table 1: List of eucalypt species fed to koalas, the subgenus they belong to and the tree use score in the Central and Southern Tablelands KMA. Usage was classified based on relative koala tree ‘use levels’ for feeding, shelter and/or social purposes (State of NSW and Office of Environment and Heritage 2018).

Tree species	Subgenus	Tree use score
<i>E. bridgesiana</i>	<i>Symphyomyrtus</i>	Irregular
<i>E. mannifera</i>	<i>Symphyomyrtus</i>	High
<i>E. rubida</i>	<i>Symphyomyrtus</i>	Irregular
<i>E. viminalis</i>	<i>Symphyomyrtus</i>	High
<i>E. dives</i>	<i>Eucalyptus</i>	High
<i>E. macrorhyncha</i>	<i>Eucalyptus</i>	High
<i>E. radiata</i>	<i>Eucalyptus</i>	Irregular
<i>E. rossii</i>	<i>Eucalyptus</i>	High

The experiments were conducted as a series of 4 x 4 Latin squares, in which four sets of trees were offered to four koalas over four consecutive nights (Table 2). No koala received the same set of trees on the same night as any other koala, and all koalas were offered all four sets of trees for one night each. For all species except *E. mannifera* and *E. rubida*, there were three individual trees in each set. Each set always contained the same three trees, so that 12 individual trees were used for each experiment. The purpose of this was twofold: first, to encompass and control for potential variation between individual trees within species, and, second, to ensure that sufficient leaf could be harvested to provide in excess of voluntary intake for all four koalas.

For *E. mannifera* and *E. rubida*, we were unable to find 12 trees with enough epicormic growth for four consecutive nights. Instead, we used a modified version of the 4 x 4 Latin square (Table 2) with four trees that had enough growth for the four-

night period and eight other trees that we broke into groups of two, whereby koalas received trees from the first group for the first two nights and trees from the other group for the last two nights. For those two species, we have four observations per tree for four trees and two observations per tree for the other eight trees. Therefore, for these two species, each koala received two individual trees per night rather than three.

Table 2: Experimental design for feeding experiment. For *Eucalyptus bridgesiana*, *E. macrorhyncha*, *E. radiata*, *E. rossii*, *E. viminalis* and *E. dives*, each treatment consisted of a group of three trees that were always offered together: A = trees 1, 2 and 3; B = trees 4, 5 and 6; C = trees 7, 8 and 9; D = trees 10, 11 and 12. For *E. mannifera* and *E. rubida*, the first tree from each treatment was the same across all four nights. For the first two days, the second tree of the treatment was offered in conjunction with the first tree (e.g. A = trees 1 and 2) and on the last two days the last tree of the treatment was offered (e.g. A = trees 1 and 3).

Koala	Day			
	1	2	3	4
1	A	B	D	C
2	B	C	A	D
3	C	D	B	A
4	D	A	C	B

3.3.3 Leaf collection

For each group of three trees, we selected a central tree, and two other trees that were within 25m of the central tree. There was a minimum distance of 60m

between each group of trees to reduce the chance of genetic and chemical similarity between groups (Andrew *et al.* 2007). Individual trees were selected based on whether they had sufficient epicormic growth to feed four koalas for four consecutive nights in addition to providing enough leaf for control bunches and samples for nutritional analysis.

Leaf collection was undertaken between 0930 and 1230 h on each day of the experiment. Epicormic growth was collected by either pulling it off of the trunk by hand, or by using an extendable cutting pole if it was higher in the tree. Stems were placed into buckets of water within 30 minutes of collection and stored indoors until feeding in the late afternoon.

3.3.4 Leaf preparation and koala feeding

The epicormic growth from each tree was divided into two bunches: one to feed to koalas and the other served as a control to identify any weight changes in the absence of herbivory. At 1600 h, bunches were weighed and the stems were placed into labelled plastic bags (35cm x 25cm) and filled with water so all stems were covered.

Each koala was provided with three separate bunches of epicormic leaves comprising their allocated set of trees for that day. Koalas were also offered their regular diet, which consisted of branches of mature and young leaves from a minimum of three of the following species: *E. bridgesiana*, *E. rossii*, *E. mannifera*, *E. rubida* and occasionally *E. viminalis*, *E. pauciflora* and *E. dives*. The control bunches were placed outside under cover to mimic the conditions in the koala enclosures.

The epicormic leaf bunches were removed from the koala enclosures the following morning between 0800 h and 0830 h and reweighed to determine each

koala's wet matter intake. Occasionally, a feeding bunch was tipped during the night so that stems were no longer sitting in water. These leaves were likely to have lost water mass during the night, and so when this happened, we removed all of the leaves from the stems before weighing only the stems. The leaves that had been removed and any other leaves that koalas had dropped onto the ground while feeding were collected into paper bags and dried at 60 °C until constant mass to determine their dry weight. Control bunches were also reweighed, and a sample resembling that eaten by koalas (e.g. tips, leaves, stems or a combination) was removed from each control bunch and dried to constant mass to determine the percentage dry matter (% DM). A separate sample was collected and frozen at -20 °C for later chemical analysis. Dry matter intake (DMI) per tree was calculated by multiplying the % DM by apparent wet matter intake and then subtracting the dry weight of dropped or removed leaves for each tree. We also calculated nightly DMI of epicormic leaves for each koala by adding together the DMI of the two or three trees that were offered together.

3.3.5 Chemical analysis

Leaf samples were freeze-dried and then ground in a Foss Tecator 1093 Cyclotec sample mill (Foss Tecator, Höganäs, Sweden) with a 1mm particle sieve. For all dried, ground foliage samples, we collected reflectance spectrum from 400 to 2498 nm using a lab-based Foss XDS NIR spectrometer (Foss-NIRsystems Inc., Silver Spring, MD, USA).

Total and available N were determined in a subset of samples (n = 73) using a modified version of the method of DeGabriel *et al.* (2008) as outlined in Marsh *et al.* (2014b). For both digested and undigested leaves, the N concentration was

determined in 0.100 ± 0.020 g of sample using a Leco Truspec C/N analyser (Leco Corporation, Sydney, NSW, Australia). These values were used to calculate the *in vitro* N digestibility and available N.

We used Win ISI II, Version 10.2 (Intrasoft International, Port Matilda, Pennsylvania, USA) to develop calibrations from the NIR spectra to predict total and available N in all epicormic leaf samples using the lab values in the subset of chemically analysed samples for model training and testing. We applied a standard normal variate and detrend scatter correction to raw spectra to minimise noise and then used modified partial least square regression with cross-validation and further independent validation. The resulting calibration accurately predicted concentrations of both total N ($r^2 = 0.995$, SECV = 0.063) and available N ($r^2 = 0.974$, SECV = 0.174) in the training data set and in the independent validation set for N ($r^2 = 0.978$, slope = 0.971, standard error of prediction (SEP) = 0.094) and available N ($r^2 = 0.869$, slope = 0.998, and SEP = 0.331). Concentrations of UBFs and FPCs were predicted using the pre-existing calibrations made with mature eucalypt foliage described in Au (2018) and Marsh *et al.* (2019).

3.3.6 Comparison with mature leaves

Au (2018) measured concentrations of total N, available N, UBFs and FPCs in mature leaves of 2368 individual eucalypt trees, from 63 species across the range of the koala. Five of the sites included in that study covered the region from which the koalas in our study were rescued. We collated the data from those five sites and plotted the mean and range of concentrations of each nutritional constituent as an indication of the potential differences in nutritional composition between mature and epicormic leaves of each species. We did not statistically compare the chemical

composition of epicormic leaves with mature leaves from the same species because the measurements could be partially influenced by the different individual trees sampled and slight differences in the analytical methods.

3.3.7 Data analysis

All analyses were completed using R statistical Software (v4.2.2) (R Core Team 2022). We tested for differences in daily DMI between eucalypt species and subgenera using a linear mixed effects model with the *lme4* package (v1.1.31) (Bates *et al.* 2015) and *LmerTest* (v3.1.3) (Kuznetsova *et al.* 2017). Daily DMI was the dependent variable, and the independent variables were eucalypt species and subgenera. We included koala and tree group as random effects to account for the experimental design. We used ANOVAs for comparisons between species and subgenera using the *car* package (v3.1.2) (Fox and Weisberg 2018). For post-hoc analyses we used the *emmeans* package (v1.8.5) (Lenth 2023) to generate pairwise comparisons between the different eucalypt species and also to see the differences between the two subgenera. We used the square root of DMI so the residuals were normally distributed.

To look at chemistry differences in epicormic leaves between species, we ran linear regression models using the *stats* package (v4.2.2) (R Core Team 2022). We ran separate models with available N, FPCs and UBFs as the dependent variables. Eucalypt species and subgenus were the independent variables in the available N model, whereas species was the only independent variable in the FPC and UBF models.

3.4 Results

Koalas ate between 0 and 95 g DM of epicormic leaves per night. Koalas ate more from some eucalypt species compared to others ($F_{6,24} = 11.90$, $p < 0.001$). On average, they ate most per day from epicormic *E. viminalis* and *E. bridgesiana* and the least from epicormic *E. radiata* and *E. macrorhyncha* (Figure 1). There was also an effect of subgenus, with koalas eating over four times more from symphyomyrtle species than monocalypt species ($F_{1,24} = 58.53$, $p < 0.001$). All four koalas chose not to eat epicormic leaves from two individual *E. radiata* trees, eight *E. macrorhyncha* and one *E. rubida*.

3.4.1 Chemistry of epicormic leaves

Total N concentrations in epicormic leaves ranged from 1.46 % DM in an *E. rossii* individual to 3.97 % DM in an individual *E. rubida*, whereas available N concentrations ranged from 0.07 % DM in an *E. macrorhyncha* to 3.44 % DM in an *E. viminalis*. Some eucalypt species contained higher concentrations of total N ($F_{6,87} = 12.54$, $p < 0.001$; figure 2a; Supplementary material 1) and/or available N than others ($F_{6,87} = 16.84$, $p < 0.001$; figure 2b; Supplementary material 1). In particular, on average, the epicormic leaves from symphyomyrtle species had higher concentrations of both total and available N than epicormic leaves from monocalypts (total N: $F_{2,87} = 2745.08$, $p < 0.001$; available N: $F_{2,87} = 1245.20$, $p < 0.001$; figure 2a and 2b).

In the symphyomyrtle species, FPC concentrations ranged from 22 to 84 mg g⁻¹ DM across all individuals of all species. In the monocalypt species, UBF concentrations ranged from 0 to 38 mg g⁻¹ DM across all species (figure 2c; Supplementary material 1). Both FPC concentrations in symphyomyrtles and UBF

concentrations in monocalypts differed between tree species ($F_{4,44} = 323.35$, $p < 0.001$ and $F_{4,43} = 155.84$, $p < 0.001$ respectively; figure 2c).

When comparing epicormic leaves to mature leaves of the same species from an earlier study, the general pattern suggests that epicormic leaves may contain more total and available N than mature leaves (figures 2a and 2b), but that the effect for available N may be stronger in symphyomyrtle species than in monocalypts. Most symphyomyrtle species appeared to have slightly higher FPC concentrations than mature leaves, but the pattern was less clear for UBF concentrations in monocalypts.

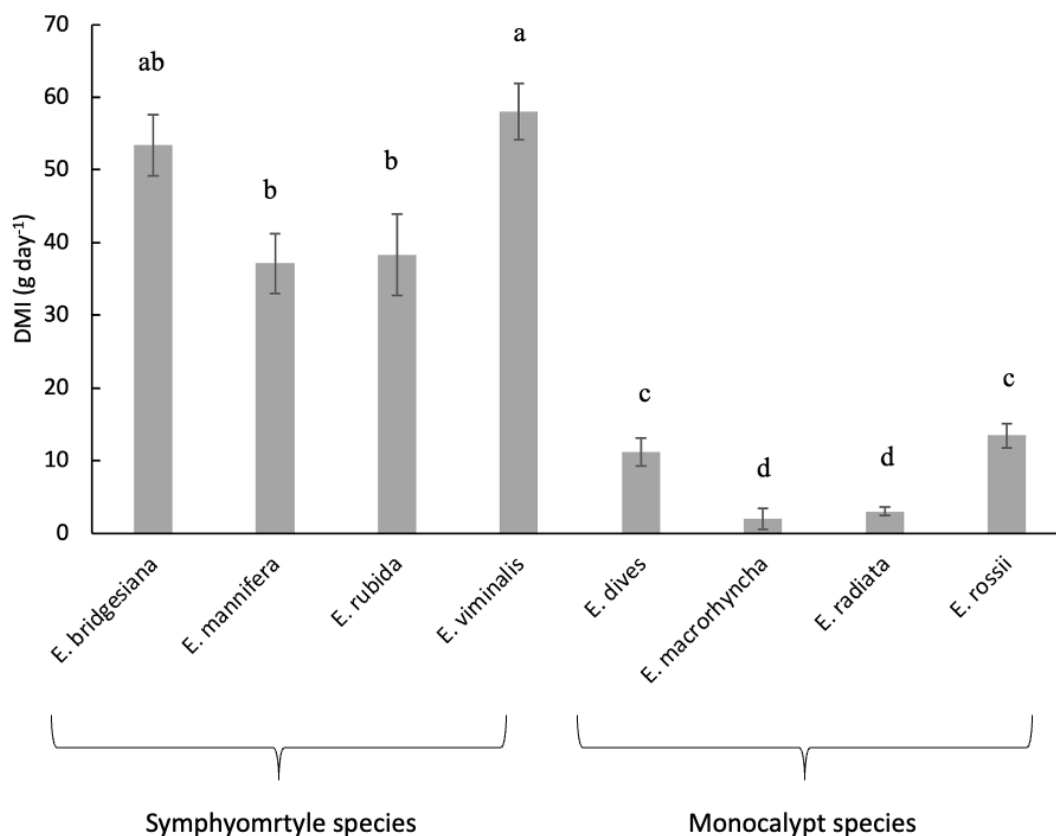


Figure 1. Mean $\pm$ SE daily dry matter intake (DMI) of epicormic leaves from eight eucalypt species ($n = 4$ sets of trees offered to 4 koalas per species). Bars with different letters are significantly different from one another in post-hoc testing ($p < 0.05$).

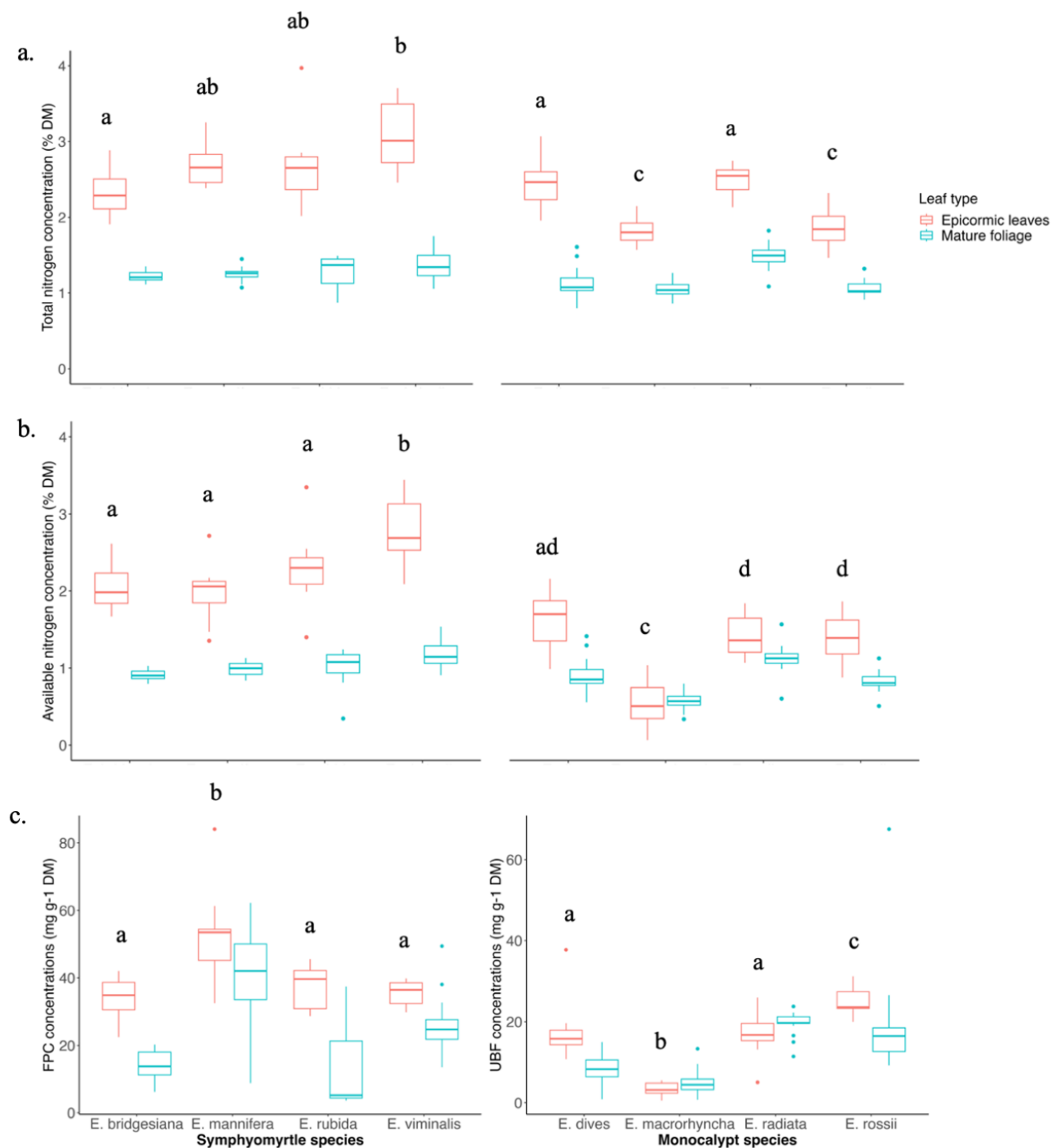


Figure 2. Mean concentrations of total N (a), available N (b) and FPCs and UBFs (c) in epicormic leaves (this study) and mature foliage (from Au 2018) of symphyomyrtle and monocalypt species. Different lettering denotes significance between epicormic leaves of eucalypt species ($p < 0.05$).

3.5 Discussion

This is the first study to measure both digestible N and PSM concentrations in epicormic eucalypt leaves and to investigate whether koalas will eat them. We have definitively shown that koalas will eat some epicormic leaves, although the amount depended on the species and the individual trees offered. Notably, koalas ate at least four times more epicormic leaves from species from the *symphyomyrtle* subgenus compared to monocalypt species. As discussed below, these findings substantially increase our understanding of the post-fire ecology of koalas and their habitats.

Our findings demonstrate that captive koalas in the NSW Southern Tablelands can consume the epicormic growth of several eucalypt species, suggesting that they could potentially utilise these leaves in the wild as well. This supports previous observations of radio-tracked koalas in burnt eucalypts, with koalas documented using burnt trees for both food and shelter (Matthews *et al.* 2007). Our study also suggests that koalas may browse more readily on epicormic leaves from *symphyomyrtle* species than from monocalypts. It is likely that the chemistry of epicormic leaves played a strong role in driving differences in intake between groups of trees, eucalypt species, and subgenera.

Koala feeding decisions are influenced by variation in nutritional composition and subgenera-specific plant secondary metabolites within and between eucalypt species (Lawler *et al.* 1998; Lawler *et al.* 2000; Marsh *et al.* 2003b; Marsh *et al.* 2018). For example, koalas have previously been shown to have a relatively high threshold and tolerance for FPCs, and as a result tend to favour and consume more foliage from *Symphyomyrtus* species (Lawler *et al.* 1998; Moore and Foley 2005). In contrast, they seem to be deterred by much lower concentrations of UBFs compared to FPCs, which may contribute to their avoidance of some monocalypt trees and

species (Marsh *et al.* 2021). As these subgenera have different PSM profiles, it is not possible to directly compare concentrations of FPCs in symphyomyrtle species to UBF concentrations in monocalypt species. Our findings support the above pattern, with captive koalas consuming more epicormic foliage from the symphyomyrtle species compared to the monocalypts.

Another difference that has previously been noted between the subgenera is that monocalypt species typically contain lower available N concentrations compared to symphyomyrtle species, although total N concentrations are generally similar (Wallis *et al.* 2010). We found that epicormic leaves also displayed this pattern. Animals require sufficient dietary protein to meet their daily maintenance requirements ($283 \text{ mg kg}^{-0.75} \text{ d}^{-1}$ for total N and $271 \text{ mg kg}^{-0.75} \text{ d}^{-1}$ for digestible N, in the case of the koala; Cork 1986), and diets higher in digestible protein can improve health (French *et al.* 2017), reproductive capacity (DeGabriel *et al.* 2009), and the capacity to metabolise PSMs (Au *et al.* 2013; Marsh *et al.* 2005). Thus, the higher consumption of symphyomyrtle species could be partly due to selection for a diet higher in digestible protein. This could also mean that the epicormic leaves from symphyomyrtle species may be of greater nutritional value to koalas after fire than monocalypt species, due to the higher concentrations of available N from the trees within this subgenus. It is also worth noting that epicormic foliage of some monocalypt species was higher in available nitrogen than the mature foliage of some symphyomyrtle species. This might explain why koalas still choose to eat some epicormic foliage of monocalypt species when there is a choice.

Although the epicormic regrowth from a few symphyomyrtle species had higher concentrations of a well-known herbivore deterrent PSM (FPCs) compared to mature leaves from the same species, the epicormic regrowth from all measured

symphyomyrtles was substantially higher in both available N and total N. Similarly, limited studies on epicormic leaves from mature *E. blakelyi* trees, a symphyomyrtle species, found they contained higher concentrations of total N than mature foliage (Landsberg 1990; Landsberg and Wylie 1983). However, in the monocalypt species, concentrations of total N, available N and UBFs in the epicormic leaves were more similar to that of the mature foliage, with a few exceptions. This preliminary data suggest that koalas may face trade-offs between acquiring extra nutrients and avoiding higher intakes of potentially toxic PSMs when choosing whether to feed on mature or epicormic leaves, especially for symphyomyrtle species. It is important to note, however, that epicormic and mature leaves were collected from different trees and at different points in time, which can influence leaf chemistry (Moore *et al.* 2005; Wallis *et al.* 2011). Future research should prioritise comparing leaf chemistry of epicormic growth and mature foliage from the same trees and/or locations at consistent points in time and investigate how PSM and protein concentrations affect intake of epicormic growth. It would also be interesting to compare the nutritional composition of epicormic growth and young adult-phase leaves from the same species to better understand whether there are nutritional similarities between the effects of fire and flushes of new adult-phase growth on unburnt trees.

Although our results suggest that koalas can find some food in burnt areas, koalas in this study consumed epicormic leaves in conjunction with mature foliage, which raises questions about the extent to which koalas and other marsupial folivores can utilise epicormic leaves in their diet. Captive koalas readily eat 150-250 g DM on average per night (Marsh *et al.* 2021; Marsh *et al.* 2014a; Marsh *et al.* 2007). At most, the koalas in our study ate an average of 60 g DM epicormic leaves from the “best” species and as little as 2 g DM from the “worst” species. This fits with

our observations that they always ate mature leaves in addition to epicormic leaves. It also raises questions about whether free-ranging koalas could depend entirely on epicormic leaves in burnt habitat with no intact canopy, or whether epicormic leaves are only a supplementary food source. Ideally, we would have liked to measure absolute intake of epicormic leaves in the absence of mature foliage to clarify the extent to which koalas could be sustained with epicormic leaves. However, it was unethical to restrict rescued koalas to diets that may potentially limit their intake. This should be a priority for investigation in future studies to better understand whether koalas can eat enough to meet their nutritional requirements in burnt areas that contain only epicormic leaves. It may also be useful to observe whether captive koalas choose to consume epicormic or mature foliage first and how often they switch between them, or to record how often free-ranging koalas are feeding in stands dominated by epicormic growth during the night.

Interestingly, the amounts of epicormic leaves that koalas ate from each species did not necessarily align with perceptions of their “use” by free-ranging koalas. For example, *E. rossii* is considered “high use” in the Southern Tablelands of NSW (State of NSW and Office of Environment and Heritage 2018) and the mature leaves are often highly preferred by captive koalas (K. Marsh and M. Lane personal observation). However, koalas ate little epicormic regrowth of *E. rossii*. It would be useful to determine whether the apparently higher UBF concentration in epicormic leaves in *E. rossii* contributed to this, given that koalas can be deterred from feeding as UBF concentrations increase (Marsh *et al.* 2019). Although PSM concentrations are known to affect how much koalas eat (Marsh *et al.* 2007; Moore and Foley 2005; Moore *et al.* 2005; Moore *et al.* 2004b), it was not possible to relate the chemistry of individual trees in this study to food intake because koalas had a choice between

multiple options of epicormic and mature leaves each night. Future research should prioritise examining the effects of leaf chemistry on intake of epicormic leaves because this would assist with understanding how fire affects food quality more broadly across the range of the koala.

Unlike *E. rossii*, we observed that captive koalas rarely ate either epicormic or mature leaf from *E. macrorhyncha* and *E. dives*, even though both of these species are also considered “high use” in the study area (KMA5) based on sightings of koalas in trees and the presence of scats under trees (State of NSW and Office of Environment and Heritage 2018). Both *E. macrorhyncha* and *E. dives* are abundant in the Southern Tablelands region and may therefore be mostly used during the day rather than for feeding at night. It is well recognised that koalas do not necessarily feed from the trees in which they sit during the day (Marsh *et al.* 2014a; Matthews *et al.* 2007; Moore and Foley 2005; Phillips and Callaghan 2000). Thus, observations of tree use may be poorly correlated with diet (Marsh *et al.* 2014a). We suggest that *E. macrorhyncha* and *E. dives* are less important as browse species than their “use” by koalas suggests, either before or after fire.

In the same vein, *E. rubida* and *E. bridgesiana* are considered to be used irregularly by free-ranging koalas in KMA5 (State of NSW and Office of Environment and Heritage 2018), but captive koalas regularly ate substantial amounts of mature *E. rubida* and *E. bridgesiana* leaves in addition to epicormic leaves when they were offered. This suggests that free-ranging koalas in the NSW Southern Tablelands may incorporate *E. rubida* and *E. bridgesiana* into their diet when they encounter them, even though they have not been commonly reported to sit in these species (State of NSW and Office of Environment and Heritage 2018).

3.6 Conclusions

Wildfires are predicted to increase in frequency and severity in the future due to climate change (Lucas *et al.* 2007). This study provides important baseline data on the chemistry of epicormic leaves 2-6 months post fire and describes some trends in the feeding behaviour of captive koalas offered a choice between mature and epicormic leaves after fire. This information is critical for understanding some of the indirect effects of fire on koalas and their habitat, and for informing post-fire conservation and management decisions. For example, bushfire events are often patchy, creating a mosaic of different burn severities in many areas (Burrows *et al.* 2021), which means that koalas may have choices between both mature foliage and epicormic growth. In this instance, the higher available N content of epicormic growth, particularly in *symphyomyrtle* species, may provide additional nutritional support to their diet during post-fire recovery, as was previously hypothesised by Moore *et al.* (2004a). It is also important to consider that the preferences for epicormic leaves might differ from preferences for mature leaves of the same species. This means that the species typically preferred by koalas when mature foliage is present may be different when trees are producing epicormic regrowth. These differences should be considered when examining preferred trees from a management perspective (e.g. for tree retention during development or during revegetation activities) and by ensuring that preferred tree data is based on feeding observations and not just on sightings of koalas and the presence of scats under trees.

Although this example is for koalas in the NSW Southern Tablelands, there may be similar patterns in other areas. It is important to learn more about the nutritional quality of epicormic leaves in other areas known to be used by koalas, as the quality

of burnt habitat is likely to depend on which species of eucalypts are present and the proportions of those species. Our results suggest that after fire, epicormic regrowth from symphyomyrtle species may be more readily browsed than monocalypts by koalas in the NSW Southern Tablelands. Although this matches the known preferences of koalas for symphyomyrtle species more broadly (Moore *et al.* 2004a), monocalypt species can form an important component of the diet of koalas in some locations (Brice *et al.* 2019; Marsh *et al.* 2021), including the Southern Tablelands (K. Marsh and M. Lane personal observation). Therefore, it is particularly important to understand nutritional preferences following fire. Taking this into consideration, some burnt areas are likely to be better quality habitat than others, revealing additional complexities in the spatial and temporal distribution of browse quality, which may influence koala feeding, movement behaviour and habitat requirements after fire.

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3.8 Supplementary material

Supplementary material 1: Summary of the nutritional composition of mature leaves from the eucalypt species from Au (2018) and from the epicormic leaves used in this study. The mean $\pm$ SE and range are presented for each constituent for each species.

Species	Leaf type	Total N (% DM)	Available N (% DM)	UBFs (mg.g ⁻¹ DM)	FPCs (mg.g ⁻¹ DM)
<i>E. bridgesiana</i>	Mature (n=14)	1.24 $\pm$ 0.04 (1.06 - 1.76)	0.94 $\pm$ 0.04 (0.66 - 1.28)	0	10 $\pm$ 2 (0 - 24)
	Epicormic (n=12)	2.35 $\pm$ 0.09 (1.91 - 2.88)	2.06 $\pm$ 0.09 (1.67 - 2.61)	0	33.71 $\pm$ 1.87 (22.49 - 41.99)
<i>E. mannifera</i>	Mature (n=12)	1.12 $\pm$ 0.02 (0.99 - 1.25)	0.89 $\pm$ 0.03 (0.61 - 1.07)	0	23 $\pm$ 7 (0 - 62)
	Epicormic (n=12)	2.69 $\pm$ 0.08 (2.39 - 3.25)	1.97 $\pm$ 0.11 (1.35 - 2.72)	0	52.45 $\pm$ 3.59 (32.51 - 84.06)
<i>E. rubida</i>	Mature (n=7)	1.30 $\pm$ 0.08 (1.00 - 1.60)	1.01 $\pm$ 0.06 (0.80 - 1.21)	0	6 $\pm$ 2 (0 - 16)
	Epicormic (n=12)	2.66 $\pm$ 0.14 (2.02 - 3.97)	2.29 $\pm$ 0.13 (1.40 - 3.35)	0	37.60 $\pm$ 1.72 (28.70 - 45.52)
<i>E. viminalis</i>	Mature (n=32)	1.38 $\pm$ 0.03 (1.05 - 1.80)	1.17 $\pm$ 0.04 (0.61 - 1.53)	0	22 $\pm$ 2 (0 - 50)
	Epicormic (n=12)	3.06 $\pm$ 0.12 (2.46 - 3.70)	2.77 $\pm$ 0.12 (2.09 - 3.44)	0	35.74 $\pm$ 0.98 (29.82 - 39.80)
<i>E. dives</i>	Mature (n=9)	1.17 $\pm$ 0.07 (0.95 - 1.68)	0.84 $\pm$ 0.09 (0.60 - 1.41)	6 $\pm$ 1 (0 - 9)	0
	Epicormic (n=12)	2.43 $\pm$ 0.09 (1.96 - 3.07)	1.63 $\pm$ 0.11 (0.99 - 2.16)	17.41 $\pm$ 1.97 (10.79 - 37.72)	0
<i>E. macrorhyncha</i>	Mature (n=32)	1.13 $\pm$ 0.03 (0.84 - 1.44)	0.83 $\pm$ 0.04 (0.42 - 1.17)	4 $\pm$ 1 (0 - 13)	0
	Epicormic (n=12)	1.82 $\pm$ 0.05 (1.57 - 2.15)	0.53 $\pm$ 0.09 (0.07 - 1.04)	2.99 $\pm$ 0.56 (0 - 5.54)	0

<i>E. radiata</i>	Mature (n=17)	1.55 ± 0.06 (1.12 – 1.87)	1.25 ± 0.06 (0.95 – 1.63)	19 ± 1 (0 – 24)	0
	Epicormic (n=12)	2.50 ± 0.05 (2.13 – 2.75)	1.41 ± 0.08 (1.07 – 1.84)	16.97 ± 1.5 (5.05 – 25.95)	0
<i>E. rossii</i>	Mature (n=17)	1.13 ± 0.03 (0.95 - 1.44)	0.82 ± 0.05 (0.51 - 1.37)	16 ± 1 (9 – 26)	0
	Epicormic (n=12)	1.85 ± 0.08 (1.46 – 2.32)	1.39 ± 0.09 (0.88 – 1.86)	25.21 ± 1.08 (20 – 31.17)	0

Chapter 4: Temporal changes in the chemical composition of eucalypt foliage after fire

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4.1 Abstract

Food is essential for animal survival; however, both anthropogenic and natural landscape disturbance can affect food availability and quality. Bushfires are a natural disturbance process in many landscapes and are increasing in frequency and severity due to climate change. The 2019–2020 megafires in Australia burned over 78,000 ha of eucalypt woodland and forest, drastically altering the availability of browse for arboreal folivore species. A key fire adaptation strategy of many eucalypts is their ability to resprout epicormically, producing new shoots from aerial stems rapidly after fire. However, very little is known about the chemistry of epicormic foliage, and the implications for the survival and recovery of arboreal folivores populations after fire. Small changes in nutrient availability and herbivore-deterrent plant secondary metabolites (PSMs) are known to have a substantial influence on diet selection and landscape use for many eucalypt folivores. In this study, we measured the nutritional composition of epicormic foliage from six common eucalypt species in a burnt landscape and how they changed over a one-year period. We also sampled adult-phase foliage from the same species in a nearby unburnt area for comparison. In most species, total and available nitrogen concentrations (proxies for protein) were initially higher in epicormic foliage compared to adult phase leaves but decreased over time. Of two types of PSMs known to influence feeding behaviour of marsupial folivores, formylated phloroglucinol compound (FPC) concentrations were higher, on average, in epicormic growth compared to adult-phase foliage, while the opposite pattern was observed for foliar concentrations of unsubstituted B-ring flavanones (UBFs). This study shows that the chemistry of epicormic foliage changes over time after fire and differs to adult-phase foliage, which could influence folivore diet selection and diet quality. Marsupial folivores likely face a trade-off

between higher nitrogen and FPC concentrations in epicormic growth. The ability to obtain adequate nutrition after fire will influence the health, survival and persistence of marsupial folivores in burnt landscapes.

4.2 Introduction

Adequate nutrition is essential for animals, ensuring species survival, reproduction and growth and development (Wu 2018). Feeding choices are influenced by numerous factors including foraging strategies, food abundance, food quality and both the survival and reproductive requirements of individuals and species (Barboza *et al.* 2009). Changes to food availability (including both quality and quantity) can affect animal movements, growth, reproduction, and survival, and can therefore impact population dynamics (Birnie-Gauvin *et al.* 2017). Human-induced disturbance processes that fragment a landscape can negatively impact wildlife species by reducing the availability of food, and affecting foraging behaviour (Birnie-Gauvin *et al.* 2017). Bushfires, both natural and anthropogenic, are also a major process of disturbance in many terrestrial landscapes (Bowman *et al.* 2009; Keeley *et al.* 2011), and they can influence food availability (MacGregor *et al.* 2013; Sutherland and Dickman 1999; Vieira and Briani 2013). However, the effects of fire can be complex (Doherty *et al.* 2022). Some animal species benefit; for example, predatory species often move into burnt areas where prey species are more exposed (McGregor *et al.* 2015; McGregor *et al.* 2014). However, for other animals, fire can reduce the availability of resources, causing declines in populations (Sutherland and Dickman 1999).

The dry sclerophyll forests of south-eastern Australia are fire-prone and are subjected to regular fire events (Sawyer *et al.* 2018). These forests are dominated by

the *Eucalyptus* genus (Ladiges *et al.* 2011; Prober *et al.* 2017), which comprises over 900 species that are widely distributed throughout the Australian landscape (Bayly *et al.* 2013). Several arboreal marsupial folivores rely on eucalypt forests for food and shelter (Crowther *et al.* 2014; McAlpine *et al.* 2015), including the koala (*Phascolarctos cinereus*), the common brushtail possum (*Trichosurus vulpecula*), the common ringtail possum (*Pseudocheirus peregrinus*) and greater glider species (*Petauroides spp.*) (Cork and Catling 1996).

Eucalypt foliage, like other plant material, is often low in essential nutrients (such as protein) and high in particular herbivore deterrent plant secondary metabolites (PSMs) (Külheim *et al.* 2011; Marsh *et al.* 2017a; Marsh *et al.* 2020). PSMs are structurally diverse compounds that play a major role in plant growth and survival by defending against biotic and abiotic stressors, including herbivory (Böttger *et al.* 2018; Kaur and Suseela 2020; Mazid *et al.* 2011). When ingested by herbivores, PSMs can cause a range of physiological effects (Iason 2005; Palo and Robbins 1991), and can act directly as toxins or as feeding deterrents, reduce the palatability of food, or affect the digestibility of nutrients (DeGabriel *et al.* 2009; Iason 2005; Moore *et al.* 2004b). Herbivores tend to consume less from individual plants with high PSM concentrations relative to conspecifics with lower concentrations (Marsh *et al.* 2007).

Protein is an important dietary requirement for animal species (Wu *et al.* 2014), and it is generally considered a limiting nutrient for herbivores due to its relatively low availability in plant foliage. The concentration of foliar nitrogen (N) is often used as a proxy for protein, as N gives a relative measure of amino acids. Thus, when assessing the quality of browse for marsupial folivores, eucalypt foliage high in nitrogen is often be considered better quality food (Moore *et al.* 2004b). However, the

ability for marsupial folivores to acquire protein can be reduced by tannins, which are present in high concentrations in eucalypt foliage (Marsh *et al.* 2017a; Marsh *et al.* 2020). Consequently, when studying the feeding behaviour of marsupial folivores, concentrations of total N are not always a reliable indicator of how much protein an animal can obtain (Marsh *et al.* 2003a), and instead, available nitrogen (or digestible protein) is a better measure (Wallis *et al.* 2010). When selecting food, herbivores must balance the quantity and quality of nutrients and PSMs present in the plant to meet their nutrient requirements while avoiding any detrimental effects of PSMs (Villalba *et al.* 2002).

Marsupial folivore feeding behaviour is strongly influenced by foliar chemistry, particularly by the concentrations of herbivore-deterrent PSMs (Jensen *et al.* 2014; Lawler *et al.* 1998; Moore *et al.* 2004a). The types and concentrations of PSMs present in eucalypt foliage differ between eucalypt species and between the subgenera within the *Eucalyptus* genus (Goodger *et al.* 2016; Heskes *et al.* 2012; Tucker *et al.* 2010). For example, formylated phloroglucinol compounds (FPCs) are known to occur in species from the *Symphyomyrtus* subgenus (common name: symphyomyrtle) (Eschler *et al.* 2000; Tucker *et al.* 2010) and unsubstituted B-ring flavanones (UBFs) have been detected only in species within the *Eucalyptus* subgenus (common name: monocalypt) (Goodger *et al.* 2016; Marsh *et al.* 2019; Tucker *et al.* 2010). Both of these groups of compounds are known to deter marsupial folivore feeding behaviour (Jensen *et al.* 2015; Lawler *et al.* 2000; Marsh *et al.* 2015; Wallis *et al.* 2002), although some folivore species are more tolerant than others (Jensen *et al.* 2014). Despite numerous studies on the effects of PSM concentrations on marsupial folivore feeding behaviour, there is very little information

about how fire affects the nutritional composition of eucalypt foliage and how this may influence the quality of food for eucalypt folivores after fire.

Eucalypts are well adapted to tolerate and resist fire and have evolved several important strategies to do so, with adaptive traits selected for both low and high severity fire (Burrows 2002; Burrows 2013; Burrows *et al.* 2010; Pausas and Keeley 2017; Waters *et al.* 2010). The ability to resprout epicormically is an important adaptation that allows rapid recovery after fire (Pausas and Keeley 2017). Epicormic resprouting is the emergence of new shoots from above-ground stems and branches, unlike basal sprouting which occurs below ground (Pausas and Keeley 2017; Waters *et al.* 2010). Many species from the *Eucalyptus* genus are considered to be some of the most successful and prolific epicormic resprouters (Burrows 2013). Epicormic growth may be the first available food source for marsupial folivore species in areas that have experienced wide-spread crown-scorching fire.

Marsupial folivores have been documented to eat epicormic growth and utilise burnt areas (Kavanagh 1987; Lane *et al.* 2024; Matthews *et al.* 2007). Young juvenile foliage is known to be higher in nitrogen (Kavanagh and Lambert 1990; Landsberg 1990; Landsberg and Wylie 1983), however, few studies have measured the nutritional quality of epicormic growth after fire. Despite these observations and the regular occurrence of fire, relatively little is known about the nutritional composition of epicormic leaves, how it changes over time and how it compares to the composition of adult-phase foliage.

The aim of this study was to measure how the nutritional and chemical composition of epicormic growth changes over time in six species of eucalypts known to be used by koalas in the New South Wales (NSW) Southern Tablelands, including three species from the *Symphyomyrtus* subgenus (*Eucalyptus mannifera*,

E. viminalis and *E. rubida*) and three from the *Eucalyptus* subgenus (*E. rossii*, *E. dives*, and *E. macrorhyncha*). We also compared the nutritional composition of epicormic growth to that of adult-phase foliage of the same species in an unburnt area. We measured the concentrations of total and available nitrogen, FPCs (in symphyomyrtle species) and UBFs (in monocalypt species). We had several expectations for this study. Firstly, we expected post-fire epicormic regrowth in this study to have higher concentrations of total and available nitrogen compared to adult-phase foliage. Secondly, we expected epicormic growth to have higher concentrations of the target PSMs, as young, immature eucalypt leaves are often better defended to deter herbivory (McKiernan *et al.* 2014; Silvestre *et al.* 1997). Thirdly, we expected that the foliar chemistry of adult-phase foliage in the unburnt landscape would remain relatively constant over time but that epicormic foliage may change over time as it matures. Given that bushfire events are likely to become more severe and regular with a changing climate (Lucas *et al.* 2007; Steffen *et al.* 2019), understanding how the composition of epicormic growth differs from adult-phase foliage and changes over time provides valuable information about the impact of fire on food quality, and how this may affect factors such as foraging behaviour, landscape use, and population recovery of arboreal marsupial folivores.

4.3 Materials and methods

4.3.1 Study sites

Leaf collection for this study was undertaken at a burnt and adjacent unburnt site in the NSW Southern Tablelands region, within and around the Macanally-Numeralla Ranges in the Monaro region. The sites and region are described in more detail in Lane *et al.* (2023). These sites were selected in the same areas known to be

utilised by radio-tracked koalas, outlined in chapters 1 and 2, as I wanted to obtain an overview of the nutritional landscape being used by koalas living in burnt and unburnt habitat.

4.3.2 *Eucalypt species*

We selected six species within the *Eucalyptus* genus. Three species were monocalypt species and included *E. dives*, *E. macrorhyncha* and *E. rossii*. The other three species were symphyomyrtle species, and included *E. viminalis*, *E. rubida* and *E. mannifera*. The selected species were abundantly available at both the burnt and unburnt site and are used to varying degrees by koalas in the Central and Southern Tablelands of NSW for feeding, shelter and/or social interactions (Lane *et al.* 2024; State of NSW and Office of Environment and Heritage 2018).

4.3.3 *Sampling locations*

Initially, 50 locations were generated at both the burnt and unburnt sites using the Halton Iterative Partitioning (HIP) sampling method (Robertson *et al.* 2018), which provides a well-distributed spatial sample and ensures equal probability random sampling. This selection process was implemented in R (v4.2.2) (R Core Team 2022), using the *SDraw* package (v.2.1.13) (McDonald and McDonald 2020), and the locations were generated within a polygon that outlined the study sites. All of these locations were visited and surveyed in numerical order, with one tree of each of the selected species marked at each location (where available) until ten trees from each of the six species were marked at both the burnt and unburnt sites. We selected multiple trees per species because individual trees, even within the same species, can differ in concentrations of foliar chemical compounds (Lawler *et al.*

1998; Marsh *et al.* 2018; Steinbauer *et al.* 2015b). Only one tree per species was selected at any one location to reduce the chances of having closely related trees within the same species, as trees that are close in proximity may have similar genetic, and therefore chemical, profiles (Andrew *et al.* 2007a). Sampling from more genetically diverse trees allowed us to obtain a more representative sample of the chemical variation within species. Unused locations were eliminated from the study. We did not encounter 10 *E. rubida* trees at the 50 allocated locations at the burnt site, so we selected some additional trees from known locations of occurrence, ensuring that there was at least 60 m between each tree to again reduce the chances of genetic similarity (Andrew *et al.* 2007a). At the end of this selection period, we had 33 locations in the burnt landscape and 31 locations in the unburnt landscape (figure 1). Two locations in the burnt landscape were removed after the second sampling round due to loss of access. This mean that two *E. mannifera* trees and one *E. rossii* tree were removed.

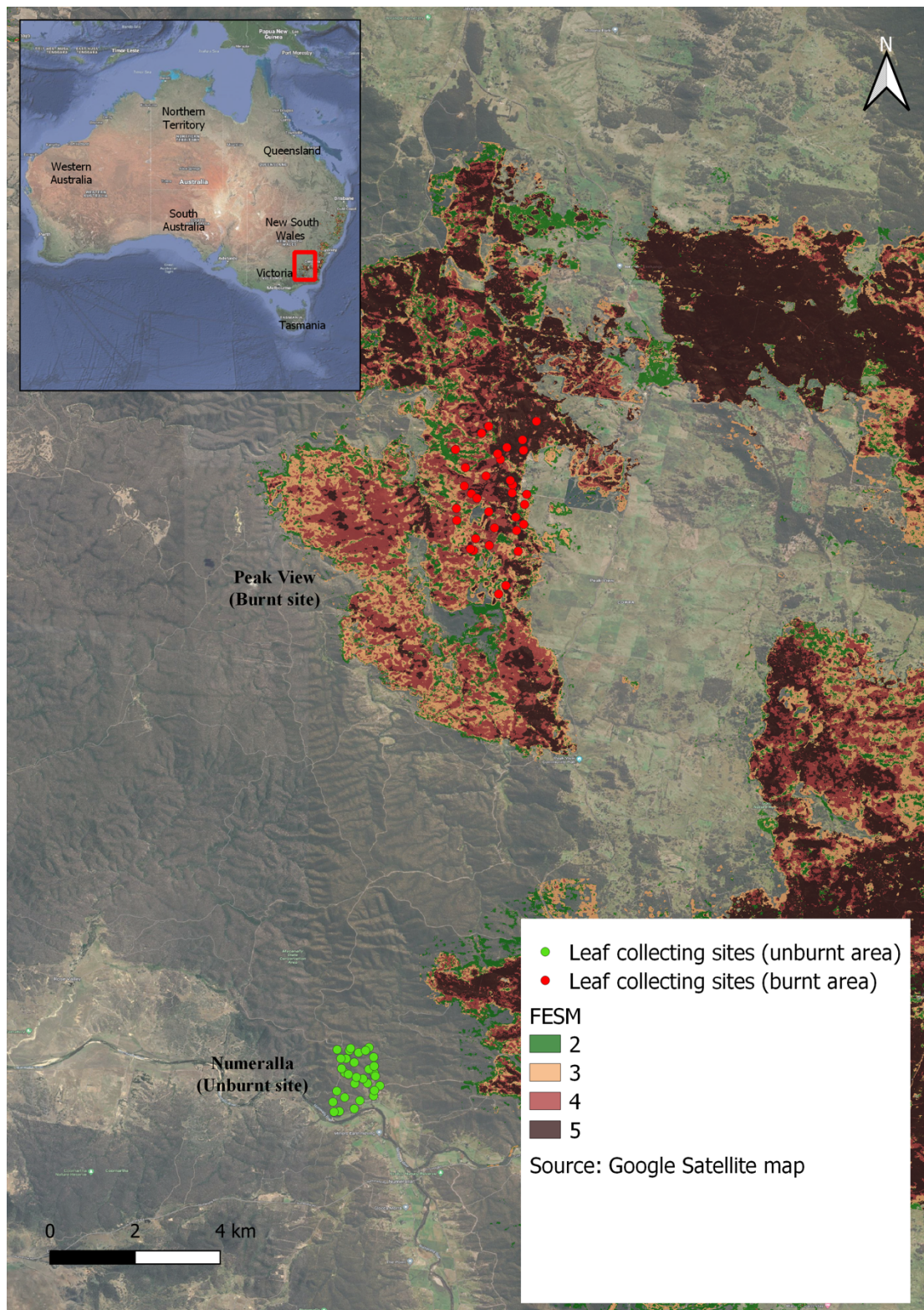


Figure 1: leaf collecting sites in burnt (red circles) and unburnt (green circles) forest overlaid onto a fire extent and severity mapping (FESM) layer (State Government of NSW and Department of Planning and Environment 2020), with 2 representing the lowest severity (burnt understorey with unburnt canopy), 3 representing moderate

(partial canopy scorch), 4 representing high (completed canopy scorch, +/- partial canopy consumption) and 5 representing extreme (complete canopy consumption) (map data ©Google). The inset shows the location of the study area within Australia.

4.3.4 Leaf collection

Leaf collection commenced in November 2020 and ended in November 2021, with a total of seven rounds being completed. At the beginning of the first leaf collection round, we recorded the GPS locations of each tree and measured the diameter at breast height (DBH). Leaf collection rounds were done over the following time periods:

- Round 1: 26th November 2020 – 10th December 2020
- Round 2: 6th January 2021 – 1st February 2021
- Round 3: 23rd February 2021 – 9th March 2021
- Round 4: 27th April 2021 – 18th May 2021
- Round 5: 14th June 2021 – 1st July 2021
- Round 6: 26th July 2021 – 6th August 2021
- Round 7: 24th October – 9th November 2021 (this round was delayed due to Covid-19 restrictions in the Australian Capital Territory (ACT) and NSW).

In the burnt landscape, epicormic growth was collected from all trees in all collection rounds. At the unburnt site, mature (i.e. fully expanded), adult-phase foliage was collected. We collected epicormic growth at least 1 m above the base of the trunk and either used our hands to remove growth from the trunk or a saw attached to an extendable pole if it was higher in the tree. Adult-phase foliage was collected using an arborist throw-line launcher (methods outlined in Youngentob *et*

al. (2016)). Approximately 100 g of leaf was collected per tree by individually removing each leaf from the stems. At the end of each day, samples were frozen at -20 °C.

4.3.5 Sample preparation

Leaf samples were freeze-dried and then ground in a Foss Tecator 1093 Cyclotec sample mill (Foss Tecator, Höganäs, Sweden) with a 1mm particle size sieve. For all dried, ground foliage samples, we collected reflectance spectrum from 400 to 2498 nm using a lab-based Foss XDS near-infrared reflectance (NIR) spectrometer (Foss-NIRsystems Inc., Silver Spring, MD, USA). This range (400–2498) covers the full visible (400–700 nm) and near infrared (700–2498) spectrum (Pan *et al.* 2022; Rossel *et al.* 2006).

4.3.6 Total and Available N

Total and available N were determined in the first round of collected leaf samples ($n = 160$) using a modified version of the method of DeGabriel *et al.* (2008) as outlined in Marsh *et al.* (2014) and also used in (Lane *et al.* 2024). This method involved a two-step digestion process, after which the N concentration was determined in 0.100 ± 0.020 g of both the digested sample using a Leco Truspec C/N analyser (Leco Corporation, Sydney, NSW, Australia). These values were used to calculate the *in vitro* N digestibility and available N.

4.3.7 Quantification of UBFs

We measured the total concentration of UBFs in all 80 monocalypt samples from round 1. All solvents were LCMS grade. The procedure for extraction followed

Marsh *et al.* (2019). Specifically, 20.5 ± 0.5 mg of dried, ground leaf material was extracted twice in 1 mL methanol by sonicating for 10 minutes and then centrifuging at 2500 rpm for 10 minutes each time. The supernatant from both extractions was combined and filtered through a 0.2 μ m syringe filter. Prior to analysis, all extracts were diluted into 50% methanol containing 0.5 μ M naringenin as an internal standard.

5 μ L of sample extracts were analysed using a Dionex Ultimate 3000 High Performance Liquid Chromatography (HPLC) system coupled to an Orbitrap Fusion Mass Spectrometer (Thermo Fisher Scientific, USA). The order in which samples were run was randomised, with a quality control standard mix inserted randomly at different points within the order. Samples were chromatographically separated on a Waters Acquity Premier BEH C18 column with dimensions of 50 mm length x 2.1 mm ID and particle size of 1.7 μ m (part number 186009455, Waters, Milford, USA). The solvents were Milli-Q water (A), and methanol (B), both containing 0.1% formic acid. A flow rate of 0.3 mL min⁻¹ was used. The LC gradient consisted of: 50% B from 0-1.3 min, a linear gradient from 50-95% B from 1.3-13.3 minutes, 95% B from 13.3-16.3min, 95-50%B from 16.3-16.4 min and 50% B from 16.4-20 min. The Orbitrap Fusion was operated with a heated electrospray ionisation (ESI) source under the following conditions: vaporizer temperature of 350 °C, an ion transfer tube temperature of 325 °C, and a positive ion spray voltage of 3500 V. The instrument was operated in a Data Independent Acquisition (DIA) mode consisting of a Full MS scan followed by 12 subsequent MS/MS scans spanning an m/z range of 195-437 with a window size of 23 m/z and an overlap of 3 m/z between each MS/MS scan. Full MS scans were acquired in the orbitrap detector with a resolution of 120 000, scan range of 180-440 m/z, normalised AGC target of 25%, and maximum injection

time of 50 ms with quadrupole isolation selected. MS/MS scans were acquired in the orbitrap detector using quadrupole isolation with a resolution of 30 000, Higher Energy Collisional Dissociation (HCD) energy of 35%, maximum injection time of 54 ms and an automatic scan range.

Data were analysed using the mass spectrometry software Skyline (Henderson *et al.* 2018; Pino *et al.* 2020). Compounds were identified and quantified based on their retention times, mass spectra, and area under the curve (MS1) relative to the authentic standards outlined in Marsh *et al.* (2019). Stock solutions were prepared by dissolving each standard in methanol, after which they were combined to form a standard mix. Standard curves were prepared in duplicate by serially diluting the standard mix in 50% methanol. We developed a linear calibration curve for each standard with 10 points ranging from 0 to 2.4 μM and a 2.5-fold dilution each time.

4.3.8 Quantification of FPCs

We measured the total concentration of FPCs in all 80 symphyomyrtle samples from round 1. All solvents were LCMS grade. 50.0 ± 0.5 mg of dried, ground leaf material was extracted once in 5.0 mL of acetonitrile:water (93:7 v/v) with 0.1% trifluoroacetic acid for one hour with occasional sonication. The extract was filtered through a 0.2 μm nylon syringe filter prior to injection.

20 μL sample extracts were analysed on an Agilent 1100 HPLC with an Agilent 1260 Infinity Diode Array Detector (Agilent Technologies, USA). Samples were chromatographically separated on a 4.6 x 250 mm Waters XBridge C18 3.5 μm column (part number 186003943, Waters, Milford, USA). The solvents were Milli-Q water (A), and acetonitrile (B), both containing 0.1% trifluoroacetic acid. A flow rate of 1.0 mL min^{-1} was used. The LC gradient consisted of: 60% B from 0-5 min, a linear

gradient from 60-90% B from 5-60 min, 90% B from 60-70 min, 90-60% B from 60-61 min and 60% B from 71-80 min. Absorbance was read at 275 nm.

Raw chromatographic data were analysed using the package ChromatographR (v0.7.1) (Bass 2023) in the R Statistical Environment, version 4.3.1 (R Core Team 2023). Processing steps included interpolation and baseline correction followed by variable penalty dynamic time warping to achieve peak alignment across chromatograms, peak fitting and peak table assembly. Known compounds were identified based upon retention times and similarity of UV spectra to authentic standards where available. Quantification was made on the basis of previously established standard curves where authentic standards were available for known compounds. Additional minor peaks were classified as macrocarpal-type meroterpenoid formylated phloroglucinol compounds on the basis of spectral similarity to known macrocarpals, and a standard curve for Macrocarpal G was used for their quantification.

4.3.9 Analysis by near-infrared spectroscopy (NIRS)

We used the subset of leaves for which we had obtained foliar chemical values as described above to develop calibrations to predict total and available N, FPCs and UBFs in all epicormic and mature leaf samples from their NIR spectra. Using Win ISI II, Version 10.2 software (Intrasoft International, Port Matilda, Pennsylvania, USA), we applied a standard normal variate and detrend scatter correction to untransformed spectra to minimise noise and then used modified partial least square regression with cross-validation and further independent validation as outlined in (Lane *et al.* 2024). For total N, the final model included 119 samples and had an r^2 of 0.988 with a standard error of cross validation (SECV) of 0.057. For available N, the

final model included 117 samples and had an r^2 of 0.970 with a SECV of 0.105. For FPCs, the final model included 53 samples and had an r^2 of 0.953 with a SECV of 2.446. Lastly, for UBFs, we developed a model using 221 samples from a variety of species (including the round 1 samples), with an r^2 of 0.929 and a SECV of 3.848.

4.3.10 Data analysis

All analyses were completed using R statistical Software (v4.2.2) (R Core Team 2022). To determine whether the chemical constituents differed between the eucalypt species and foliage type (epicormic in burnt vs. adult-phase foliage in unburnt locations), we developed a linear regression model for each chemical constituent (total N, available N, FPCs and UBFs) using the mean concentration of each constituent for each individual tree sampled across the entire sampling period. Tree species and foliage type and the interaction between them were independent variables and we used an ANOVA for each model to determine if there were any differences in mean constituents between species and foliage type. For post-hoc analysis, we used the *emmeans* package (v1.8.5) (Lenth 2023) in R to conduct pairwise comparisons of foliage types within each eucalypt species. Pairwise comparisons were only conducted when the overall effect of the factor was statistically significant ($p < 0.05$). For FPCs, our data was restricted to symphyomyrtle species only, and for UBFs, data were restricted to only monocalypt species.

We generated another linear regression model for each constituent to determine whether the concentrations changed over time. The data used to fit this model included all of the measurements for each tree at different times, unlike the first model which used means for each tree averaged over time. The model included tree species and foliage type and the interaction between them, and also included

the interactions between tree species, foliage type and the covariate time, which was measured as each leaf collection round (1–7). This is equivalent to fitting a linear model of each concentration against time for every species and foliage type. The aim was to see how the changes of the response over time differed between species and foliage types. We used the *emmeans* procedure to generate pairwise comparisons between the foliage types for each species. Two-sided t-tests were conducted to determine whether the slopes of the response against time were significantly different from zero (i.e., did the response change over time) for each species and foliage type. We also tested whether the slope was different between foliage types for each species. These comparisons were all carried out using the *emmeans* procedure in R.

For all models, we ran residual histograms and residual vs. fitted values plots. All models, apart from the change over time models for total and available N, were consistent with the model assumptions. There was a visible outlier in the residual vs fitted values plot for change over time for total and available N, which showed that the only contrast affected was the burnt vs unburnt comparison for *E. mannifera*. We re-ran the models with the outlier removed to check if the conclusions were sensitive to that outlier. The results remained the same, showing that our conclusions were not sensitive to the outlier.

4.4 Results

4.4.1 Total N

Concentrations of total N depended on the interaction between eucalypt species and foliage type ($F_{5,111} = 8.399$, $p < 0.001$). Post-hoc comparisons showed that total N was higher, on average, in epicormic growth compared to adult-phase

foliage for all six eucalypt species analysed ($p < 0.05$ for all pairwise comparisons; figure 2; table 1).

For change over time, the response also depended on the interaction between eucalypt species and foliage type ($F_{5,110} = 3.496$, $p = 0.006$. When running post-hoc analyses, total N decreased over time in epicormic growth and increased over time in the adult-phase foliage for *E. dives*, *E. mannifera* and *E. viminalis* (see table 1). For *E. rubida*, total N decreased over time in the epicormic growth, but did not change in adult-phase foliage. For *E. rossii* and *E. macrorhyncha*, there was no change in total N concentrations in either epicormic or adult-phase foliage over time (table 1; figure 2).

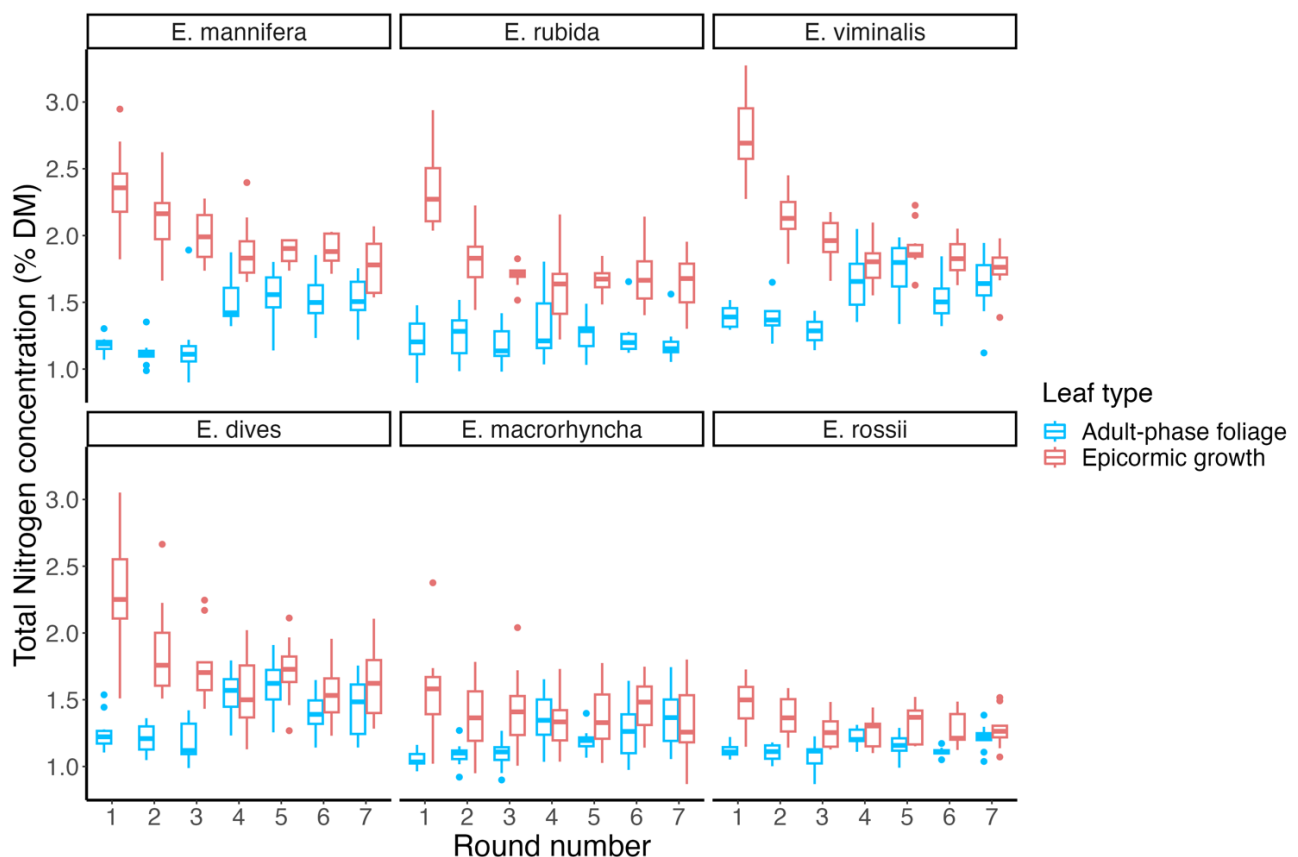


Figure 2: total nitrogen concentrations for each collection round in each eucalypt species for epicormic growth and adult-phase foliage. Species in the top row belong to the symphyomyrtle subgenus, while those in the bottom row are monocalypts.

Table 1. Pairwise comparisons within eucalypt species for total and available N, FPCs and UBFs. Mean concentration per tree compares constituent concentrations between epicormic and adult-phase leaves. Change over time tests whether the response over time differed between epicormic and adult phase leaves. Epicormic change over time and adult phase change over time test whether the slope of the response differs from zero (i.e. there is a change over time). Values in bold indicate a significant difference at the 0.05 level.

Total N (n = 113)								
Variable	Mean concentration per tree		Change over time		Epicormic change over time		Adult phase change over time	
Comparison	Foliage type		Foliage type		Slope of zero		Slope of zero	
	t	p	t	p	t	p	t	p
<i>E. mannifera</i>	9.839	<0.001	-5.910	<0.001	-5.364	<0.001	2.992	0.003
<i>E. rubida</i>	7.770	<0.001	-1.854	0.066	-3.224	0.002	-0.598	0.551
<i>E. viminalis</i>	7.638	<0.001	-4.903	<0.001	-4.926	<0.001	2.020	0.046
<i>E. dives</i>	5.997	<0.001	-4.313	<0.001	-3.785	<0.001	2.356	0.020
<i>E. macrorhyncha</i>	3.032	0.003	-1.945	0.054	-0.826	0.410	1.956	0.053
<i>E. rossii</i>	2.828	0.006	-1.534	0.128	-1.630	0.106	0.541	0.590
Available N (n = 113)								
Variable	Mean concentration per tree		Change over time		Epicormic – change over time		Adult phase – change over time	
Comparison	Foliage type		Foliage type		Slope of zero		Slope of zero	
	t	p	t	p	t	p	t	p
<i>E. mannifera</i>	8.337	<0.001	-4.778	<0.001	-3.996	<0.001	2.741	0.007
<i>E. rubida</i>	7.576	<0.001	-1.499	0.1368	-2.826	0.006	-0.709	0.480
<i>E. viminalis</i>	7.834	<0.001	-5.233	<0.001	-4.513	<0.001	2.873	0.005
<i>E. dives</i>	3.910	<0.001	-4.208	<0.001	-3.170	0.002	2.790	0.006
<i>E. macrorhyncha</i>	-2.217	0.0286	0.870	0.3861	3.361	0.001	2.266	0.025
<i>E. rossii</i>	-0.131	0.896	-0.048	0.962	0.721	0.473	0.785	0.434
FPCs (n = 56)								
Variable	Mean concentration per tree		Change over time		Epicormic – change over time		Adult phase – change over time	
Comparison	Foliage type		Foliage type		Slope of zero		Slope of zero	
	t	p	t	p	t	p	t	p
<i>E. mannifera</i>	5.593	<0.001	-5.777	<0.001	-1.871	0.067	6.312	<0.001
<i>E. rubida</i>	2.450	0.018	-0.988	0.327	-1.283	0.205	0.117	0.907
<i>E. viminalis</i>	2.300	0.025	-1.872	0.067	1.060	0.294	3.710	<0.001

UBFs (n = 59)								
Variable	Mean concentration per tree		Change over time		Epicormic – change over time		Adult phase – change over time	
Comparison	Foliage type		Foliage type		Slope of zero		Slope of zero	
	t	p	t	p	t	p	t	p
<i>E. dives</i>	-4.053	<0.001	5.495	<0.001	7.083	<0.001	-0.831	0.409
<i>E. macrorhyncha</i>	-4.210	<0.001	0.567	0.573	0.950	0.348	0.170	0.862
<i>E. rossii</i>	-2.141	0.037	2.693	0.009	3.486	<0.001	-0.310	0.757

4.4.2 Available N

Mean concentrations of available depended on the interaction between eucalypt species and foliage type ($F_{5,111} = 20.830$, $p < 0.001$). In post-hoc analyses, available N was higher in epicormic growth compared to adult-phase foliage in four of the six species analysed, and lower in one (figure 3; table 1).

For change over time, the response also depended on the interaction between them ($F_{5,110} = 6.828$, $p < 0.001$). In post-hoc analyses, the responses were similar to what was observed with total N. Available N decreased over time in epicormic growth and increased over time in mature foliage for *E. dives*, *E. mannifera* and *E. viminalis* (table 1; figure 3). For *E. rubida*, available N decreased over time only in epicormic leaves. For *E. macrorhyncha*, available N increased in epicormic growth and adult-phase leaves at a similar rate. For *E. rossii*, the concentration was stable in both epicormic growth and adult-phase foliage over time (table 1).

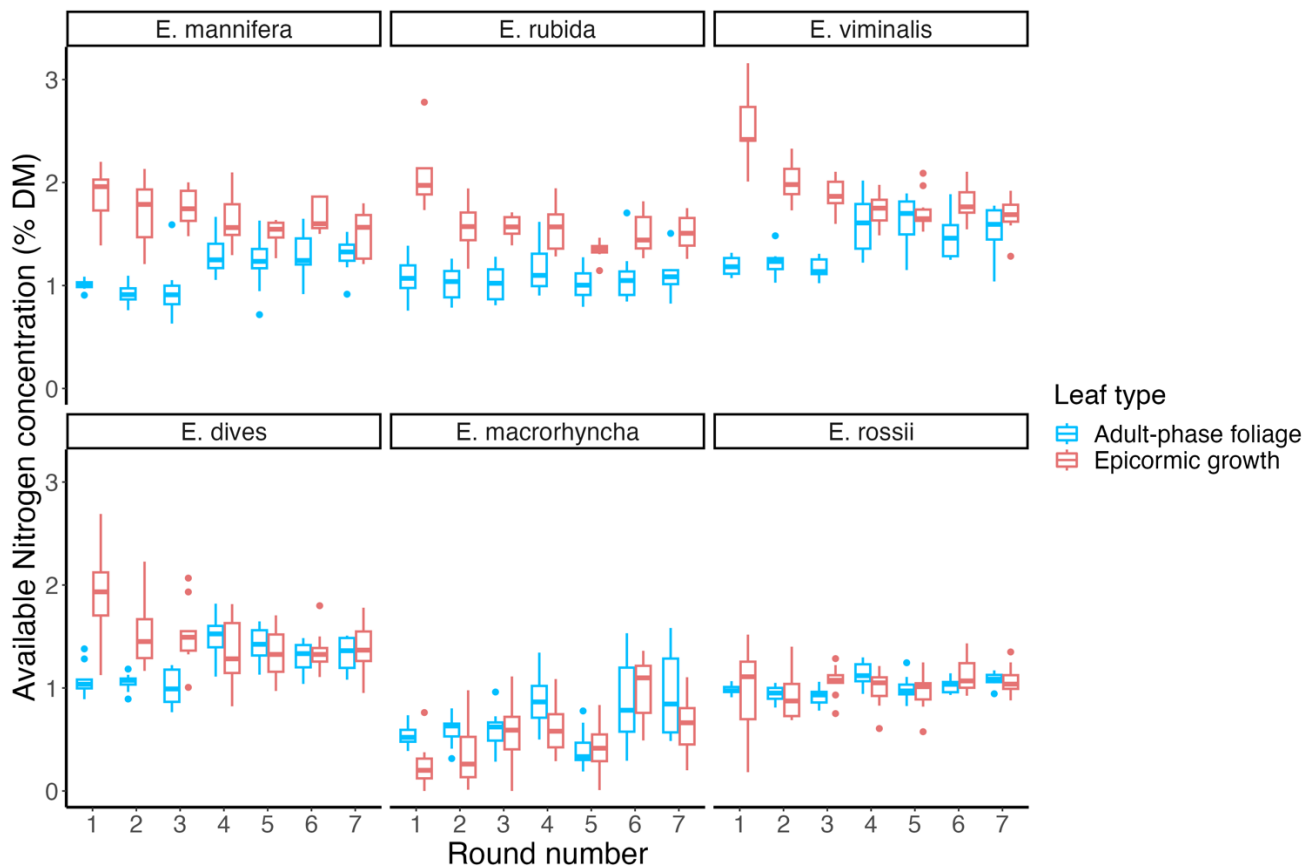


Figure 3: Available nitrogen concentrations for each collection round in each eucalypt species for epicormic growth and adult-phase foliage.

4.4.3 FPC concentrations

Mean FPC concentrations depended on the interaction between eucalypt species and foliage type ($F_{2,54} = 3.754$, $p = 0.030$). Pairwise comparisons showed that FPC concentrations were higher, on average, in epicormic growth compared to adult-phase foliage in all three symphyomyrtle species (figure 4; table 1).

Similarly, for change over time, the response depended on the interaction between eucalypt species and foliage type ($F_{2,54} = 6.494$, $p = 0.003$). Post-hoc analyses showed that, for *E. mannifera*, and *E. viminalis*, FPC concentrations increased over time in adult-phase foliage, while there was no change in either leaf type for *E. rubida* (table 1; figure 4).

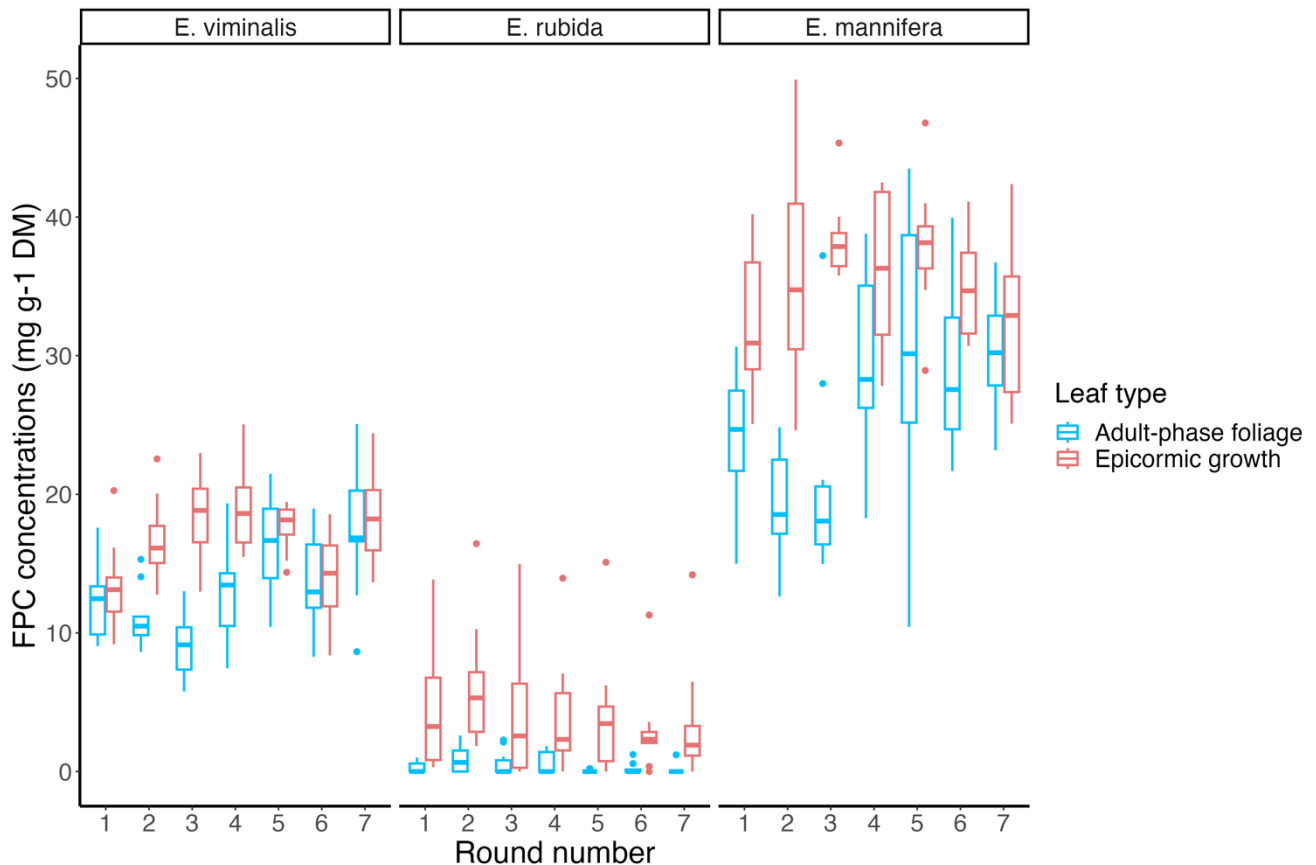


Figure 4: FPC concentrations for each collection round from each eucalypt species belonging to the *Symphyomyrtus* subgenus in epicormic growth in the burnt landscape and adult-phase foliage in the unburnt landscape.

4.4.4 UBF concentrations

Mean UBF concentrations differed between eucalypt species ($F_{2,57} = 193.359$, $p < 0.001$) and foliage type ($F_{1,57} = 36.082$, $p < 0.001$). In pairwise comparisons, concentrations of UBFs were higher, on average, in adult-phase foliage compared to epicormic growth in all three monocalypt species (figure 5; table 1).

For change over time, the response depended on the interaction between eucalypt species and foliage type ($F_{2,56} = 6.097$, $p = 0.004$). Post-hoc analyses showed that the only changes in UBF concentrations were increases in epicormic growth in *E. dives* and *E. rossii* over time (table 1; figure 5).

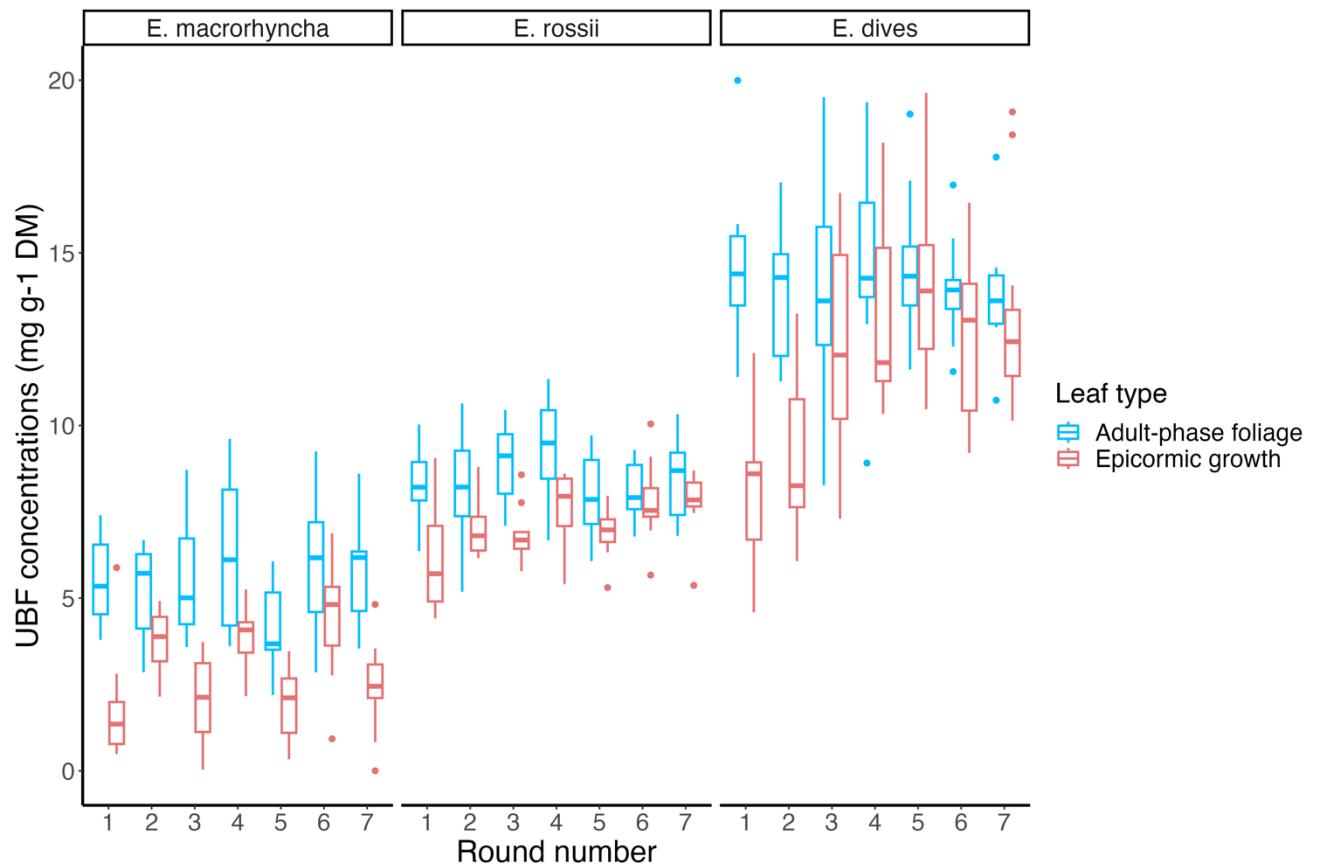


Figure 5: UBF concentrations for each collection round from each eucalypt species belonging to the *Eucalyptus* subgenus in epicormic growth and adult-phase foliage.

4.5 Discussion

This study ultimately shows that the nutritional quality (total N, available N and PSM concentrations) of post-fire epicormic growth differs from adult-phase foliage in multiple eucalypt species, and that the magnitude of these effects depends on time since fire. It is the first study to rigorously quantify temporal changes in foliage composition post-fire in a eucalypt forest, and the findings have important implications for marsupial folivores that feed on eucalypts. This is because individual tree and landscape nutritional composition influences the presence, abundance, feeding behaviour, and reproductive capacity of marsupial folivores (Au *et al.* 2019;

DeGabriel *et al.* 2009; Lawler *et al.* 1998; Wallis *et al.* 2012). The differences in leaf chemistry were most apparent soon after fire and epicormic leaves became more similar to adult-phase leaves as time progressed. Our findings provide valuable information about the ecological recovery of a burnt landscape and the timeframe of the effects of bushfires on the nutritional composition of the landscape for resident herbivores.

Younger leaves often contain higher concentrations of nitrogen, which contributes to rapid leaf expansions. It is therefore not surprising that total N concentrations were higher, on average, in the epicormic growth of all eucalypt species we studied, and that concentrations decreased in epicormic leaves over time since fire. Interestingly, however, the available N concentration, which is an *in vitro* measure that incorporates the effect of fibre and tannins on protein digestibility (Wallis *et al.* 2010), was not always higher in epicormic leaves. This finding is important because most studies only consider the effect of fire on total N concentrations in herbivore food plants (Reich *et al.* 2001; Song *et al.* 2022). Depending on the herbivore and plant species of interest, our findings show that total N concentrations may not adequately represent the effects of fire on food quality.

Animals need a certain amount of protein per day to meet maintenance requirements (Mattson Jr 1980). For this reason, the concentration of digestible protein in foods can influence diet selection and the amount eaten (Jensen *et al.* 2015; Marsh *et al.* 2003a; Marsh *et al.* 2014; Stalenberg *et al.* 2014; Troyer 1984). Higher concentrations of digestible protein also reduce the costs of PSM detoxification (Au *et al.* 2013). In our study area, animals such as insects and common ringtail possums that have adaptations to either hinder the formation of protein-tannin complexes or to minimise their effects (Barbehenn and Constabel

2011; Fox and Macauley 1977; Marsh *et al.* 2003a; Marsh *et al.* 2003b), are likely to obtain more protein by feeding on epicormic leaves than adult phase leaves, regardless of the eucalypt species. In contrast, the outcomes for other folivores, for which available N may be a better measure of digestible protein than total N, will depend on which eucalypt species they eat. Our findings suggest that symphyomyrtle species are more likely to be beneficial in terms of protein provision than monocalypt species, which is also consistent with findings from Lane *et al.* (2024).

Another important component of understanding the quality of food for herbivores post fire is to consider what happens with herbivore-deterrent PSMs. Some plants produce or accumulate higher concentrations of these constituents in young leaves to protect them from herbivory (Coley 1983; Coley and Kursor 1996). For this reason, we expected that FPC and UBF concentrations would be higher in epicormic leaves. This was true for FPCs in symphyomyrtle species, but not for UBFs in monocalypts. The findings for FPCs align with research on other PSMs in juvenile phase foliage on eucalypt seedlings, with total phenolics and terpenes reported to be higher in seedlings compared to adult-phase foliage (Goodger *et al.* 2013; Li *et al.* 1994). O'Reilly-Wapstra *et al.* (2007) also observed that concentrations of sideroxylonal A (an FPC), were higher in coppice foliage (i.e., foliage that had resprouted from tree stumps after felling) compared to the adult-phase foliage in *E. globulus*.

Marsupial folivore feeding behaviour is strongly influenced by the concentrations of many herbivore-deterrent PSMs, with increasing concentrations linked to reduced and slower intake, and reduced visitation to particular trees (Marsh *et al.* 2007; Moore and Foley 2005; Moore *et al.* 2005). The preferences for eucalypt

foliage also differs between marsupial folivore species and this is, in part, thought to be due to differing tolerances for PSMs (Jensen *et al.* 2014). For example, common ringtail possums can tolerate high concentrations of UBFs and feed more extensively from monocalypt species than do other marsupial folivores (Beale *et al.* 2022), while koalas appear to have a high tolerance for FPCs compared to other marsupial folivores and therefore tend to prefer foliage from *Symphyomyrtus* species (Lawler *et al.* 1998; Moore and Foley 2005). Given that, in this study, symphyomyrtle epicormic growth was higher in nitrogen but also in FPCs, marsupial folivores may face a trade off when consuming this type of foliage. This could ultimately influence feeding choices for different eucalypt species and individual trees. A useful next step would be to determine whether the diet composition of free-ranging folivores differs between burnt and unburnt habitat. Together with the nutritional data, this would assist with understanding whether some eucalypt species become disproportionately more valuable to folivores after fire.

Interestingly, in this study, we observed that concentrations of UBFs were higher in adult-phase foliage compared to epicormic growth in all monocalypt species. Furthermore, two of the three monocalypt species showed UBF concentrations increasing over time in epicormic foliage. The quantification of UBF types and concentrations in eucalypt species is relatively new (Marsh *et al.* 2019) and the effect of UBFs on marsupial feeding has also received less attention than FPCs. Nevertheless, UBFs are known to deter marsupial folivore feeding above certain concentrations, even though tolerances differ between folivore species (Beale *et al.* 2022; Marsh *et al.* 2021; Marsh *et al.* 2015). If our pattern of lower UBF concentrations in epicormic leaves holds true in other eucalypt species, it suggests that epicormic monocalypt leaves could be less deterrent to eucalypt folivores than

adult phase monocalypt leaves. Lane *et al.* (2024) found that koalas avoided monocalypt epicormic leaves in favour of adult phase foliage from a variety of species, but this could reflect their preferences for the alternative species offered, some of which were symphyomyrtles. Thus, it is important to not only consider the differences in composition between epicormic and adult leaves, but also the context in which leaves are available. Since koalas and many other eucalypt folivores have a large distribution across Australia and tree species composition across their range can vary substantially, it would be worth repeating our study in other eucalypt forest types with different eucalypt species to determine whether the findings for both monocalypt and symphyomyrtle species are largely consistent or context specific.

Although we expected to see changes in nutritional composition over time in epicormic leaves, we predicted that the composition of adult phase leaves would remain relatively stable during our study. This is because changes in leaf composition are often related to leaf ontogeny (Goodger *et al.* 2013; Li *et al.* 1994; Mediavilla *et al.* 2018; Ochoa-López *et al.* 2015), but eucalypt trees are non-deciduous and often retain their leaves for several years (Bowman and Prior 2005; Williams *et al.* 1997). We attempted to collect mature leaves during all sampling rounds to minimise potential effects of comparing very new or very old leaves across sampling rounds. Despite this, in two of the three symphyomyrtle species, concentrations of FPCs increased over time in the adult-phase foliage. UBF concentrations were also noticeably variable across time periods in some species, and total and available nitrogen concentrations increased over time in the adult-phase foliage of some species. Steinbauer *et al.* (2015b) found that total FPC and nitrogen concentrations in mature leaves of some eucalypt species varied between sampling periods, probably reflecting the diversion of precursors to other structures

or pathways during flowering and leaf flushing and the impact of climatic conditions. Given that leaves were sampled across a one-year period, the trees in this study were subjected to variable environmental conditions (e.g. temperature and rainfall), variable insect herbivory and pulses of new leaf growth and flowering. Future research is needed to determine if there are particular environmental conditions or other external factors that can influence foliar chemistry in adult-phase leaves across different tree species, as evidence for this in eucalypts is equivocal.

Our findings also raise several other considerations for further research. Firstly, in this study, we focussed on epicormic leaves when sampling in the burnt area. Fire severity can vary within landscapes, leaving some eucalypts in burnt areas with intact canopy. It would be useful to confirm whether adult-phase foliage in burnt areas follows the same pattern as adult-phase foliage in unburnt areas to differentiate environmental effects of fire (e.g., changes in soil composition or water availability) on leaf composition from the effects of leaf type (i.e., epicormic vs adult-phase). Studies in some other plant systems have shown that post-fire changes in soil properties can influence leaf nutrient value (Huang and Boerner 2007). Some glasshouse fertilisation studies suggest that changes to soil properties can influence leaf chemistry in eucalypt seedlings (Meiners *et al.* 2017; Seneviratne *et al.* 1999), but the effects of changing soil composition on eucalypt leaf chemistry in mature trees is unclear (Cochrane *et al.* 2023). Additional research investigating the effects of fire on the chemical composition of adult foliage would assist with understanding how lower severity fire, including hazard reduction burns, affect the quality of available food for eucalypt folivores.

Secondly, this study began sampling 9 months post-fire. Given that the largest differences observed between epicormic growth and adult-phase foliage were

generally at the first round of leaf collection, there may have been even larger differences in early regrowth that were not detected in this study. Future studies could prioritise sampling sooner after fire to better determine the full extent of foliar chemistry changes in epicormic growth. This would provide a more complete understanding of the nutritional trade-offs facing eucalypt folivores in the initial post-fire recovery period.

4.6 Conclusions

Bushfires are of global significance and are likely to increase in severity and frequency as a result of anthropogenic climate change (Lucas *et al.* 2007). Animals within fire-prone landscapes are subjected to disturbance from these events, and therefore, understanding how fire affects their habitat quality is critical for their conservation now and in the future. Fire can influence the type of browse available for leaf eating animals and its nutritional composition. Our results suggest that epicormic regrowth after fire could be a valuable source of food for some eucalypt folivores, including koalas, but that its nutritional quality varies depending on tree species and subgenus. The overall nutritional quality of post-fire landscapes is therefore likely to be influenced by the available tree species and the amount of browse. We also found that the nutritional composition of epicormic foliage tends to become more like that of adult-phase foliage over time and, to a lesser extent, there may be temporal changes in adult-phase foliage, which warrants further investigation. These findings demonstrate the value of undertaking temporal studies.

Understanding the nutritional quality of foliage from the perspective of browsing herbivores can help us to better manage and conserve habitats and wildlife populations. The relative importance of particular tree species for folivores, like

koalas, is often determined by their perceived 'use' based on observations of animals in the tree, whether that be for shelter or food (State of NSW and Office of Environment and Heritage 2018). Although this is valuable information, the changes in chemistry over time and in different foliage types, as demonstrated by this study, suggest that our understanding of how folivores use trees for food may be more variable than anticipated, and may differ after a major fire event when the available browse differs chemically from the mature foliage typically present. Although our analyses have focused on constituents known to be important to marsupial folivores, our findings are also likely to be useful for understanding how fire can affect the nutritional landscape for a range of other insect folivores that are responsive to the same foliar chemical constituents measured in this study (Andrew *et al.* 2007b; Fox and Macauley 1977; Joseph *et al.* 1993; Marsh *et al.* 2017b; Steinbauer *et al.* 2015a). Broadly this research demonstrates the potential influence of fire on nutritional landscapes for browsing herbivores and the need to incorporate information on plant-herbivore systems into efforts to understand the effects of fire on habitat quality for wildlife populations.

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Synthesis

Research summary and conservation implications

Obtaining post-fire data on animal species and the habitats they live in can provide valuable information about their health, survival and resilience. This information can help guide effective conservation efforts in the future. Despite their iconic status, there is a significant knowledge gap in the literature about how koalas respond to fire and the nutritional changes in the landscape. This knowledge gap, and the need for research on this topic to improve koala conservation and management, has been recognised at both State (Department of Environment Water and Natural Resources 2016; NSW Department of Planning and Environment 2022; Office of the NSW Chief Scientist & Engineer 2016; State of NSW and Office of Environment and Heritage 2019; State of Victoria Department of Energy 2023) and Federal levels (Department of Agriculture Water and the Environment 2022b). In this thesis, I investigated how surviving bushfires and living in burnt landscapes affected koala health and movements in both rehabilitated and non-rehabilitated individuals (chapters one and two). I also addressed key knowledge gaps in our understanding of whether koalas will eat post-fire eucalypt epicormic regrowth and its nutritional quality (chapters three and four).

I will use these concluding remarks, not to reiterate the results and discussions of each previous chapter, but to connect the findings, discuss the conservation implications of this research, and highlight areas for future investigation. My thesis is the most comprehensive study ever undertaken on the effects of fire and post-fire conditions on koalas and their habitat. In the region in which I undertook my research I found that, 1) burnt forests can successfully support healthy koalas, and 2) koalas living in burnt forests do not necessarily need to be rescued or rehabilitated,

provided there are adequate food resources to support them. These observations are based on the findings of chapters one and two, where, within the period of 4 months to 16 months post fire, non-rehabilitated koalas living in burnt and unburnt habitat had similar home range sizes and travelled similar distances each day. Furthermore, rehabilitated and non-rehabilitated koalas were also in similar health and body condition. This should reassure wildlife carers, and those invested in koala welfare and management, that these burnt areas can remain suitable habitat.

Many marsupial folivores are reliant on eucalypt foliage for both food and shelter (Crowther *et al.* 2014; Crowther *et al.* 2022; McAlpine *et al.* 2006b). Although the quantity of food available to koalas in burnt habitat decreased immediately after the megafires (Gallagher *et al.* 2021), diet quality in the burnt area, given the particular composition of eucalypt species available, was likely higher due to epicormic leaves having more digestible protein despite sometimes higher foliar concentrations of defensive chemicals (chapters three and four). My data on leaf composition may help to explain my findings on movement and health. Koalas living in burnt areas of the Southern Tablelands of NSW could probably meet their nutritional requirements by feeding on the available epicormic leaves and did not need to move larger distances between pockets of less fire-affected trees to feed on adult leaves in intact canopy.

Interestingly, although the health and body condition of rehabilitated koalas matched their non-rehabilitated counterparts (chapter one), they moved farther from their release sites and generally had larger home ranges (chapter two). Although studies have shown that rehabilitated and translocated animals can often disperse farther when released at a different location (Beaman *et al.* 2023; Whisson *et al.* 2012), this was not the case in this study. I suspect that the larger movements and

home ranges observed may have been due to animals trying to rehabilitate to such a physically changed landscape or because they were competing with non-rehabilitated koalas. However, as most koalas were in good health nine months after their release, it demonstrates that rehabilitated koalas can be released into and survive in burnt landscapes. These findings fill important knowledge gaps about post-rehabilitation release success of koalas. Wildlife rescue and rehabilitation will continue to be an ongoing and important practice, particularly following major disturbance events, such as fire. The results outlined in this thesis can be used to refine koala release policies, such as in the NSW Code of Practice for Injured Sick and Orphaned Koalas (NSW Government Department of Planning and Environment 2023).

Because they have been prepared as standalone publications for scientific journals, most of my chapters have focused on the broad implications of the findings for koala health and ecology after fire. However, it is important to acknowledge that my work also has substantial value for koala conservation and management in the Monaro region. There is no historical information on the health, incidence of disease, movements, and home range size of koalas in this region. My data will therefore be highly valuable in aiding the conservation of koalas in this area, particularly if it is incorporated into a planned Comprehensive Koala Plan of Management for the Snowy Monaro Shire. My research provides a baseline for ongoing monitoring and helps to define the amount of habitat required to support koalas. It also provides important information about the nutritional quality of local eucalypt species to inform a detailed and comprehensive review of koala habitat in this region.

Limitations and future research directions

The data obtained in this study are essential for understanding the impact of fire on koalas and their habitat, offering valuable insights into their response to extreme fire events. It is likely that some of the responses I recorded in chapters one and two are context dependent. Koalas occupy a large geographic range, from islands off the coast of southern Australia through to northern Queensland (Lee and Martin 1988; Melzer *et al.* 2000). Their habitat, diet, and the environmental conditions they are exposed to vary substantially across this range (Moore and Foley 2000). This thesis focuses on a population of koalas in the NSW Southern Tablelands. Even within this region, eucalypt species responded differently to fire in terms of the nutritional value of their epicormic leaves (chapter four). Preliminary work in other eucalypt forests post fire in northern NSW and Victoria suggests that it is common to find this variability in species responses (Marsh *et al.* 2021; B. Wagner, personal communication). Depending on the proportional availability of different eucalypt species, some habitats may provide substantially more nutritional value to koalas after fire than others, and this could potentially flow through to affect the movement ecology and health of koalas. My study significantly improves our understanding of the effects of fire on koalas and their habitat. However, it is important to note that any koala conservation strategies that incorporate these findings recognise that the effects of fire on landscapes and wildlife are complex, and there may be situations where these findings don't apply. Further research is needed to identify these scenarios and understand the conditions under which differences may occur, for example, where eucalypt species decline in nutritional quality after fire. This could impact how koalas, and other marsupial folivores, utilise burnt landscapes, and affect population resilience.

Some of my findings may also be influenced by the fire extent and degree of severity at my study sites. Fire severity can affect animal abundance, with arboreal mammal abundance lower at severely burnt sites than in unburnt habitat and forests that only had understorey burn (Chia *et al.* 2015). Fire severity can also influence the amount of epicormic and adult leaves that are available (Keeley 2009). This variation should be kept in mind because it could lead to differences in wildlife movement behaviour. Nevertheless, given that responses of koala movements and their health were similar between both the burnt (which had areas of extreme fire severity) and unburnt landscape, my findings may be reasonably robust to differences in fire extent and severity.

Through my tracking work in chapter two, one knowledge gap that I identified was whether koalas actively seek out burnt areas for feeding. Not only did koalas have home ranges entirely within burnt areas, but I also found that several koalas residing in an unburnt area moved into burnt areas. These koalas stayed in burnt areas for the remainder of the study, and were presumably feeding there. It would be beneficial to conduct targeted tracking studies of koalas living on the fringes of burnt habitats. This would determine if the patterns we observed with koalas moving from unburnt areas into burnt areas, were normal after a major fire event, and whether this plays a role in population recovery post fire. Given the higher nutritional quality of epicormic growth shortly after fire (chapters three and four), it is possible that koalas are attracted to post fire regrowth, similar to grazing animals that move into burnt areas to access new fresh growth (Allred *et al.* 2011; Archibald and Bond 2004). This strengthens arguments against devaluing burnt landscapes as potentially valuable habitat for koalas simply due to a recent or even severe fire.

Koalas are known to prefer some tree species over others (State of NSW and Office of Environment and Heritage 2018). However, the need to better understand the interactions between diet quality, tree species composition and the type of browse available (epicormic and adult-phase mature foliage) is becoming more widely recognised. My results from chapters three and four show that koalas consume more epicormic leaves from certain eucalypt species (chapter three), and the nutritional composition of these leaves change over time (chapter four). This suggests that some eucalypt species may become more prominent in koalas' diets after fire, leading to a different diet quality compared to koalas in unburnt habitats, particularly in the early months post-fire. If I had additional capacity during my research program, I would have liked to look more in-depth at how diet composition and diet quality differs between burnt and unburnt habitat.

My temporal analysis of leaf chemistry in chapter four focussed on epicormic leaves in burnt habitat and adult leaves in unburnt habitat. Future studies should observe whether adult-phase foliage present in burnt landscapes follow a similar trajectory to what was observed for adult foliage in unburnt landscapes, or whether there are fire-related effects on the chemical composition of adult leaves in burnt areas. Fire can affect a variety of soil properties, including water retention (Alkin and Kinoshita 2020), pH (Agbeshie *et al.* 2022), porosity (Certini 2005) and available nutrients (Certini 2005; Verma and Jayakumar 2012). In some plant species, these types of changes in soil profiles can affect leaf nutritional quality (Sariyildiz and Anderson 2005). Although I clearly demonstrated that habitat nutritional composition changes after fire when epicormic leaves are produced, analysing the composition of adult leaves in burnt habitat would give a more complete overview of landscape nutritional quality when there are patches of trees available with intact canopy. It

would also help to understand the nutritional consequences of low severity fire, such as cool hazard reduction burns or cultural burning, which do not always stimulate the production of epicormic leaves.

The large dataset I collected on koala behaviour and landscape characteristics could also be used by future researchers to address some additional questions about koala ecology. For chapters one and two, I tracked over 30 koalas for up to nine months, and collected five GPS points per night. While I was able to generate valuable home range and movement data from this, it would also be highly beneficial to visit the points used by koalas during the night to assess what eucalypt species they are likely browsing on. Although I did not use it for this thesis, I also obtained daytime data on tree use through checking koalas weekly to meet ethics requirements. It is well known that the trees koalas occupy during the day are not necessarily good indicators of their diet preferences (Marsh *et al.* 2014), so it would be an interesting comparison to see how daytime tree use differed from the species they use at night. Observing the trees that koalas in this area are likely feeding on would provide more valuable insights into this understudied population. Combining this knowledge with the data on leaf chemistry obtained in chapter four could indicate which areas of the landscape may be of better quality.

After fire, structural changes to the forest may also affect how koalas use the landscape. For example, fires can cause significant canopy loss (Bowman *et al.* 2021), which removes important shelter for koalas during the day, potentially impacting their ability to thermoregulate (Cristescu *et al.* 2023; Crowther *et al.* 2014). In this study, the burnt site at Peak View had variable topography, with an elevation range of 800 to 1233m. Future studies should investigate whether koalas position themselves in the landscape based on temperature and aspect and whether this is

significantly different after fire when canopy cover is reduced. This information would further our understanding of koala behaviour after fire and may explain their movement behaviour in the months after fire. For example, if koalas stay at lower altitudes or choose different trees to shelter in, it shows that living in burnt landscapes influence their behaviour. While it was out of scope for this thesis, it would be valuable to have temperature loggers at various positions in the landscape to see how the temperature varies and whether it influences landscape use for koalas. It would also be worth conducting studies on whether koalas preferentially use certain eucalypt trees species after fire for improved thermoregulation. Koalas are known to rest against tree trunks during hot weather, using large lower branches, as they tend to be cooler than the surrounding air temperature (Briscoe *et al.* 2014). Both rough and smooth bark trees were available to koalas throughout this study, and it would be interesting to observe whether bark temperature differs between eucalypt species and whether this impacts koala daytime tree preferences after fire. Understanding these preferences could help identify critical habitat features that support koala thermoregulation and recovery in fire-affected landscapes, improving our understanding of how koalas recover post-fire. My data on daytime tree species use in burnt and unburnt habitat could be used to inform such a study.

Conclusion

Disturbance processes, both human-induced and natural, can impact many of Australia's wildlife species and the habitats they live in (Bowd *et al.* 2021; Doherty *et al.* 2021; Driscoll *et al.* 2021; Lindenmayer *et al.* 2021). Fire intensity and frequency are likely to increase with climate change (Jones *et al.* 2022), therefore, identifying how bushfire events can impact our native wildlife species, and their habitat, is

crucial for enhancing their survival into the future. The information provided in this thesis offers valuable insights into the ecology of koalas after fire that could improve management and rehabilitation processes that aid their conservation. Furthermore, the information obtained on the nutritional composition of a landscape after fire will help guide the management of other marsupial folivores living in eucalypt forests, as we will be able to better determine important habitat areas after fire and can predict how the landscape might change over time. Understanding nutritional changes can improve our understanding of animal behaviour as well, allowing us to predict how they may respond after fire and as the landscape recovers.

There is no doubt that fires can have substantial effects on koala mortality (Van Eeden *et al.* 2020), but my research provides reassurance that the effects of fire on habitat nutritional quality are transient and may even be positive in the short term. Koalas can live wholly within burnt habitat and rehabilitated koalas can reintegrate into these areas. These results can be incorporated into koala conservation and policy documents to inform the management, rehabilitation and release of koalas in the future.

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