

Investigating the conservation requirements of the endangered  
Tasmanian wedge-tailed eagle (*Aquila audax fleayi*)



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10<sup>th</sup> August 2019

### *Declaration of originality*

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

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The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University.

All wildlife research carried out by the candidate was conducted with approval from the University of Tasmania Animal Ethics Committee (approval number A0015930 and A0015422) and the Department of Primary Industries, Parks, Water and Environment (permit number 17328 and 16312).

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## *Statement of contribution*

The candidate was lead researcher and author of all work presented in this thesis. The following people and institutions contributed to the preparation of the four data chapters, which are written as scientific journal papers intended to be submitted for publication:

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Chapter 2 was conceived by JP, TK, JW, and EC. Carcasses were acquired by WB and NM. JP collected samples from the eagle carcasses. JP, JW and TK collected blood samples. JP prepared all samples for lab analysis. JP analysed the data with assistance from TK. JP led writing of the manuscript. EC, CH, AK and TK contributed to revisions of the manuscript.

Chapter 3 was conceived by JP, TK, JW, CH and EC. Carcasses were acquired by WB and NM. JP collected samples from the eagle carcasses and prepared them for lab analysis. JP analysed the data with assistance from LB, TK and EC. JP led writing of the manuscript. CH, TK and EC contributed to revisions of the manuscript.

Chapter 4 was conceived by all authors. JP, JW and TK attached GPS transmitters. JP analysed the data with assistance from TP, TK and KP. JP led writing of the manuscript. EC, CH, and AK contributed to revisions of the manuscript.

Chapter 5 was conceived by JP, TK and JW. Carcasses were acquired by WB and NM. JP collected all measurements. KP and JP performed molecular sexing. JP analysed the data. JP led writing of the manuscript. EC, CH and TK contributed to revisions of the manuscript.

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Image T. Katzner

## *Abstract*

Predators play a key role in maintaining ecosystem integrity in terms of ecosystem functions, long term ecological stability and even species composition. Raptors are important predators in many ecosystems, yet they are disproportionately at risk of extinction. Threats to these species include habitat loss and degradation, illegal persecution, unintentional poisoning and collisions with anthropogenic structures such as powerlines and wind turbines. The impacts of each threat will vary between populations due to inter- and intra-specific differences in behaviour as well as regional differences in landscapes and anthropogenic modification. An understanding of both the behavioural ecology and the specific combination and/or extent of threats faced by a raptor species is therefore crucial to the planning of effective in situ conservation management.

The overarching goal of the research described in this thesis was to inform the conservation management of the Tasmanian wedge-tailed eagle (*Aquila audax fleayi*), an endangered subspecies endemic to the island of Tasmania. Prior to this study, conservation efforts had focussed on the threats of breeding habitat loss and disturbance to nesting eagles, but little is known about the habitat requirements of other life stages, or the extent of other threats that may impact survival. Addressing these knowledge gaps will be important for future successful conservation management.

Within this overall goal, the first aim, addressed in Chapter 2, was to identify the extent of lead exposure in Tasmanian wedge-tailed eagles and explore patterns in exposure detected. Avian predators and scavengers are known to be susceptible to lead poisoning through the ingestion of lead-based bullet fragments in shot animals. Herbivores are shot widely in Australia, including over one million macropods annually just in Tasmania, yet lead exposure is not presently recognised as a major threat to any Australian predator. I detected elevated lead levels in 10.4% of 106 liver samples and 3.7% of 108 femur samples opportunistically collected from dead wedge-tailed eagles across the island. I also detected lead in 95.9% of blood samples taken from 24 eagle chicks captured at the nest, 8.3% of which had levels indicative of potential clinical poisoning. Femur sample lead levels were higher in adults than in immature birds, suggesting chronic exposure. Isotopic similarities found between lead from these samples and lead from ammunition supported my interpretation that lead ammunition may be a significant

source of exposure. My results indicate that lead exposure is an overlooked threat to the Tasmanian wedge-tailed eagle and highlight the need for regulation of lead-based ammunitions and use of lead-free alternatives.

In Chapter 3 I tested the extent to which Tasmanian wedge-tailed eagles are exposed to anticoagulant rodenticides, and investigated which factors influence exposure within the population. Anticoagulant rodenticides are used worldwide to control rodent pests, but non-target exposure to raptors is known to occur through predation of poisoned animals. In Australia, many anticoagulant rodenticides are unrestrictedly available for private and commercial use, but there has been little work evaluating the impacts of anticoagulant rodenticides on native predatory species. Anticoagulant rodenticide residues were detected in 74% of 50 dead eagles, with 16% having liver concentrations that were lethal. Anticoagulant rodenticide levels and probability of exposure increased with proportion of agricultural habitat and human population density in the estimated home range. I identify anticoagulant rodenticide exposure as a previously unrecognised threat to the Tasmanian wedge-tailed eagle population, and indicate a need to review the current registration of these compounds for residential and agricultural use.

The third aim, addressed in Chapter 4, was to provide a detailed understanding of juvenile Tasmanian wedge-tailed eagle behaviour and habitat use through GPS-tracking of 25 birds. The pre-dispersal period (147-575 days) was much longer than previously predicted. The birds exhibited three distinct behavioural states: perching, short flights and long flights. During all behaviours, the eagles selected for areas less than 75 m from forest edges and with topographic slopes over 15°. Long flights occurred more frequently over forests, whilst open habitats were generally avoided regardless of behaviour type. Cross-validated habitat selection models performed well in predicting the areas where short flights and long flights occur in Tasmania. These models can be developed into management tools, to estimate impacts of land-use changes and predict high risk areas for collisions with powerlines and wind turbines on a life stage about which very little was previously known.

In Chapter 5, I describe the development of a morphological method to sex Tasmanian wedge-tailed eagles. An individual's behaviour, and thus the impacts of different threats to it, are likely to differ between sexes; the demographic consequences of these threats are therefore likely to depend on the population sex ratio. Yet molecular analyses are costly, and there is currently no

validated method to sex Tasmanian wedge-tailed eagles through morphology. Using my method 100 free-flying birds could be sexed with 97.6% accuracy using forearm, tarsus width and hallux length, whilst 25 late-stage nestlings could be sexed with 95.4% accuracy using hallux width, hallux breadth and tarsus breadth. I present the corresponding equations as tools for use in future research and management of the subspecies.

In this thesis, I took a multi-dimensional approach to the conservation ecology of the Tasmanian wedge-tailed eagle. Combined, this research provides new guidance and tools for the conservation of the population. I have identified previously unknown threats which may also apply to other species both in Tasmania and more widely in Australia. In addition, I have developed novel habitat selection models for different flight behaviours, and a low-cost morphological tool for identifying sex. These tools will assist managers to better predict, understand and mitigate the effects of unnatural mortalities on this endangered raptor population. Future research priorities to build on this work include the incorporation of my findings into a population model and the development of new techniques to fill life-history knowledge gaps still present. Such a model will guide, and support conservation managers needing to plan, assess and regulate the impacts of proposed new human activities on the population.



Image J. Wiersma

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# Chapter 1

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## General introduction



Image J. Pay

Apex predators are important for ecosystem function, as they help maintain trophic dynamics through multiple ecological pathways and interactions (Estes *et al.*, 2011; Sergio *et al.*, 2006). Changes in apex predator abundance can have dramatic effects on the populations and behaviour of prey species and smaller predators, due to deviations in the predation pressure and fear they instil (Ale and Whelan, 2008; Ritchie and Johnson, 2009). These population and phenotypic changes can propagate indirect effects in the wider food-chain, with impacts reaching plant communities and the composition of soils (Leo *et al.*, 2019). For example, the loss of apex predators, such as wolves (*Canis lupus*) in Yellowstone National Park, jaguars (*Panthera onca*) and harpy eagles (*Harpia harpyja*) in the Lago Guri islands and the great shark species in the coastal oceans of the United States, have resulted in a cascade of effects causing dramatic changes in the overall structure and biomass of the associated ecosystems (Berger and Conner, 2013; Myers *et al.*, 2007; Terborgh *et al.*, 2001). Most significantly, the reorganisation of ecosystems resulting from the loss of top predators has been associated with reductions in both faunal and floral biodiversity (Ale and Whelan, 2008; Estes *et al.*, 2011). Re-establishing and preserving the function of top predators are therefore key conservation goals across a variety of ecosystems (Letnic *et al.*, 2012; Paviolo *et al.*, 2016; Sergio *et al.*, 2005).

Despite their recognised conservation importance, top predators are threatened at a high rate due to intrinsic characteristics that make them more vulnerable than species at lower trophic levels (Cardillo *et al.*, 2004; Estes *et al.*, 2011). Top predators have naturally low population densities, high energetic requirements and range widely over extensive foraging areas (Carbone *et al.*, 1999; Cardillo *et al.*, 2004; Ripple *et al.*, 2014). These high metabolic demands and wide-ranging behaviours facilitate conflict with humans through the loss of livestock (Newsome *et al.*, 2015; Ogada *et al.*, 2003), competition for shared wild prey (i.e. through consumptive and

recreational hunting; Thirgood *et al.*, 2000; Thompson *et al.*, 2009) or the direct injury and death of people (Packer *et al.*, 2019; Penteriani *et al.*, 2017). As a result, top predators are commonly the target of human persecution (St John *et al.*, 2012), with impacts often exacerbated by the slow reproductive rates of top predators, so populations are less able to tolerate the increased mortality (Ripple *et al.*, 2014).

Predatory species can also be more sensitive to broader scale indirect anthropogenic impacts. The large habitat areas and stable availability of prey species required by top predators, makes them especially vulnerable to habitat loss and depletion in prey populations (Crooks and Soule, 2010; Paviolo *et al.*, 2016; Wolf and Ripple, 2016). Furthermore, the high trophic position of predators makes them vulnerable to environmental contaminants. The persistent nature of some chemicals can allow them to move and accumulate through several levels of a food chain, causing unintentional poisonings in predatory species (Grier, 2006). As a consequence of these sensitivities to multiple threats and increasing pressure from the growth in human populations, recent vertebrate extinctions have been biased towards apex consumers (Estes *et al.*, 2011).

As predators occupying high trophic positions, raptors are susceptible to the same threats as other predatory species, including illegal persecution (Villafuerte *et al.*, 1998), habitat loss (Kendall *et al.*, 2018; Reynolds *et al.*, 2007), climate change (Phipps *et al.*, 2017) and naturally slow life histories. Birds of prey are also particularly vulnerable to the bioaccumulation of toxins and unintentional poisonings (Blus, 2011; Espín *et al.*, 2016; Garbett *et al.*, 2018), and suffer from additional threats, such as collisions with anthropogenic structures and electrocution (Hernández-Lambrano *et al.*, 2018; Phipps *et al.*, 2013; Smallwood and Thelander, 2008). Consequently, raptors are recognised as more sensitive to extinction than other bird species (Bennett and Owens, 1997). A recent review of the conservation status of raptors determined that

18% of extant raptor species ( $n = 557$ ) are listed as threatened (vulnerable, endangered or critically endangered) by current IUCN guidelines, and 52% have populations that are estimated to be in decline; by contrast 13% of all bird species are threatened, with 44% thought to be declining (McClure *et al.*, 2018). Thus, raptors are of particular conservation concern and their decline may be associated with the loss of ecosystem functioning seen with other, better studied, top predators.

Habitat loss and alteration has long been recognised as a primary threat to biodiversity around the world (Hanski, 2011). More than half of the world's known threatened and near-threatened species are considered to be threatened by anthropogenic land modification (Maxwell *et al.*, 2016). Raptors are no different, with habitat loss identified as the primary threatening process in almost half of all raptor species (McClure *et al.*, 2018). The main drivers of the loss and degradation of suitable habitat for predatory birds are the homogenisation of landscapes by agriculture and the conversion or harvest of native forests (Bildstein *et al.*, 1998; Carrete *et al.*, 2002; McClure *et al.*, 2018; Thiollay, 1998). Furthermore, anthropogenically disturbed environments are not effective breeding habitat in many raptor species, particularly large *Aquila* spp., as they are shy nesters and will abandon breeding attempts and nest sites when human activities get too close (González *et al.*, 2006; Pauli *et al.*, 2016; Spaul and Heath, 2016).

Our understanding of the impacts of habitat loss for raptor species is typically biased towards breeding requirements (González *et al.*, 1992; Kendall *et al.*, 2018; Reynolds *et al.*, 2007). Habitats used away from nesting sites and during different life stages are less well-understood, particularly since raptors are inherently difficult to study, owing to their high vagility, low population densities, avoidance of humans and cryptic nature (Newton, 1979). New research techniques, such as high frequency Global Positioning System (GPS) tracking

technologies, are now able to provide detailed insights into their behavioural ecology (Braham *et al.*, 2015; Carter *et al.*, 2019). The use of such techniques can clarify key life history information, which is a recognised research priority in 129 raptor species (McClure *et al.*, 2018). This information will enable the identification of specific habitat requirements for conservation, even in anthropogenically modified landscapes.

Habitat modification can also impact raptors less directly, particularly through human infrastructure which is pervasive in raptor habitats. For example, both electrocutions and collisions with power poles and overhead power lines are a significant source of raptor mortality (Bernardino *et al.*, 2018; Mojica *et al.*, 2018; Phipps *et al.*, 2013). Raptors use power line poles and pylons as perches to hunt from (Hernández-Matías *et al.*, 2015) and their often large wing spans increase the risk of making a connection between live and grounded components of electrical infrastructure. The power lines themselves are a collision risk since birds can collide with the conductors and suffer physical trauma or electrocution (Rollan *et al.*, 2010). Population declines have been attributed to power line infrastructure in several raptor species (Angelov *et al.*, 2013; Galmes *et al.*, 2018; Hernández-Matías *et al.*, 2015) and may have caused the local extinction of eagle owls (*Bubo bubo*) from low altitude regions in the Italian Alps (Sergio *et al.*, 2004).

Collisions with wind turbines are increasingly recognised as a threatening process to many raptor populations, and their impact is likely to increase with wind-energy representing one of the fastest growing renewable industries (Bilgili *et al.*, 2015). While numerous bird (Smallwood and Thelander, 2008) and bat (Cryan and Barclay, 2009) species are affected, raptors and soaring birds are at particular risk of collisions (Smallwood and Thelander, 2008). Large raptors are reliant on wind conditions associated with certain topographic features that are

also favoured for wind turbine placement (Braham *et al.*, 2015; Miller *et al.*, 2014; Reid *et al.*, 2015). The impacts can be dramatic; a single wind farm in Altamont Pass, California, was estimated to cause the deaths of 1,127 raptors a year (Smallwood and Thelander, 2008). While there is increasing research and management effort in mitigating the impacts of both power lines and turbines (Chevallier *et al.*, 2015; Katzner *et al.*, 2012), they are likely to worsen due to the growing needs for greener energy and for electrical infrastructure to service expanding anthropogenic footprints.

Other aspects of the anthropogenic landscape also pose risks for raptors. Since the mid-20<sup>th</sup> century, environmental contaminants have been increasingly recognised as a key threatening process. The high trophic position of raptors combined with their physiological susceptibility to some pollutants makes them particularly vulnerable to contaminants (Swan *et al.*, 2006; Walker, 1983). Famously, Dichlorodiphenyltrichloroethane (DDT), an organochlorine pesticide used to control invertebrate populations, bioaccumulated and caused unprecedented declines in bald eagle (*Haliaeetus leucocephalus*) and peregrine falcon (*Falco peregrinus*) populations (Grier, 2006; Newton, 1988). Although DDT is now increasingly regulated worldwide, other less regulated contaminants still pose a threat to raptors.

Raptors are particularly susceptible to lead poisoning through the ingestion of bullet fragments (Fisher *et al.*, 2006; Garbett *et al.*, 2018; Golden *et al.*, 2016; Pain *et al.*, 2019). When a lead bullet hits a target animal, it can fragment into small pieces that can be inadvertently ingested by scavengers (Church *et al.*, 2006). Many raptors are facultative scavengers and are especially prone to ingesting bullet fragments, as they are often the first species to locate a carcass and the bullet wound provides an easy access point for feeding (Nadjafzadeh *et al.*, 2015). Furthermore, their highly acidic digestive tracts break down lead effectively resulting in

increased lead absorption (Beasley *et al.*, 2015). Since lead poisoning was identified as the main threat to the Californian condor (*Gymnogyps californianus*) in the 1980s (Finkelstein *et al.*, 2012; Walters *et al.*, 2010), concern has been growing over the conservation implications of lead exposure. Lead poisonings have been identified in 34 raptor species (Pain *et al.*, 2009, 2019), with population growth of at least two species limited by this threatening process (Finkelstein *et al.*, 2012; U.S. Fish and Wildlife Service, 2016). Despite these issues, the use of lead-based ammunitions remains prevalent worldwide (Hampton *et al.*, 2017).

Anticoagulant rodenticides (ARs) also represent a particular threat to raptors through non-target secondary poisoning. ARs are used worldwide to control synanthropic rodent populations, killing by inhibiting blood clotting and causing internal haemorrhaging in target rodents (Rattner *et al.*, 2014). However, poisoning is not instantaneous and so active rodents can carry high levels of rodenticides, potentially lethal to a predator if consumed (Lee *et al.*, 2006). Furthermore, behavioural changes induced by poisoning can make poisoned rodents more susceptible to predation (Brakes and Smith, 2005; Mooney, 2017). Consequently, non-target AR exposure has been detected in numerous predatory birds in Europe and North America (Christensen *et al.*, 2012; López-Perea *et al.*, 2015; Riley *et al.*, 2007; Shore *et al.*, 2003; Thomas *et al.*, 2017), with population impacts estimated in at least one species (Thomas *et al.*, 2011). The more chemically persistent and toxic rodenticides are now subject to increasing regulation in North America and Europe, however, beyond these regions, they remain mostly unmonitored and unregulated (Lohr and Davis, 2018).

Although raptors are generally susceptible to the threats outlined here, empirical data is required on the specific threats faced by different populations, especially those that are threatened. Conservation priorities must respond to inter- and intra-specific differences in

behaviour as well as regional differences in landscapes and anthropogenic impacts. For example, vultures and other obligate soaring raptors are at higher risk of wind turbine collisions than raptors with lower wing loadings and higher manoeuvrability (de Lucas *et al.*, 2008). Despite this need for species-specific information, there is an absence of data on the threats faced by different raptors, with the International Union for the Conservation of Nature (IUCN) recommending the understanding of threats as a research priority in 141 raptor species worldwide (McClure *et al.*, 2018). Information on threatening processes is required to both implement management of recognised threats (Braham *et al.*, 2015; Dwyer *et al.*, 2014) and measure the efficacy of management implemented (Chevallier *et al.*, 2015; Murray, 2017). Furthermore, species-specific research is key to identifying new and potentially unexpected threats, such as the recent identification of diclofenac exposure as a threat in the endangered steppe eagle (*Aquila nipalensis*), a compound previously only thought to affect vultures of the genus *Gyps* (Sharma *et al.*, 2014). Understanding the specific combination and extent of threats faced by a raptor species is therefore crucial to the effective planning of *in situ* conservation management.

## *Study location – Tasmania*

Tasmania is an island state of Australia located 240 km south of the Australian mainland. The island covers a land area of 68,150 km<sup>2</sup> between latitudes -40°S and -43°S and longitudes 144°W and 149°W. This temperate maritime location and the diverse topographies of the island translate to a range of climatic conditions. Coastal and low altitude regions of the island experience mean monthly temperatures ranging from 8 to 25°C and approximately 560 mm annual precipitation, whilst the central highlands experience mean monthly temperatures ranging

from -2 to 18.6°C and over 1,000 mm of rainfall per year (Bureau of Meteorology, 2019). This climatic variability combined with diverse soil types found across the island have resulted in a complex range of vegetation communities. Wetter regions, such as those in the west of the island, are characterised by wet sclerophyll eucalypt-dominated forests, temperate rainforests and button grass (*Gymnoschoenus sphaerocephalus*) moorland (Harris and Kitchener, 2005).

Regions in the east and middle of the island are dryer, and dominated by dry eucalypt-dominated forests and native grasslands (Harris and Kitchener, 2005). A large proportion of Tasmania comprises native habitats, with areas of minimal land use, nature conservation and other protected areas accounting for 50.3% (34,280 km<sup>2</sup>) of the island land area (DPIPWE, 2015a). Anthropogenic landscapes, predominantly comprising grazing and modified pastures, occupy 27.7% (18,900 km<sup>2</sup>; DPIPWE, 2015), primarily in the north and east of the state. The estimated human population on the island is 520,830, which is mostly localised to towns and cities, with approximately 50% of the population living in the areas surrounding the state capital Hobart in the southeast (Australian Bureau of Statistics, 2018).

The island is currently separated from mainland Australia by the Bass Strait, a 240 km wide seaway. The shallow depth of the Bass Strait means that there has historically been a land bridge between Tasmania and mainland Australia during periods of glaciation and lower global sea levels. The most recent land bridge was flooded after the Last Glacial Maximum, separating Tasmania from mainland Australia for the last 14,000 years (Waters, 2008). This isolation has both facilitated speciation and protected native animals from threatening processes present on mainland Australia (e.g. the presence of the European red fox, *Vulpes vulpes*). Presently 527 endemic species of plant (De Salas and Baker, 2014) have been identified, together with five

endemic mammal species (DPIPWE, 2015b), over 650 invertebrate species (DPIPWE, 2014) and 12 endemic bird species (Stattersfield *et al.*, 1998).

## *Study species – the Tasmanian wedge-tailed eagle*

The wedge-tailed eagle (*Aquila audax*) is a large *Aquila* species found throughout Australia and southern New Guinea (Olsen, 2005). Weighing 3–5.5 kg and with a wingspan of 1.9–2.3 m, the wedge-tailed eagle is the largest bird of prey throughout its range, serving an important ecological function as an apex predator (Bell and Mooney, 1998; Debus, 2012). Two subspecies are recognised; *A. a. audax*, found throughout most of the species' distribution, and the Tasmanian wedge-tailed eagle *A. a. fleayi*, which is endemic to the island of Tasmania (Commonwealth of Australia, 1999; see Figure 1.1). Thought to be isolated since the Last Glacial Maximum (Bell and Mooney, 1998), the Tasmanian wedge-tailed eagle has specific morphological and life-history traits: a paler colouring to the nape and upper wings (Marchant and Higgins, 1993), a larger body size (Nankervis, 2010), smaller clutch sizes (Olsen, 2005), and a higher sensitivity to disturbance during the breeding season (Mooney and Holdsworth, 1991). However, genetic work suggests the separation of the populations is more recent and that the trait divergence may be due to founder effects (Burridge *et al.*, 2013).

The ecological function of the Tasmanian wedge-tailed eagles' top predator role is heightened by the threatened status and ongoing population declines of other predatory species on the island. The thylacine (*Thylacinus cynocephalus*) is extinct, while all three remaining large marsupial carnivores are listed as threatened, with the formerly widespread Tasmanian devil (*Sarcophilus harrisii*) suffering ongoing declines (Lazenby *et al.*, 2018). Despite this ecological importance, the Tasmanian wedge-tailed eagle is listed as endangered under Schedule 3.1 of the

Tasmanian Threatened Species Protection Act (State Government of Tasmania, 1995) and Schedule 1 of the Commonwealth Environment Protection and Biodiversity Conservation Act (Commonwealth of Australia, 1999).



Figure 1.1. Distribution of the two subspecies of wedge-tailed eagle. *A. a. audax* is found throughout mainland Australia and southern New Guinea. *A. a. fleayi* is confined to the island of Tasmania.

The population size is constrained by the size of Tasmania and the areas required to support breeding territories. Although the population is distributed widely across the island, densities appear to vary considerably. Territory sizes, estimated from the distributions of known nests, range from 20–30 km<sup>2</sup> in the dryer, more fertile and open habitats of the lowlands, to 1,200 km<sup>2</sup> in the highlands and western regions (Bell and Mooney, 1998). Based on these evaluations and observations of a subset of nesting eagles, it was estimated that there are 220 territories state-wide and the total population size is less than 1000 individuals (Bell and Mooney, 1998). However, more recent estimates have ranged from 426 to 457 territories and the total population between 1,000 and 1,524 individuals (Mooney, 2005; Threatened Species Section, 2006).

Habitat loss is recognised as a major threat to the Tasmanian wedge-tailed eagle, though this is based exclusively on our understanding of nesting habitat requirements. Breeding eagles rely primarily on large emergent trees in sheltered areas of old growth forest for nesting (Mooney and Holdsworth, 1991), a habitat type patchily distributed throughout the state (Forest Practices Authority, 2013). It is estimated that 40% of the forest cover on the island was lost between 1803 and 1964 (from 48,600 to 24,400 km<sup>2</sup>; Bradshaw, 2012), and 60–170 km<sup>2</sup> continued to be cleared annually until 1988 (Kirkpatrick, 1991). Although the rate of deforestation has subsequently slowed, the continued loss of nesting habitat is still recognised as a primary threatening process, as only 22% of known nests occur on protected land (DPIPWE, 2016, 2015a; Threatened Species Section, 2006).

Nest disturbance is also a significant threatening process that impacts the breeding success of the subspecies. The Tasmanian wedge-tailed eagle is highly sensitive to disturbance during the breeding season (Mooney and Holdsworth, 1991; O’Sullivan, 2014; Threatened

Species Section, 2006). Breeding adults readily abandon their nests if human activities (e.g. forestry, recreation, development, research) occur nearby. There is also evidence that Tasmanian wedge-tailed eagles sensitise rather than habituate to repeated disturbance (O’Sullivan, 2014), suggesting that ongoing disturbances could have cumulative effects on nesting adult behaviour. This sensitivity to disturbance is thought to be linked to decreasing breeding success in the population (Bell and Mooney, 1998; Threatened Species Section, 2006), with breeding failure estimates ranging 50–60% (Mooney and Holdsworth, 1991; O’Sullivan, 2014; Tasmanian Forest Practices Authority unpublished data). This sensitivity to disturbance is a trait shared by other large *Aquila* spp. and a key focus of their conservation management (González *et al.*, 2006; Pauli *et al.*, 2016; Spaul and Heath, 2016). It is thought that Tasmanian wedge-tailed eagles are more sensitive to disturbance than the subspecies present on mainland Australia and southern New-Guinea (Mooney and Holdsworth, 1991). However, this reported trait divergence requires systematic study.

Causes of unnatural mortality through collisions with power lines and turbines are suspected to be increasing threats to the Tasmanian wedge-tailed eagle. Since reporting began in 2001, there have been over 100 eagle deaths attributed to electrocutions and power line collisions in the state (TasNetworks unpublished data). Over half of these mortalities have occurred in the last four years, with 29 eagle deaths reported in 2018 (TasNetworks, 2018). Although this suggests a growing impact, improved incident reporting may contribute to the recent increase in discovered eagle carcasses. Wind turbines also cause unnatural mortality with five wedge-tailed eagle strikes reported in 2018 at one of the operational farms in the state (Woolnorth Wind Farm Holding, 2018). These impacts from both turbines and power lines will potentially worsen, due

to an increased focus on utilising wind energy in Tasmania and a growing need for electrical infrastructure to service expanding developments (Bell and Mooney, 1998).

There are also other recognised causes of mortality in Tasmanian wedge-tailed eagles. Illegal persecution is a continuing threat, predominantly motivated through conflict over livestock. Of 175 eagles found injured or dead between 1980 and 1995, 47 were identified as shot (Mooney, 1996). Although such conflict continues to occur (Dolan, 2018), data from eagles found between 1995 and 2018 suggest lower rates of persecution (Tasmanian Museum and Art Gallery unpublished data). Vehicle collisions are another anthropogenic cause of mortality, as the scavenging behaviour of the eagles brings them down to roadkill, making them vulnerable to collisions.

Such additional unnatural mortalities may be having significant impacts on the Tasmanian wedge-tailed eagle population. For species with slow life histories, enhancing adult survival has higher conservation priority than promoting reproduction (Crone, 2001). Furthermore, the demographic consequences of unnatural mortalities can be influenced by the sex of individuals and the population sex ratio. For example, the larger size of female eagles can make them more vulnerable to collisions and electrocutions from power lines than males (Ferrer and Hiraldo, 1992), potentially resulting in increased impacts to the population due to a resulting sex-bias. Identifying the sex of injured and dead eagles is therefore important to clarifying the nature of conservation threats faced by a population. However, no simple method has been available to identify the sex of dead Tasmanian wedge-tailed eagles and consequently sex biases in mortality have not been investigated.

Concern over the long-term viability of the Tasmanian wedge-tailed eagle population has translated to costly management strategies to address some of the recognised threats.

Conservation management is predominantly focused on protecting nesting habitat and reducing human disturbance around nests. Current prescriptions define that a 10 ha reserve (where no forestry activity is allowed) is implemented around all known nest sites and activities are restricted within 500 m/1 km line-of-sight of nests with breeding eagles (FPA, 2014). Although these regulations have been developed for the Tasmanian forestry industry, they are used by regulators to manage a multitude of other residential, recreational and industrial activities. With over 2,000 eagle nests identified across the state, eagle nest management poses a significant cost to Tasmania's primary industries (DPIPWE, 2016). Managing mortalities caused by power line and wind farm infrastructure is also an area of increasing focus. TasNetworks, the state-owned company responsible for managing the power lines in Tasmania, spends AU\$180,000 annually on mitigating infrastructure (e.g. flight diverters, pole top perches; TasNetworks, 2016). Whereas wind farms are required to financially offset any eagles found dead as a result of a turbine strike, usually through the protection (via covenants) of eagle nest sites (Keserue-Ponte *et al.*, 2011; Woolnorth Wind Farm Holding, 2013).

To guide effective conservation management of the Tasmanian wedge-tailed eagle, our understanding of their ecology and threatening processes needs to be improved. With much of our knowledge of the habitats required by the species focused at nesting sites, there is a need to understand how eagles use the landscape in more detail and during different life stages. Such information will both help inform the protection of important habitats and aid the management of unnatural mortalities. For example, preferred landscape characteristics can be used to reduce the ecological impacts of power lines and wind turbines (Miller *et al.*, 2014). Furthermore, threatening processes apparent in other raptor populations may constitute presently unrecognised threats to the Tasmanian wedge-tailed eagle. In particular, over one million macropods are

estimated to be shot annually in Tasmania, yet there has not been a formal investigation into lead levels in the species. Additionally, anticoagulant rodenticides are widely available, and their use is largely unmonitored throughout Australia (Lohr and Davis, 2018). The conservation value of the extensive efforts in place to protect breeding eagles from disturbance will be limited if such unrecognised threats are impacting the survival of adults and immatures post-fledging.

## *Aims and thesis structure*

The overarching aim of this thesis is to provide information to inform the conservation management of the Tasmanian wedge-tailed eagle, by addressing knowledge gaps on the behavioural ecology and threats faced by the population. The thesis consists of four data chapters with the following aims:

Chapter 2: Determine the degree to which the Tasmanian wedge-tailed eagle is exposed to lead and to explore the likely routes of exposure.

Chapter 3: Determine the degree to which the Tasmanian wedge-tailed eagle is exposed to ARs, and to identify the intrinsic and extrinsic drivers of the AR exposure evident.

Chapter 4: Identify habitats used by juvenile Tasmanian wedge-tailed eagles for different behaviours and predict where different behaviours are likely to occur in the Tasmanian landscape.

Chapter 5: Assess whether Tasmanian wedge-tailed eagles of different age classes can be sexed accurately using morphological measurements.

Each chapter is written as a self-contained manuscript intended for publication. In Chapter 6 I bring the conclusions of all the manuscripts together, and then discuss the findings in relation to the overarching conservation of the Tasmanian wedge-tailed eagle. Integrating the major findings, I propose future research directions and innovations to the population management approach.

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## Chapter 2

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High frequency of lead exposure in an endangered Australian top predator,  
the Tasmanian wedge-tailed eagle (*Aquila audax fleayi*)



Image D. James

## Abstract

Lead toxicity caused mainly by the ingestion of lead-based bullet fragments in carcasses is increasingly recognised as a threat to scavenging bird species worldwide. However, the prevalence in Australia is unknown, despite the widespread use of non-consumptive shooting for animal control and a range of native scavenging species susceptible to exposure. We aimed to determine the degree to which the Tasmanian wedge-tailed eagle (*Aquila audax fleayi*), an endangered Australian raptor, showed evidence of lead exposure, and to explore the likely routes of exposure. We detected lead in all but one of 132 individuals sampled. Levels were elevated in 10.4% of 106 liver ( $> 6$  mg/kg) samples and 3.7% of 108 femur ( $> 10$  mg/kg) samples from carcasses opportunistically collected throughout Tasmania. Levels in two blood samples taken from 24 live nestlings were also elevated ( $> 10$   $\mu$ g/dL). Femur lead levels were significantly higher in adults than in immature birds ( $W = 1361$ ,  $p = 0.007$ ), suggesting chronic exposure. Hepatic lead isotope signatures ( $\text{Pb}^{207/206}$ ) of the eagles with elevated lead levels were significantly different from those with background levels ( $W = 802$ ,  $p = 0.004$ ). Furthermore, 73% of samples with elevated liver lead levels had isotope signatures ( $\text{Pb}^{207/206}$ ) within the range of lead-based bullets. Our results represent the first data on lead exposure for an Australian raptor species and are comparable to those for raptor studies elsewhere that identify lead-ammunition exposure as a conservation threat. In concluding that lead-based ammunition is a probable cause of lead intoxication of Tasmanian wedge-tailed eagles, we emphasise the need to determine the extent and impacts of lead contamination in other species of Australian scavengers.

## Introduction

Lead is a toxic element that can negatively affect a range of physiological systems, thereby threatening susceptible animal populations and ecosystems (Finkelstein *et al.*, 2012; Goyer and Clarkson, 2001). Clinical symptoms of lead poisoning include ataxia, impaired mobility, lowered sensorial ability, vomiting, anaemia, lethargy, gastrointestinal stasis, weakness and mortality (Fallon *et al.*, 2017). Although low levels of lead occur naturally as part of the geological cycle (Turekian and Wedepohl, 1961), most bioavailable lead is brought into the environment through anthropogenic activities, such as mining, sewage treatment, paint, ammunition and leaded gasoline (Behmke *et al.*, 2015; Finkelstein *et al.*, 2012; Jenni *et al.*, 2015). The worldwide distribution of this array of anthropogenic sources of lead has resulted in the documented exposure of over 120 bird species (Haig *et al.*, 2014).

One reason that avian predators and scavengers are susceptible to lead poisoning is that they ingest lead-based bullet fragments (Fisher *et al.*, 2006; Golden *et al.*, 2016; Pain *et al.*, 2019). While this has long been known, the use of lead-based bullets remains prevalent worldwide, since the physical properties of lead make it a suitable projectile that is also inexpensive and easy to process. When a lead-based bullet hits a target animal the bullet can fragment into small pieces that can be inadvertently ingested by scavengers (Church *et al.*, 2006). Scavenging bird species are particularly prone to ingesting lead from spent ammunition, as they are often the first species to locate a carcass, the bullet wound provides an easy access point for feeding, and their highly acidic digestive tracts break down lead effectively (Beasley *et al.*, 2015; Nadjafzadeh *et al.*, 2015).

Lead poisoning has been detected in 34 raptor species (Pain *et al.*, 2009, 2019), with the link to lead ammunition evidenced by seasonal patterns in exposure (Garbett *et al.*, 2018) and similarities in lead stable isotopes (Behmke *et al.*, 2015). Seasonal patterns in lead exposure detected in wildlife have been linked to seasonal changes in lead ammunition use; for example, increased lead concentrations in scavenging birds during the hunting season (Cruz-Martinez *et al.*, 2012; Garbett *et al.*, 2018; Kelly and Johnson, 2011). Patterns in the stable isotopes of lead can also be used to link lead exposure to its source. There are four stable isotopes of lead ( $\text{Pb}^{204}$ ,  $\text{Pb}^{206}$ ,  $\text{Pb}^{207}$ , and  $\text{Pb}^{208}$ ) and the ratios of these isotopes differ depending on the origin, creating a signature that differentiates between sources of lead. For example, lead isotopes from lead ammunition differ from those of other environmental and anthropogenic sources of lead (Behmke *et al.*, 2015; Ishii *et al.*, 2017). These patterns in lead isotope ratios have been used to investigate the source of exposure detected in raptors (e.g. by assessing differences in isotopes between individuals with different levels of lead exposure (Legagneux *et al.*, 2014) or by comparing overlap in isotopic ratios with different sources of lead (Finkelstein *et al.*, 2012)).

Lead can be detected in a range of tissue types, indicating different types of exposure. Following lead particle ingestion, lead is transported by the blood stream and deposited in different tissues, primarily liver, kidney and bone (Pain *et al.*, 2005). However, the persistence of lead in each tissue varies. The relatively short persistence of lead in blood ( $\approx 13$  days; Fry *et al.*, 2009) and liver (days to months; Fisher *et al.*, 2006) provides a measure of short-term exposure, whereas bone lead is an aggregate of lifetime exposure (Fisher *et al.*, 2006). These differences have been exploited to investigate acute and chronic exposure in raptor populations worldwide (Behmke *et al.*, 2017; Ganz *et al.*, 2018; Jenni *et al.*, 2015).

The absence of peer-reviewed study into the impacts of lead on scavenging species in Australia is of particular concern, contrasting with increasing recognition of the issue in Europe, North America, Asia and Africa. In Australia, a range of native scavengers are susceptible to exposure (Hampton *et al.*, 2017), and use of non-consumptive shooting is widespread. For example, estimates indicate that over one million macropods (Bennett's wallaby, *Macropus rufogriseus* and Tasmanian pademelon, *Thylogale billardierii*) and 400,000 brushtail possums (*Trichosurus vulpecula*) are shot annually across the Australian island state of Tasmania (DPIPWE, 2011). Prior to the 1990s, the majority of these animals were shot for commercial harvest of skin and meat, and were thus removed from the site after shooting (DPIPWE unpublished data). However, this harvest has since declined and now these animals are primarily shot for agricultural and forestry asset protection (DPIPWE unpublished data; R. Gaffney pers. comm), whereby the standard practice is to leave entire carcasses *in situ*. These factors, and similar situations seen nationwide, combine to potentially give Australia one of the most pervasive and abundant sources of ingestible lead material in the world (Hampton *et al.*, 2017). Further, this source of lead may not act in isolation; as other anthropogenic sources of lead, including mining and the historic use of lead-based fuels and paint have also occurred in the region (Kristensen, 2015; Schneider *et al.*, 2019).

As a top avian predator and facultative scavenger, the Tasmanian wedge-tailed eagle (*Aquila audax fleayi*) shares the same characteristics as other avian species threatened by lead from spent ammunition. Endemic to the island of Tasmania, this subspecies is listed as endangered at both a state and federal level (Commonwealth of Australia, 1999; State Government of Tasmania, 1995). Conservation concern is based upon a series of known threats, including nest failures caused by anthropogenic disturbance, low breeding success rates, habitat

loss, collisions with anthropogenic structures and illegal persecution (Bell and Mooney, 1998; Mooney and Holdsworth, 1991; Threatened Species Section, 2006). Yet despite its ethological susceptibility, and the potentially large amount of ingestible lead present in Tasmania, there has been no formal research into whether lead ammunition does represent a conservation threat to the subspecies. To investigate the extent and routes of lead exposure in Tasmanian wedge-tailed eagles, we (1) assessed the concentrations of lead in multiple tissues; (2) tested for age- and sex-specific differences in lead tissue levels; (3) tested for seasonal changes in lead exposure; and (4) assessed patterns in isotope ratios in wedge-tailed eagle tissues and similarities to lead-based ammunition.

## Methods

### *Study location*

Tasmania is an island state of Australia located 240 km south of the Australian mainland, covering a land area of 68,150 km<sup>2</sup>. The isolation of Tasmania from mainland Australia for approximately 10,000 years has facilitated the evolution of an array of endemic flora and fauna (De Salas and Baker, 2014; DPIPWE, 2014; Stattersfield *et al.*, 1998). Tasmanian wedge-tailed eagles involved in this study were sampled from throughout Tasmania to obtain a state-wide understanding of lead contamination of the population (see Figure 2.1).

### *Sample collection and preparation*

Sampled eagles came from two sources; opportunistically collected eagle carcasses (broadly characterized into adults and immatures based on plumage; see Appendix A.1.) from which we sampled liver and bone, and live nestlings from which we drew blood. Carcasses were collected from throughout Tasmania and placed in -20°C freezer storage by the Department of Primary Industries, Parks, Water and the Environment (DPIPWE, Threatened Species Section, Hobart, Tasmania) and the Tasmanian Museum and Art Gallery (TMAG, Collection and Research Facility, Rosny, Tasmania) between 1996 and 2018. We defrosted the carcasses and carried out necropsies between May 2017 and March 2018. At necropsy, we collected a liver lobe and sectioned a femur diaphysis ( $\approx 3$  cm length from the middle of the femur). Nestlings were sampled prior to fledging (estimated age 65–75 days old) during 2017 and 2018. From each nestling we took a  $\approx 2$  ml blood sample from the brachial vein using a 26-gauge needle. We stored blood in low-lead vacutainers (Beckton Dickinson, Franklin Lakes, NJ, USA), which were

placed in a refrigerator until sample preparation. Blood samples and the necropsied tissues were stored at -20°C until sample preparation.

We thawed all tissues and blood samples to room temperature for preparation. For each sample, we used new gloves and scalpel blades, and washed forceps beforehand. We removed all adherent non-target tissues (e.g. muscle, connective tissue, marrow) from each sample using a stainless-steel scalpel blade. We then sectioned target tissue from the middle of each liver lobe ( $\approx 1.3$  g) and each femur diaphysis ( $\approx 460$  mg). We dehydrated femur and liver samples at 60°C to a constant weight (340–550 mg dry weight), which was recorded using a digital balance (precision  $\pm 0.0001$  g; Mettler Toledo, Columbus, OH, US). A 1 ml volume of each blood sample was also dehydrated to a constant weight (0.2–0.5 mg dry weight). We stored samples in metal free plastic containers until digestion.

### *Lead concentration and isotope analysis*

We sent all samples to Edith Cowan University Analytical Facility (Joondalup, Western Australia) for lead concentration analysis. Liver and bone samples were homogenised, and 0.4 g was aliquoted into ICP grade Teflon vessels containing 5 ml nitric acid, 0.5 ml hydrochloric acid and 3 ml hydrogen peroxide. Samples were digested for 15 minutes in a Multiwave GO microwave digestion system (Anton Paar, Sydney, Australia) set to 150°C. After digestion samples were diluted to 50 ml with Milli-Q reverse osmosis deionized (MQ) water and transferred to polypropylene tubes for analysis. Blood samples were prepared by a similar method using digestion in 2 ml of nitric acid, 0.2 ml of hydrochloric acid and 1 ml of hydrogen peroxide. Blood sample solutions were then sonicated at 60°C for 1 hour and diluted to 10 mL using MQ water.

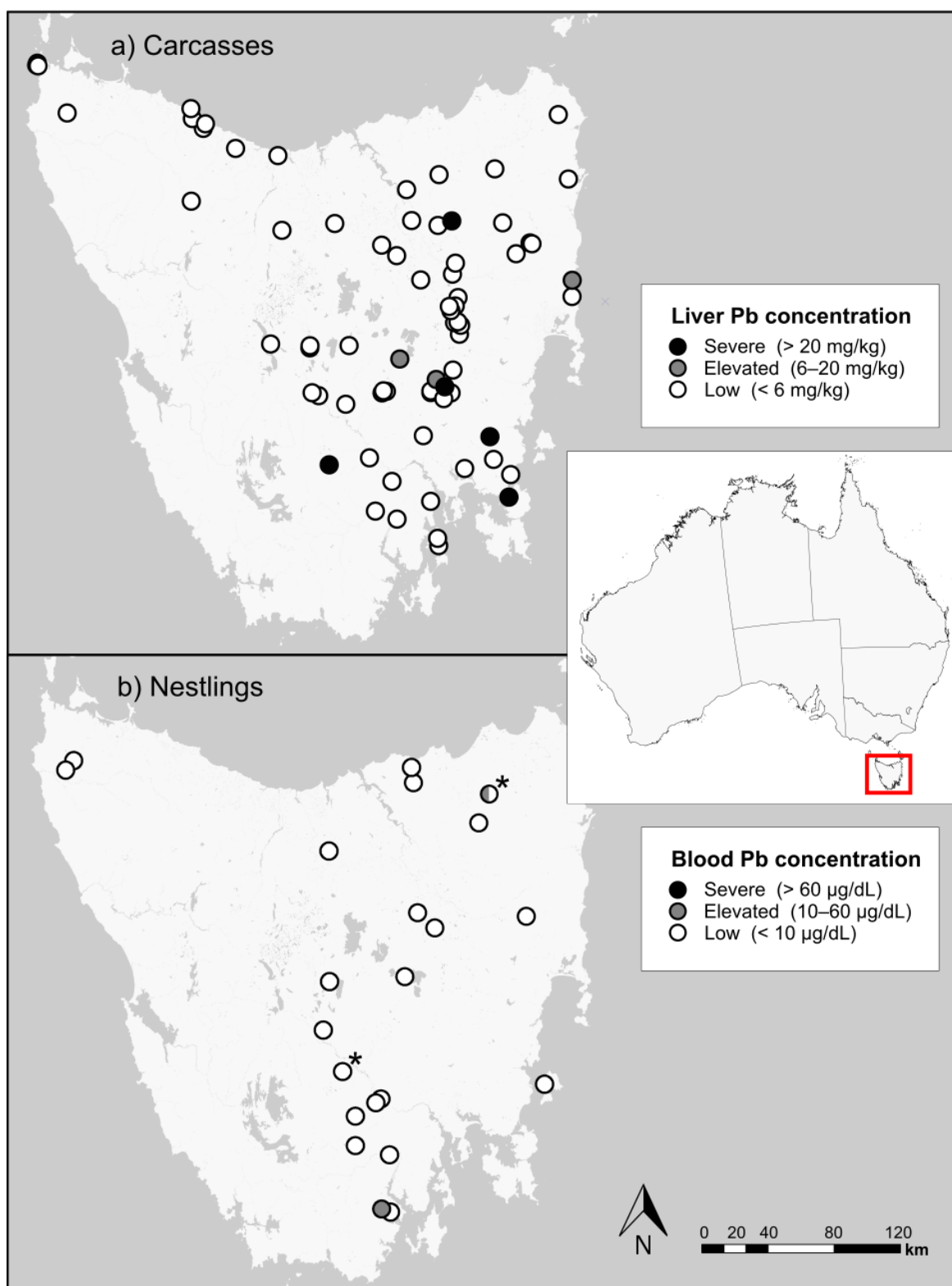


Figure 2.1. Location of eagle carcasses (a) and nestlings (b) included in the study. Location was recorded in 72 of the carcasses analysed. We analysed nestlings from the same nest in consecutive seasons at two nest sites (indicated by asterisks). Liver (carcasses) and blood (nestlings) lead concentrations are indicated by the symbols.

Lead concentrations were determined via an inductively coupled plasma mass spectrometer (ICP-MS) using an iCAP Q ICP-MS (Thermo-Fisher Scientific, Waltham, MA, USA) coupled to an ASX-520 AutoSampler (Agilent, Santa Clara, CA, USA). The instrument was calibrated using concentration ranges of iCAPQ element standards (Thermo-Fisher Scientific, NJ, USA) and ICP-MS-68A solutions (High Purity Standards, North Charleston, SC, USA) to provide standard curves before analysis. Two Certified Reference Materials (CRMs) were used as positive controls, Bone Ash Standard Reference Material 1400 (National Institute of Standards and Technology, Gaithersburg, MD, USA) and Bovine Liver Certified Reference Material BCR – 185R (Institute for Reference Materials and Measurements Geel, Belgium). Two digestions were carried out on each CRM with two ICP-MS readings of each digestion. Accuracy of CRM ICP-MS readings averaged 96.7% for femur and 104.1% for liver. Every 10<sup>th</sup> sample was re-analysed for a duplicate read (average RSD 1.8%) and duplicate blind sample digestions were carried out for 20 randomly selected samples (average RSD 5.5%). Lead concentrations were reported as mg/kg dry weight (d.w.). Limits of quantification (LOQ) and limits of detection (LOD) for the analysis were 0.005 mg/kg and 0.0015 mg/kg respectively.

Lead isotopes were determined as counts per second (cps) measured at  $m/z$  204, 206, 207, and 208. Isotope readings were adjusted according to readings from a calibration lead solution at  $m/z$  204 (1.40%),  $m/z$  206 (24.10%),  $m/z$  207 (22.10%), and  $m/z$  208 (52.40%). Analyses of isotope data focused on the lead<sup>207/206</sup> ratio. This ratio is used most commonly in research investigating sources of lead exposure in birds (Behmke *et al.*, 2015; Finkelstein *et al.*, 2012; Katzner *et al.*, 2017).

Data acquisition, element quantitation and isotope percentage analyses were carried out using Qtegra (Thermo-Fisher Scientific, Waltham, MA, USA).

### *Interpretation of lead concentrations*

Various tissue lead concentration thresholds have been proposed to provide estimates of physiological impacts. To interpret liver lead concentrations in terms of their potential impact we used previously identified categories of  $< 6$  mg/kg d.w. as evidence of low levels of exposure with limited health implications (Ishii *et al.*, 2017; Wayland *et al.*, 1999), 6–20 mg/kg d.w. as elevated with some health implications (Ishii *et al.*, 2017) and  $> 20$  mg/kg d.w. indicative of severe exposure, representing a potentially lethal dose (Pain *et al.*, 1995).

The long-term accumulation of lead in bone complicates the inference of physiological responses to concentration thresholds. We used an exposure threshold of femur lead concentrations  $< 10$  mg/kg d.w. as indicative of low exposure (reviewed in Franson and Pain, 2011), 10–20 mg/kg as elevated, and concentrations  $> 20$  mg/kg d.w. were considered severe (bone lead concentrations  $> 20$  mg/kg d.w. have been observed after lethal poisonings in raptors; Jenni *et al.*, 2015; Rodriguez-Ramos Fernandez *et al.*, 2011).

To compare our blood results to other studies, we converted dry blood results (provided in mg/kg d.w.) to wet weight ( $\mu\text{g/dL}$ ) by multiplying the dry weight concentrations by the dry to wet weight ratios (see Slabe *et al.*, 2019). Blood lead  $< 10$   $\mu\text{g/dL}$  has limited health implications (Legagneux *et al.*, 2014; Slabe *et al.*, 2019) and was categorised as low. Blood lead concentrations 10–60  $\mu\text{g/dL}$  were considered elevated and concentrations  $> 60$   $\mu\text{g/dL}$  were considered severe (Kramer and Redig, 1997).

These categories represent our best estimates of potential concern, but the true effects may be greater. Impacts can be difficult to interpret because of the paucity of experimental evidence quantifying the effects of lead concentrations. Lead concentrations we have categorised

as ‘low’ do not equate to no physiological effect (Ganz *et al.*, 2018). The thresholds used for low exposure are mostly based on a lack of apparent symptoms. However, the strong selection pressure in wild animals to hide signs of illness risks underestimating the impacts. Furthermore, lead has been shown to have physiological effects even at low concentrations (Espín *et al.*, 2015; Lanphear *et al.*, 2005), suggesting even low exposure may be of concern.

### *Data analysis*

We calculated summary statistics (mean, median, SD, SE) for liver and femur lead concentrations of all samples and also for each age class. To calculate analogous summary statistics for blood lead concentrations we used a Kaplan-Meier cumulative probability distribution (cenfit; R package ‘NADA’; Lee, 2017), to allow us to estimate means of samples with values below the ICP-MS LOD. Data did not meet assumptions for parametric analyses and so non-parametric techniques were used for statistical comparisons. We used a Wilcoxon signed rank test (wilcox.test; R Core Team, 2016) to investigate differences in femur and liver lead concentrations and the Kendall’s tau statistic to measure the correlation between liver and femur concentrations (cor.test; R Core Team, 2016). Then, to understand age- and sex-specific differences in tissue lead levels (our second research objective), we used Wilcoxon rank sum tests to compare lead concentrations in liver and femur between different age groups and between the sexes. To test for differences in lead exposure between the four seasons (our third research objective), we used a Kruskal-Wallis rank sum test (kruskal.test; R Core Team, 2016) for the samples that had the date of collection recorded.

Finally, we investigated differences in lead<sup>207/206</sup> isotopic signatures in liver samples with elevated to severe lead levels (> 6 mg/kg) to those with low levels (< 6 mg/kg) using a Wilcoxon

rank sum test. We also compared the coefficients of variation between these groups using a modified signed-likelihood ratio test (`mslr_test`; R package ‘`cvequality`’; Marwick, 2019). We used the Kendall’s tau statistic to measure the correlation between liver lead concentrations and lead isotopic signatures. To establish if ammunition was a likely source of exposure in the Tasmanian wedge-tailed eagle we calculated the overlap of published lead<sup>207/206</sup> isotopic signatures of various makes of ammunition (797 ammunitions from 30 brands; Sjøstad *et al.*, 2014) with those found in the liver samples (following methods in Behmke *et al.*, 2015 and Finkelstein *et al.*, 2012).

## Results

We analysed 113 eagle carcasses, which included 27 adults and 82 immature birds. We analysed tissue lead concentrations in 108 femur samples (26 adults and 82 immatures) and 106 liver samples (26 adults and 80 immatures). The year of carcass recovery was recorded for 68 eagles and the exact day was recorded for 62 of these eagles. We collected blood samples from 24 eagle nestlings.

### *Extent of lead contamination*

Lead levels were above the ICP-MS LOD in all femur and liver samples analysed. Mean lead levels were  $2.707 \pm 3.484$  mg/kg ( $\pm$ SD) in femur and  $6.390 \pm 22.610$  mg/kg in liver (Table 2.1). Elevated liver lead levels (6–20 mg/kg) were detected in 3.8% of the samples (one adult and three immatures), while severe levels ( $> 20$  mg/kg) were detected in 6.6% (seven immatures; see Figure 2.2a and Figure 2.2c). Elevated femur lead levels (10–20 mg/kg) were detected in 2.8% of the samples (two adults and one immature) and levels indicative of severe poisoning ( $> 20$  mg/kg) were detected in 0.9% (one immature; see Figure 2.2b and Figure 2.2d).

Lead levels were significantly higher in femur samples than in liver samples ( $Z = 1564$ ,  $p < 0.001$ ,  $n = 105$ ). However, the six highest liver lead concentrations (range: 43.9–181.6 mg/kg) were markedly higher than the highest femur lead concentration (25.6 mg/kg). Femur and liver lead concentrations were also significantly correlated ( $\tau = 0.438$ ,  $p < 0.01$ ,  $z = 6.623$ ; Figure A.2.1).

Lead levels were above the LOD (0.15 µg/dL) in 23 of 24 nestling blood samples. Mean nestling blood lead levels were  $3.083 \pm 7.731$  µg/dL (range: < LOD - 32.74 µg/dL; Table 2.1). In two nestlings (8.3%), lead levels were elevated (10–40 µg/dL; Figure 2.2e).

### *Differences between age groups and sexes*

Liver lead levels were not significantly different between the age groups ( $W = 1240$ ,  $p = 0.071$ ; Figure 2.3a). Femur lead levels were significantly higher in adult birds compared to immature birds ( $W = 1361$ ,  $p = 0.007$ ; Figure 2.3b). However, the only bird with femur lead concentrations indicative of severe exposure ( $> 20$  mg/kg) was immature. Lead levels were not different between the sexes for liver ( $W = 1469$ ,  $p = 0.542$ ; Figure 2.4a) or femur ( $W = 1445$ ,  $p = 0.911$ ; Figure 2.4b).

Table 2.1. Age-specific and total summary statistics of tissue lead concentrations for wedge-tailed eagles collected in Tasmania between 1996 and 2018.

Tissue	Sample	n	< LOD	Mean	Median	SD	SE	Min-max
Femur (mg/kg)	Adults	26	0	3.647	2.69	3.178	0.623	0.2 - 13.69
	Immatures	82	0	2.408	1.22	3.542	0.391	0.06 - 25.57
	Total	108	0	2.707	1.730	3.484	0.335	0.06 - 25.57
Liver (mg/kg)	Adults	26	0	1.975	1.205	2.506	0.491	0.23 - 17.72
	Immatures	80	0	7.825	0.68	25.864	2.892	0.1 - 181.6
	Total	106	0	6.390	0.735	22.610	2.196	0.1 - 181.6
Blood (µg/dL)	Nestlings	24	1	3.083	0.509	7.731	1.58	< LOD - 32.74

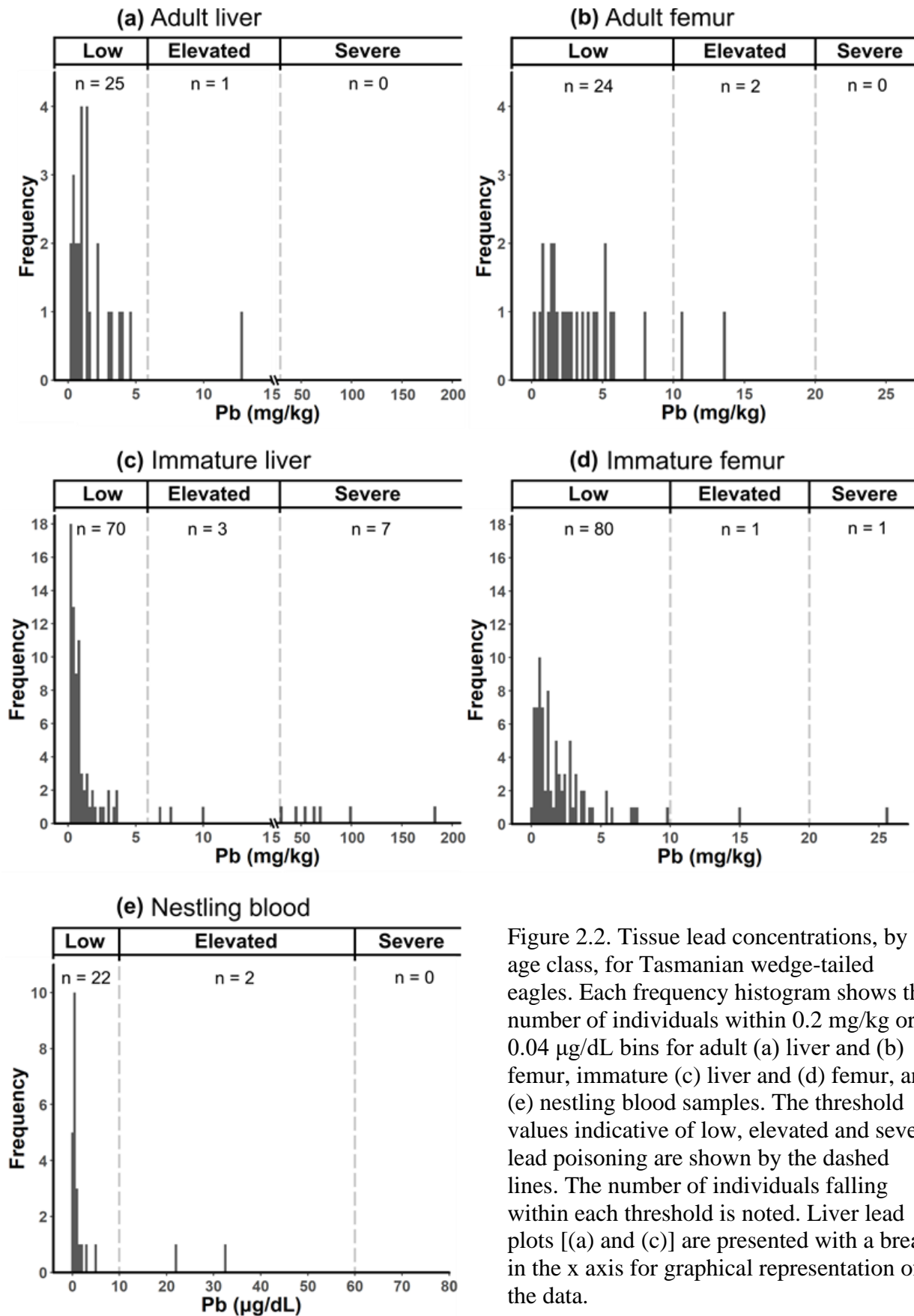


Figure 2.2. Tissue lead concentrations, by age class, for Tasmanian wedge-tailed eagles. Each frequency histogram shows the number of individuals within 0.2 mg/kg or 0.04  $\mu$ g/dL bins for adult (a) liver and (b) femur, immature (c) liver and (d) femur, and (e) nestling blood samples. The threshold values indicative of low, elevated and severe lead poisoning are shown by the dashed lines. The number of individuals falling within each threshold is noted. Liver lead plots [(a) and (c)] are presented with a break in the x axis for graphical representation of the data.

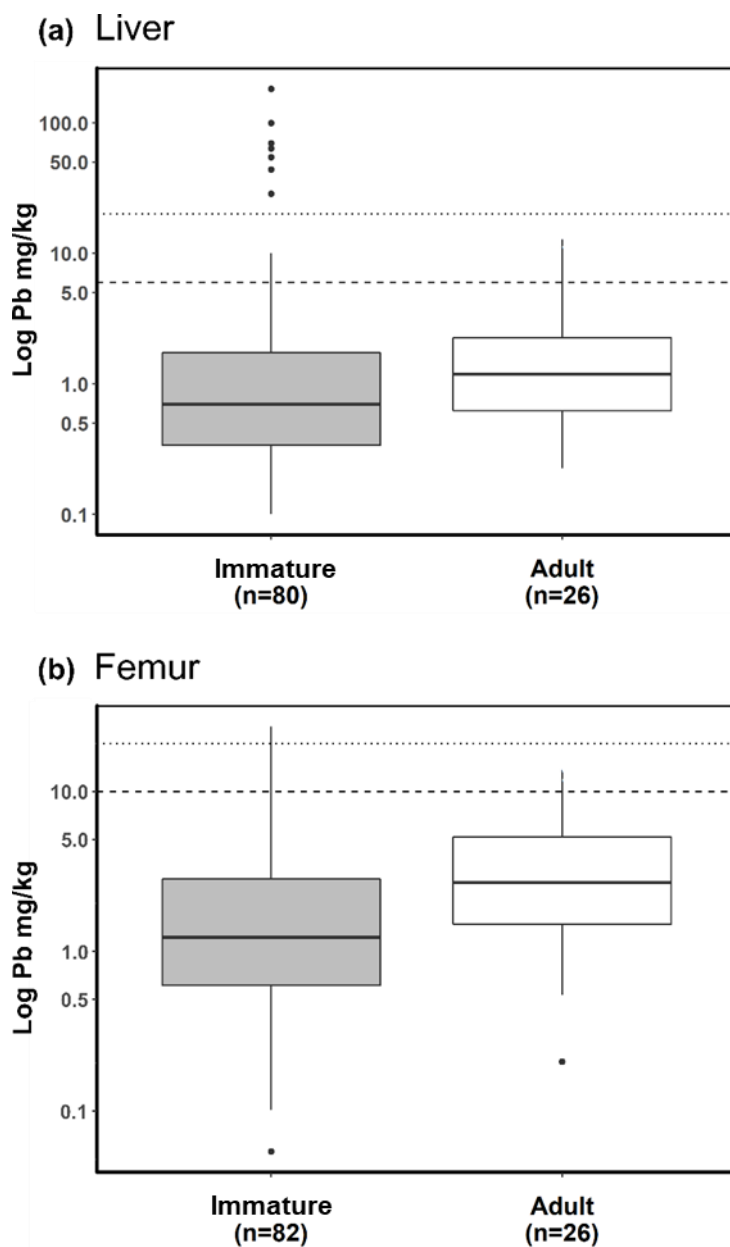


Figure 2.3. Box plots of adult and immature (a) liver lead concentrations and (b) femur lead concentrations. Plots are presented on a log scale for graphical representation of the data. The horizontal lines represent lead exposure thresholds (values above the dashed lines indicate elevated tissue lead levels, values above the dotted lines indicate severe tissue lead levels). Box plot whiskers are extended to maximum values within 150% of the interquartile range, values beyond this are plotted individually as outliers.

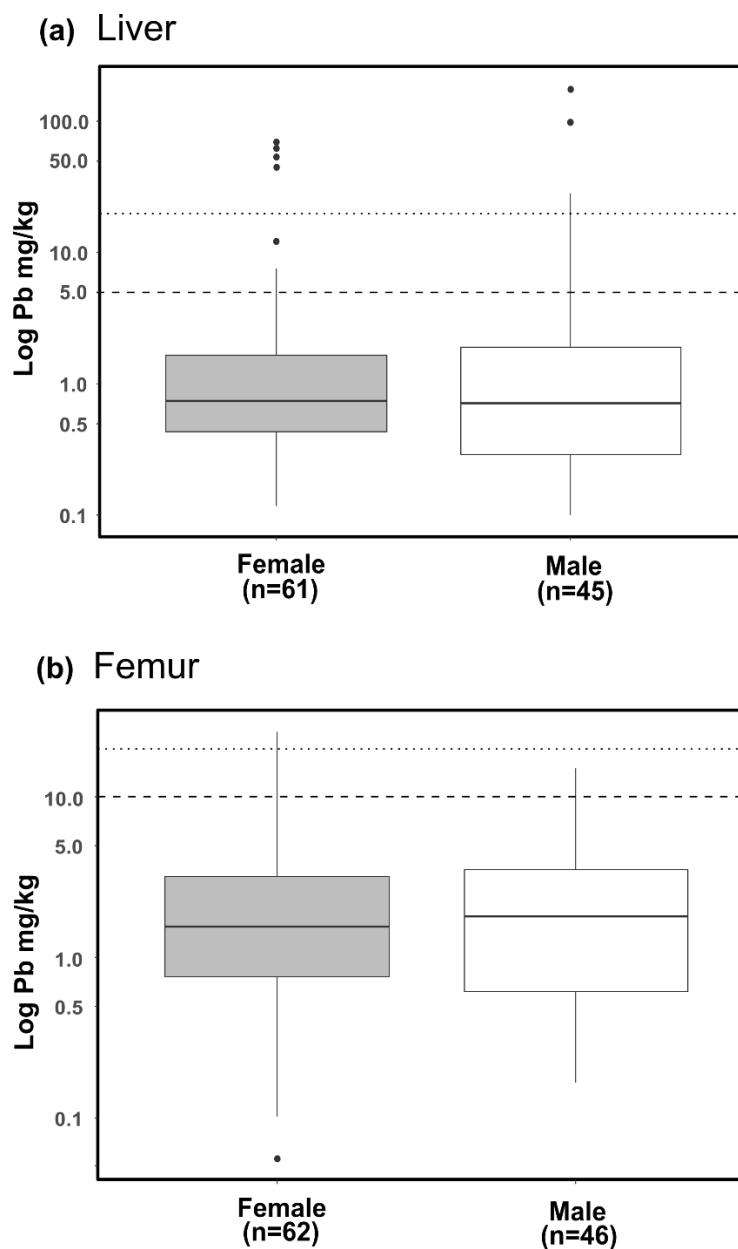


Figure 2.4. Box plots of female and male (a) liver lead concentrations and (b) femur lead concentrations. Plots are presented on a log scale for graphical representation of the data. The horizontal lines represent lead exposure thresholds (values above the dashed lines indicate elevated tissue lead levels, values above the dotted lines indicate severe tissue lead levels). Box plot whiskers are extended to maximum values within 150% of the interquartile range, values beyond this are plotted individually as outliers.

### *Seasonal differences in exposure*

The season of recovery was recorded in 61 of the liver sampled carcasses. There was no difference in hepatic lead concentrations between the seasons ( $\chi^2 = 3.55$ ,  $df = 3$ ,  $n = 61$ ,  $p = 0.31$ ; Figure A.3.1 and Figure A.3.2). That said, 7 of 9 birds with elevated hepatic lead levels and an accurate date of carcass recovery were found during the austral winter ( $n = 4$ ) or spring ( $n = 3$ ).

### *Isotopic patterns in exposure*

Mean lead<sup>207/206</sup> isotopic ratios within liver samples were  $0.8835 \pm SD 0.0539$  (range: 0.6829–0.9901;  $n = 106$ ). The lead<sup>207/206</sup> signatures of eagles with elevated hepatic lead levels ( $> 6$  mg/kg,  $n = 10$ ) were significantly different from those with low lead levels ( $< 6$  mg/kg;  $W = 802$ ,  $p = 0.004$ ; Figure A.4.1), despite the disparity in sample size reducing power to detect a difference. There was no difference in the coefficients of variation between these groups (M-SLRT = 0.726,  $p = 0.394$ ). Lead isotope signatures were also negatively correlated with liver lead concentrations ( $\tau = -0.212$ ,  $p < 0.02$ ,  $z = -3.216$ ; see Figure 2.5). Lead isotope ratios overlapped the range of published lead<sup>207/206</sup> ratios for bullets manufactured in Europe (0.8435–0.8964,  $n = 191$ ), USA (0.8070–0.9529,  $n = 99$ ), Australia (0.9195–0.9561,  $n = 100$ ) and South America (0.8306–0.8901;  $n = 96$ ). Furthermore, isotope ratios within the upper and lower quartile (25<sup>th</sup>–75<sup>th</sup> percentile) overlapped those published for ammunition manufactured in Europe and South America (Sjåstad *et al.*, 2014; Figure 2.6). The majority (72.7%) of liver samples with elevated lead levels ( $> 6$  mg/kg;  $n = 11$ ) had lead<sup>207/206</sup> ratios that were within the range of ammunition manufactured in Europe, South America or the USA.

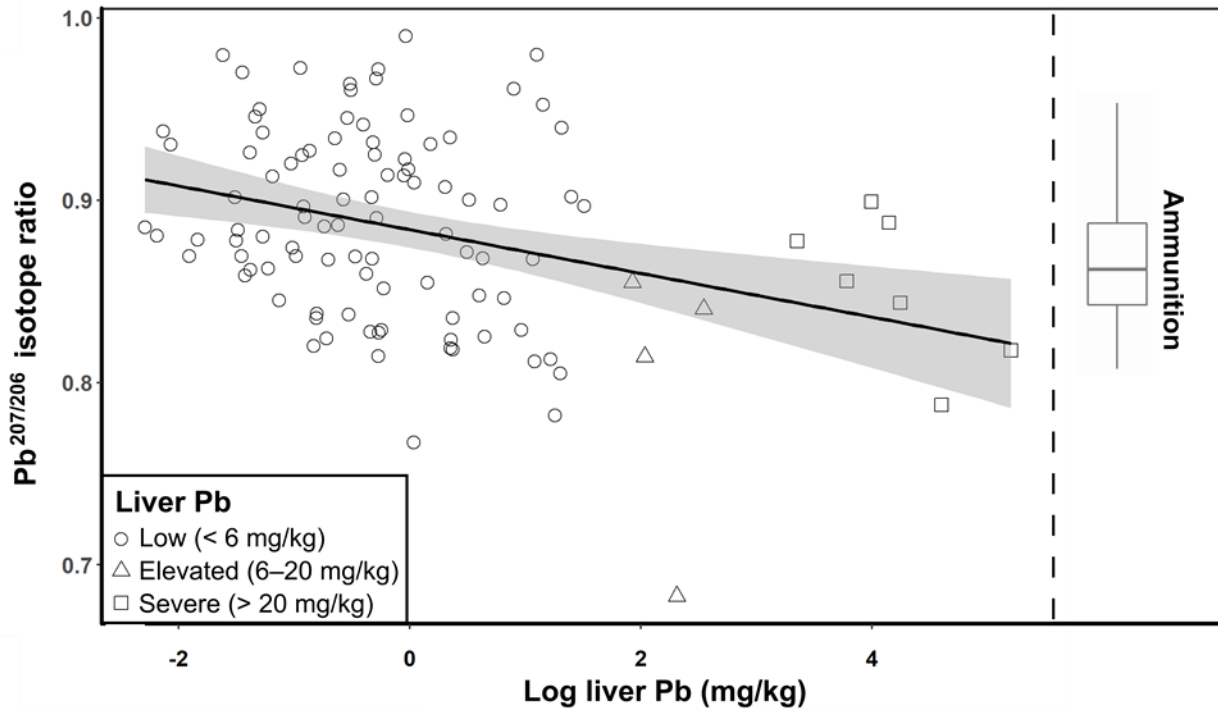


Figure 2.5. Relationship between the lead<sup>207/206</sup> isotope ratio and the concentration of lead in liver tissue. The 95% CI is indicated by the shaded area. The range in ammunition isotope signatures (Sjåstad *et al.*, 2014) is shown on the right.

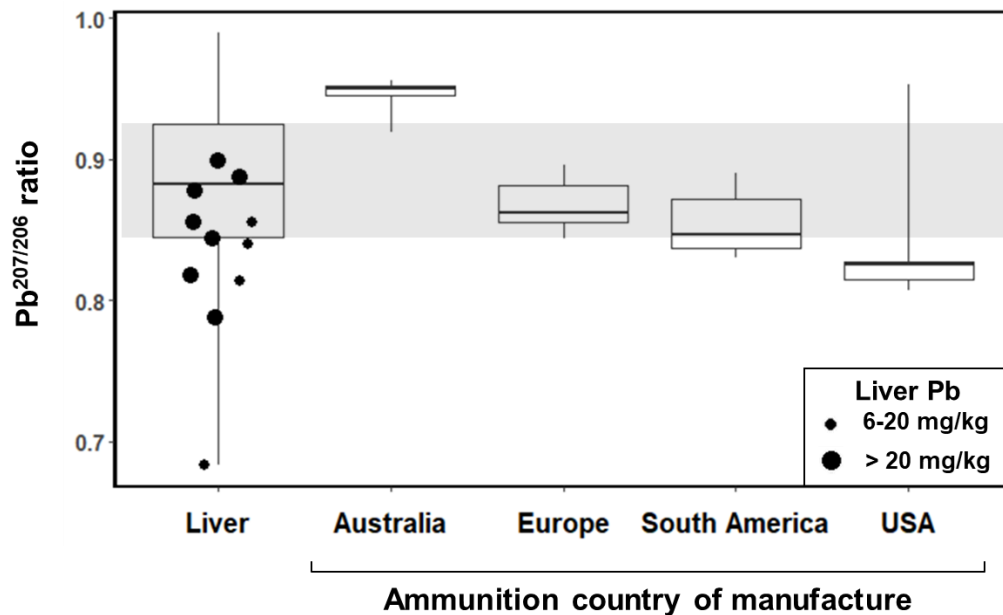


Figure 2.6. Lead<sup>207/206</sup> isotope ratios of Tasmanian wedge-tailed eagle liver lead in our study compared to ratios from lead-based bullets from various countries of manufacture (Sjåstad *et al.*, 2014). The shaded area indicates the overlap between the upper and lower quartile (25<sup>th</sup>–75<sup>th</sup> percentile) of lead<sup>207/206</sup> ratios in liver samples and ammunitions. Liver samples with elevated lead concentrations are plotted individually.

## Discussion

Our study provides the first data on lead exposure of an Australian raptor species and highlights the value of analysing different tissues when assessing the extent of lead contamination. Whilst femur levels indicated that immature Tasmanian wedge-tailed eagles are not severely exposed to lead, liver levels of birds of all ages showed more substantial evidence of lead exposure. The higher lead levels observed in femurs of older birds suggests that chronic exposure to lead is occurring. The absence of seasonal trends in hepatic lead suggests that risk of exposure does not change throughout the year. Lastly, the isotope analysis provides some evidence for an ammunition source of contamination but additional local information on isotope ratios of potential sources would be useful to clarify routes of exposure in Tasmania.

### *Extent and demographic patterns of lead exposure*

Our investigation into lead exposure of the free-flying Tasmanian wedge-tailed eagle population (i.e. liver and femur samples) relied on an opportunistic sample of birds found dead or moribund. This sample may have limitations when trying to quantify exposure rates if the sample is unrepresentative of the whole population. For example, such opportunistic samples can overestimate sub-lethal poisonings, as sick birds may be more likely to collide with anthropogenic structures and die in areas with a higher likelihood of being found (Kelly and Kelly, 2005). Equally, opportunistic sampling of carcasses may underestimate the rate of fatal poisonings caused by contaminants due to birds dying in remote areas where they are unlikely to be discovered (Newton *et al.* 1990). However, lead exposure in raptor species is commonly monitored using opportunistic samples (e.g. Ganz *et al.*, 2018; Behmke *et al.*, 2015; Carneiro *et al.*, 2014) due to the financial, ethical and logistical limitations of sampling live wild-caught

birds. To provide some context of the issue in the Tasmanian wedge-tailed eagle, it is therefore important to compare our liver and femur lead results to other raptor studies using similar sampling methods.

The femur lead concentrations we detected in the Tasmanian wedge-tailed eagle (0.06–25.57 mg/kg) covered a narrower and lower range than those of other raptor species [e.g., European golden eagles (*Aquila chrysaetos*; 0.4–54.21 mg/kg,  $n = 46$ , Ganz *et al.*, 2018), black vultures (*Coragyps atratus*; 4.5–540 mg/kg,  $n = 98$ ; Behmke *et al.*, 2015) and Spanish imperial eagles (*Aquila adalberti*; < LOD–41.68 mg/kg,  $n = 84$ ; Rodriguez-Ramos Fernandez *et al.*, 2011)]. Median femur lead concentrations were also lower in Tasmanian wedge-tailed eagles (1.73 mg/kg) compared to golden eagles (12.45–12.54 mg/kg; Ganz *et al.*, 2018; Jenni *et al.* 2015), but higher than in the Spanish imperial eagle (0.54 mg/kg; Rodriguez-Ramos Fernandez *et al.*, 2011). The maximum femur lead concentrations we detected are similar to levels observed in studies of golden and bald eagles (*Haliaeetus leucocephalus*; < LOD–18 mg/kg,  $n = 49$ ; Wayland *et al.*, 1999) and Egyptian vultures (*Neophron percnopterus*; < LOD–30 mg/kg,  $n = 39$ ; Gangoso *et al.*, 2008). Nevertheless, even in these studies, the proportion of birds with elevated femur lead is higher than we recorded (4.1% and 2.6% respectively compared to 0.9% of Tasmanian wedge-tailed eagles).

Although these results could suggest limited, chronic lead exposure in comparison to other avian scavengers, these patterns are likely driven instead by the predominance of immature birds in our sample. Bone lead concentrations reflect lifetime exposure due to the accumulative quality of lead in bone tissue (Fisher *et al.*, 2006). Older birds of many species therefore display higher bone lead concentrations if repeatedly exposed to sub-lethal levels of lead (Gangoso *et al.*, 2008; Ganz *et al.*, 2018; Rodriguez-Ramos Fernandez *et al.*, 2011). Our detection of an age-

related increase in femur lead burdens in Tasmanian wedge-tailed eagles suggests such recurrent exposure to lead occurs in the population. As a consequence, the high proportion (75.9%) of immature birds included in our study sample impedes comparisons to femur concentrations in other raptor studies and conclusions from the overall femur lead levels, as young birds have not had time to accumulate high femur lead levels.

The liver lead concentrations we detected are suggestive of frequent, widespread lead exposure of Tasmanian wedge-tailed eagles. This is consistent with the detection of an age-related increase in femur lead concentrations. The median liver lead concentration we measured (0.735 mg/kg) is comparable to the medians for other avian scavengers threatened by lead poisoning (0.23–1.38 mg/kg; Berny *et al.*, 2015; Rodriguez-Ramos Fernandez *et al.*, 2011). Furthermore, recent studies using opportunistic sampling of dead and moribund avian scavengers generally include a proportion of birds with no detectable lead in the liver (Carneiro *et al.*, 2014; Ganz *et al.*, 2018; Jenni *et al.*, 2015; Warner *et al.*, 2014). In contrast, we detected lead in every sample we analysed. Although the differing LODs complicate inter-study comparisons, the presence of lead at any given time in every single eagle suggests that lead exposure is widespread in the Tasmanian wedge-tailed eagle population. The proportion of birds we detected with severe hepatic lead levels > 20 mg/kg (6.6%) is higher than those found in congeners (0–5.5%; Ganz *et al.*, 2018; Jenni *et al.*, 2015; Madry *et al.*, 2015; Rodriguez-Ramos Fernandez *et al.*, 2011). We could also only find one report of an individual liver lead concentration higher than the 181.6 mg/kg maximum detected in our study (243 mg/kg; Wayland *et al.*, 1999). Such high levels may reflect both a) the high amount of anthropogenic lead in the Tasmanian landscape, since carcasses shot for management purposes are not removed (Hampton *et al.*,

2017), and b) the life history characteristics of wedge-tailed eagles increases their susceptibility to exposure, in that they appear to scavenge more than congeneric species.

Liver lead concentrations did not differ between the age classes suggesting there is no age-related change in short-term lead exposure risk. Due to the shorter retention time of lead in liver tissue (Fisher *et al.*, 2006), differences in hepatic concentrations between demographic groups are explained by behavioural variation (Franson and Russell, 2014). Although we did not detect a difference in lead liver concentrations between age-groups, all seven birds with severe lead liver concentrations ( $> 20$  mg/kg) were immature. This could be explained by the increased scavenging behaviour in immature birds, as they readily scavenge throughout the year whilst breeding adults reduce scavenging during the breeding season (Bell and Mooney, 1998; Olsen, 2005).

A very high proportion (95%) of nestlings had been exposed to lead, relative to proportions reported for other raptors (Bruggeman *et al.*, 2018; Harmata, 2011; Katzner *et al.*, 2017), but most exposure was at a low level, consistent with findings for other raptors (Carlson *et al.*, 2012). Several potential routes of lead exposure exist for nestlings, including inhalation of particulate emissions, ingestion of contaminated prey or maternal transfer of bone lead to the eggshell and embryo (Bruggeman *et al.*, 2018; Katzner *et al.*, 2017; Pattee, 1984). The high blood lead concentrations found in two nestlings suggest ingestion, since lead levels from both inhalation and maternal transfer normally involve less severe exposure. Wedge-tailed eagles, like other raptors, are thought to provision their nestlings with live-caught prey (Olsen, 2005), so exposure through consumption of lead ammunition fragments is unlikely. Our results suggest that either some scavenging behaviour does occur during the breeding season or that live-caught prey fed to the young contain elevated lead (e.g., potentially wounded from a non-fatal shot).

### *Seasonal patterns in lead exposure*

The absence of seasonal changes in Tasmanian wedge-tailed eagle lead exposure probably reflects the near absence of seasonality in local ammunition use. An increase in lead exposure during designated hunting seasons has been reported in scavenging bird populations in Europe (Ecke *et al.*, 2017), Africa (Garbett *et al.*, 2018), Canada (Legagneux *et al.*, 2014) and the USA (Lindblom *et al.*, 2017). In the regions of these studies, there are legally defined hunting seasons facilitating clear temporal trends in the numbers of animals that are shot. In contrast, Tasmanian legislation allows shooting throughout the year (Tasmanian Government, 2002). Therefore, although macropod shooting for agricultural asset protection in Tasmania fluctuates seasonally (generally focused around late autumn and early spring; R. Gaffney, pers. comm.), recreational shooting and browsing management for other industries occur throughout the year, and there is little evidence of a seasonal peak.

### *Ammunition as a potential source of lead exposure*

The range in isotope ratios that we detected was much greater than those documented for other avian scavengers (Behmke *et al.*, 2015; Finkelstein *et al.*, 2012; Mateo-Tomás *et al.*, 2016). This increased range could be due to either a wider variety of ammunition brands available in Australia, or sources of lead contamination other than ammunition. Although lead exposure through ammunition is likely the predominant route of exposure based on findings in other raptors (Finkelstein *et al.*, 2012; Garbett *et al.*, 2018; Legagneux *et al.*, 2014), other sources of lead can also pose a threat. For example, some California condors (*Gymnogyps californianus*) were exposed to lead both through ammunition and lead-based paint from a decommissioned fire tower used for roosting (Finkelstein *et al.*, 2012). In Tasmania there are potential sources of

anthropogenic lead for which we do not have isotopic information (e.g. mining, paint, coal emissions, leaded gasoline). Furthermore, there can also be overlap in the isotopic signatures of different sources of anthropogenic lead found in a region, making inferences on sources of exposure difficult without local isotopic information (Behmke *et al.*, 2015; Berny *et al.*, 2015; Finkelstein *et al.*, 2012). Therefore, isotopic analyses of environmental and anthropogenic lead sources in Tasmania will help clarify contamination sources.

There is some evidence that the lead exposure we recorded arose at least partly from ammunition. First, the proportion of individuals with lead isotope signatures within the range of ammunition was comparable to other studies implicating ammunition as a source of lead poisoning (e.g., 79%; Finkelstein *et al.*, 2012). Second, we detected lower isotope signatures in more highly exposed birds, similar to patterns found in other avian scavengers (Church *et al.*, 2006; Finkelstein *et al.*, 2012; Legagneux *et al.*, 2014). In these studies, the authors suggest that isotope ratios shifted downwards in acutely exposed birds due to the lower lead<sup>207/206</sup> signatures associated with ammunition (Church *et al.*, 2006; Finkelstein *et al.*, 2012; Legagneux *et al.*, 2014).

### *Conservation implications*

Our results indicate that lead exposure is likely to represent a threat to the Tasmanian wedge-tailed eagle. It is difficult to infer population level impacts since there are biases inherent with opportunistic sampling of dead and moribund eagles (see Russell and Franson, 2014). However, the hepatic lead concentrations that we detected are comparable to other raptor studies using similar sampling methods that concluded lead exposure was a conservation threat (Berny *et al.*, 2015; Ganz *et al.*, 2018; Jenni *et al.*, 2015; Madry *et al.*, 2015). As well as mortality

caused by severe poisoning, lead may negatively impact on the population in other ways. The prevalent background levels we detected may increase the susceptibility of individuals to other causes of mortality, such as collisions with anthropogenic structures and vehicles (Ecke *et al.*, 2017; Golden *et al.*, 2016; Kelly and Kelly, 2005). The detection of lead in most nestlings suggests lead may also contribute to the low fledging success rate in the population (Threatened Species Section, 2006).

Our work highlights the need for further research into lead contamination throughout the ecosystem and in other Australian regions. In Tasmania, these negative impacts are unlikely to be restricted to wedge-tailed eagles, as numerous species are known to scavenge shot carcasses, including the endangered Tasmanian devil (*Sarcophilus harrisii*; IUCN, 2018). Lead-based ammunition is used nationwide, thus similar lead exposure is expected in other susceptible species throughout Australia. However, there is a paucity of research on the ecological impacts of lead-based ammunition in Australia (Hampton *et al.*, 2017). In the USA and Europe, the environmental risks of lead ammunition have been recognised, with the promotion of lead-free alternatives and restrictions on lead-based bullet use implemented in numerous regions (Ganz *et al.*, 2018; Golden *et al.*, 2016). Reductions in use of lead ammunition can be effective in reducing ecological impacts, as scavenging bird blood lead concentrations in California significantly declined after localised bans on lead-based ammunition were implemented in 2008 (Kelly *et al.*, 2011). If lead contamination from spent ammunition is pervasive in Australia, then equivalent mitigative efforts will be critical to reducing impacts on at-risk species throughout the country.

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## Chapter 3

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Unexpectedly high exposure to anticoagulant rodenticides in endangered  
Tasmanian wedge-tailed eagles (*Aquila audax fleayi*): an over-looked  
conservation threat?



Image D. James

## Abstract

Anticoagulant rodenticides (ARs) used to control synanthropic rodent populations have been demonstrated to cause poisoning to predatory species throughout much of the world. However, much less is known about the impacts of ARs on predators in Australia. To assess long-term impacts and develop effective mitigation, the drivers of patterns of non-target AR exposure need to be identified, especially where they have not been previously investigated. Our aim was to determine if the Tasmanian wedge-tailed eagle (*Aquila audax fleayi*), an endangered top predator, showed evidence of AR exposure, and, if found, to investigate the intrinsic (age, sex) and extrinsic (habitat, season and year of death) drivers of this exposure. We detected AR residues in 74% of 50 opportunistically collected eagle carcasses, including liver concentrations in 46% that were likely to be toxic and potentially lethal ( $> 0.1$  mg/kg, as detected by LC-MS). Flocoumafen was detected at an exceptionally high exposure rate (40%); an AR that is only available from agricultural suppliers. Best performing censored and logistic models found that both the probability of AR residue detection and the AR concentrations in the liver were positively associated with the proportion of agricultural habitat and mean human population density within the estimated home range of each eagle. Our results indicate that AR exposure constitutes a previously unrecognised threat to the Tasmanian wedge-tailed eagle population. It is evident that rodenticides are heavily used in Australia, and are frequently finding their way into top predators in the ecosystems, such that both residential and agricultural use of ARs require increased monitoring and regulation to reduce impacts to Australian predators.

## Introduction

Anticoagulant rodenticides (ARs) are used worldwide to control synanthropic rodent populations. These compounds function by inhibiting blood clotting mechanisms in vertebrates, resulting in internal haemorrhaging (Rattner *et al.*, 2014). The discovery of resistance to the first-generation of ARs (FGARs) in some rodent populations led to the development of second-generation ARs (SGARs) in the 1970s (Hindmarch and Elliott, 2018). To be lethal, FGARs generally require consecutive intake over several days to accumulate sufficiently high concentrations (Erickson and Urban, 2004). Conversely, SGARs are usually lethal from a single feed and persist longer in the environment (Erickson and Urban, 2004; Van Den Brink *et al.*, 2018). The persistence of AR compounds (Horak *et al.*, 2018), the delay in mortality after bait consumption (Lee *et al.*, 2006) and the behavioural changes that occur as a symptom of poisoning (Brakes and Smith, 2005; Mooney, 2017) can make poisoned rodents AR vectors to non-target predatory species.

Detrimental non-target exposure to ARs has been shown in numerous predators in Europe and North America (Christensen *et al.*, 2012; López-Perea *et al.*, 2015; Riley *et al.*, 2007; Shore *et al.*, 2003; Thomas *et al.*, 2017). The effects can be significant, with population level impacts documented in mammals (Jacquot *et al.*, 2013) and raptors (Thomas *et al.*, 2011). It is thought that species that regularly prey upon small rodents are at higher risk of poisoning due to the likelihood of consuming AR targeted species (Hindmarch and Elliott, 2018). However, the primary consumption of AR baits by non-target species, as well as the potential for SGARs to bioaccumulate, may cause wider contamination of terrestrial food chains (Thomas *et al.*, 2011). If such broadscale contamination is apparent, species at higher trophic levels may be at increased risk of AR exposure (Riley *et al.*, 2007; Thomas *et al.*, 2011).

Understanding the drivers of patterns in non-target AR exposure is necessary to assess long-term impacts and for the development of effective mitigation. Studies have shown differences in predator AR exposure between the sexes (McDonald *et al.*, 1998), and among age groups (Christensen *et al.*, 2012; Ruiz-Suárez *et al.*, 2016) and seasons (Lohr, 2018; Serieys *et al.*, 2015). That said, the anthropogenic factors within a landscape in which a species lives are likely the most significant driver in terms of overall risk. For example, human population density and developed surface area have been linked to the probability of and levels of AR exposure in numerous predators (Lohr, 2018; Lopez-Perea and Mateo, 2018; Nogeire *et al.*, 2015; Serieys *et al.*, 2015). Agricultural AR use has also been suggested as the cause of non-target poisoning of predators (Birks, 1998; Fourel *et al.*, 2018; Hughes *et al.*, 2013) but only few recent studies have found empirical evidence of this relationship (Coeurdassier *et al.*, 2019; López-Perea *et al.*, 2018; Sainsbury *et al.*, 2018).

AR use is largely unmonitored in Australia and recent work has highlighted the need for the evaluation of impacts on Australian species (Lohr, 2018; Lohr and Davis, 2018). The Tasmanian wedge-tailed eagle (*Aquila audax fleayi*) is a subspecies of wedge-tailed eagle endemic to the island of Tasmania (Commonwealth of Australia, 1999). With the loss of the thylacine (*Thylacinus cynocephalus*), the wedge-tailed eagle serves a particularly important ecological function as one of the few remaining top predators in Tasmanian ecosystems. The subspecies is listed as endangered (Commonwealth of Australia, 1999; State Government of Tasmania, 1995), with conservation concern based upon a series of known threats, including nest failures caused by anthropogenic disturbance, low breeding success rates, habitat loss, collisions with anthropogenic structures and illegal persecution (Bell and Mooney, 1998; Mooney and Holdsworth, 1991; Threatened Species Section, 2006). ARs are not recognised as a threat to the

population, as the species generally avoids areas of high human population density, and rodents represent a very small portion of wedge-tailed eagle diet (Debus *et al.*, 2007; Olsen *et al.*, 2010). However, if bioaccumulation of ARs is occurring through Tasmania's food chains, then the high trophic position of the Tasmanian wedge-tailed eagle may increase their susceptibility to AR exposure.

Our study was designed to establish if, and to what extent, Tasmanian wedge-tailed eagles are exposed to ARs and if we could identify factors that influence AR exposure in the population. Specifically, we evaluated (1) liver tissue concentrations of individual ARs known to be used in Tasmania and the total AR burden of the eagles; (2) individual intrinsic (age, sex) and seasonal correlates of the total AR burden of the eagles; (3) how total liver AR concentrations were influenced by the covariation between intrinsic and extrinsic factors (human population density, agricultural cover, and year of death); and (4) how the probability of an eagle being exposed to ARs was influenced by the covariation between intrinsic and extrinsic factors.

## Methods

### *Study area*

The study was conducted on mainland Tasmania, an island state located 240 km south of continental Australia. Tasmania covers an area of 68,150 km<sup>2</sup>, with an estimated human population of 520,830 (Australian Bureau of Statistics, 2018; Figure 3.1b). Areas of minimal land use, nature conservation and other protected areas account for 50.3% (34,280 km<sup>2</sup>) of the Tasmanian land area (DPIPWE, 2015). Agricultural use, predominantly comprising grazing and modified pastures, occupies 18,900 km<sup>2</sup> (27.7%; DPIPWE, 2015), mostly focused in the north and east of the state (Figure 3.1c).

### *Sample collection*

Eagles were collected as fresh carcasses found opportunistically throughout Tasmania (see Figure 3.1a) by government departments, various industries and volunteers between 1996 and 2018. All carcasses were placed in -20°C freezer storage by the Department of Primary Industries, Parks, Water and the Environment (DPIPWE, Threatened Species Section, Hobart, Tasmania) and the Tasmanian Museum and Art Gallery (TMAG, Collection and Research Facility, Rosny, Tasmania). We defrosted the carcasses and carried out necropsies between May 2017 and March 2018. At necropsy, we collected a whole liver lobe and a muscle sample from each carcass. Necropsied tissues were stored at -20°C until sample preparation, when we thawed them at room temperature. We weighed out a 4 g ( $\pm 0.1$  g) wet weight sample from the middle of each liver lobe using a digital balance (precision  $\pm 0.0001$  g (Mettler Toledo, US). New scalpel blades and gloves were used between samples during necropsy and sample preparation to prevent cross contamination.

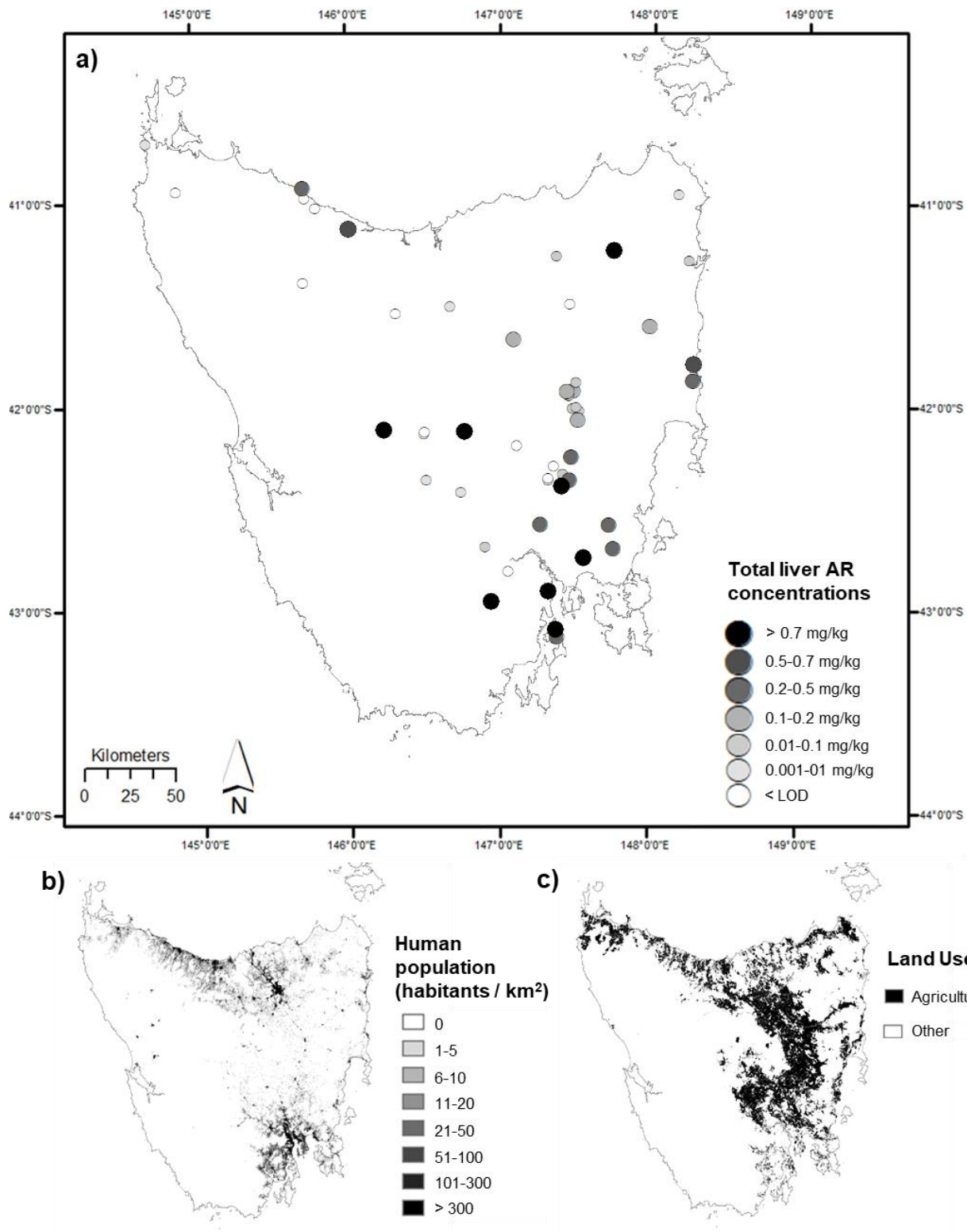


Figure 3.1. a) Spatial distribution of eagle carcasses included in the study with the respective liver AR concentration threshold that each sample fell within represented by symbol colour. Maps b) and c) indicate the general spatial distribution of the Tasmanian human population (2016 data; Australian Bureau of Statistics, 2018) and agricultural land use area (2015 data; DPIWWE, 2015) respectively.

## *Toxicological analysis*

### *Sample preparation*

All toxicological analyses were carried out at Edith Cowan University Analytical Facility (Joondalup, Western Australia). Each liver sample was freeze-dried and homogenised before being transferred to 15 ml analytical tubes with two aliquots of acetone. Analytes were extracted using 15 minutes of sonication. After extraction, samples were centrifuged at 4400 rpm for 5 minutes, transferred to a new centrifuge tube with 2 ml of hexane, vortexed for 5 minutes and centrifuged at 4400 rpm for a further 5 minutes. Each sample was then evaporated and reconstituted in 400  $\mu$ l of 50:50 ACN/H<sub>2</sub>O solution. The final extracts were transferred to 2 ml Teflon-lined vials and stored at 4°C until analysis.

### *LC-MS analysis*

Liver samples were analysed for ARs registered for use in Australia (Australian Pesticides and Veterinary Medicines Authority, 2019). Concentrations of five SGARs (brodifacoum, bromadiolone, difethialone, difenacoum and flocoumafen) and three FGARs (coumatetralyl, pindone and warfarin) were evaluated using a TSQ Quantiva triple quadrupole Mass Spectrometer (LC-MS) from Thermo Fisher (Thermo Fisher Scientific Corporation, US). Calibration curves and recovery rates for each analytical run were calculated using organic chicken livers spiked with three working solutions of each analytical standard. Recovery rates for the target ARs averaged 96.75 %, whilst limits of quantification (LOQ) and limits of detection (LOD) ranged from 0.001–0.025 mg/kg and 0.0005–0.0125 mg/kg respectively (see Table B.1). Three organic chicken liver blanks were included in each run to monitor cross-contamination. Every 10<sup>th</sup> sample was reinjected for a duplicate read (average RSD 4.1%) and duplicate blind sample extractions were carried out for five randomly selected samples (average RSD 4.1%).

*Potential drivers of AR exposure*

We evaluated potential drivers of AR exposure as a response to a suite of intrinsic and extrinsic explanatory variables. The intrinsic variables we considered were the sex of the bird (determined genetically using muscle tissue; see Appendix D.3), its age (broadly characterized into adults and immatures based on plumage (see Appendix A.1) and the season the carcass was found. Extrinsic explanatory variables were the year the carcass was found and both the mean human population density per km<sup>2</sup> (Australian Bureau of Statistics, 2018) and the proportion of agricultural area (DPIPWE, 2015) within the estimated home range of each eagle. Home ranges were estimated at two spatial scales dependent on the age of the bird. Adult carcass locations were buffered to a 25 km<sup>2</sup> area, based on estimations of adult home ranges (Bell and Mooney, 1998). Immature eagles included in the study were most likely undergoing natal dispersal; thus, we used a buffered area of 310 km<sup>2</sup> based on the mean three-month 50% movement-based kernel estimation (BRB function of adehabitatHR; Calenge, 2006) calculated from tracking data from 16 dispersing Tasmanian wedge-tailed eagles (J. Pay unpublished data). To achieve the best accuracy in estimates of spatial predictor variables, both human population density and agricultural land use area were calculated from data as close to the year the carcass was found as possible (max difference between year of death and spatial data was 6 years for human population and 3 years for agricultural land use).

*Data analysis*

We performed all statistical analyses in R, version 3.2.0 (R Core Team, 2016). Data were analysed using censored data techniques (R packages NADA; Lee, 2017, and Survival; Therneau, 2018) as AR concentrations were sometimes below the LOD of the LC-MS and

censored methods consider that the contaminants may be present below the LOD. Censored data techniques are typically applied to survival analyses, which are right-censored. However, the models can be switched to being left-censored for application to contaminant data (see Helsel 2012). For the censored analyses we assigned censored data (i.e. data <LOD) the corresponding LOD value for the AR, with an indicator variable denoting the observations as censored. Uncensored data (i.e. data >LOD) were the concentrations of the ARs detected by the LC-MS, with an indicator variable denoting the observations as not censored.

#### *Individual and total AR burden*

We used a Kaplan-Meier cumulative probability distribution ('NADA' function 'cenfit') to calculate mean, median and standard error of each AR compound and total AR burden. Analogous summary statistics were also calculated for only the eagles with detected AR concentrations. This was to facilitate comparisons with other species by matching techniques used in other raptor AR work (e.g. Hughes *et al.*, 2013; Lopez-Perea and Mateo, 2018). Total AR concentrations are commonly used in ecotoxicological studies to investigate the impacts of AR contamination, as the similar mode of action of all ARs means the toxicity of multiple ARs is expected to be roughly additive (Rattner and Mastrota, 2018). To estimate the toxicological impact of the total AR concentrations detected, we used recently published contamination categories: (i) 0.001–0.01 mg/kg, probably no toxicity; (ii) 0.01–0.1 mg/kg, unlikely lethal / possible toxicity; (iii) 0.1–0.7 mg/kg, possibly lethal / likely toxicity; (iv) 0.5–0.7, Probably lethal; (v) > 0.7 mg/kg, lethal (Lohr, 2018).

#### *Individual intrinsic and seasonal correlates of AR burden*

We used total liver AR concentrations in all statistical analyses exploring the intrinsic and extrinsic drivers of AR exposure in Tasmanian wedge-tailed eagles. We used a Peto and Peto

modification of the Gehan-Wilcoxon test to assess differences in total liver AR concentrations between the sexes, age groups and seasons the carcasses were found ('NADA' function 'cendiff').

#### *Correlates of degree of exposure*

We explored relationships between the extrinsic and intrinsic explanatory variables (age, sex, year of death and the human population density and proportion of agricultural area in the estimated age-specific home range) and total AR concentrations with left-censored regression models ('Survival' function 'survreg') assuming a lognormal distribution. The correlation of predictor variables were checked before inclusion in the models (Pearson's  $r < 0.3$ ). The dependent variable in these models was the total liver AR concentration (mg/kg) for each sample. We used Akaike information criterion with small sample size correction (AICc) to rank model performance. To interpret the effect of the explanatory variables in top ranked models ( $\Delta\text{AICc} < 2$  from the best model) we calculated the model average coefficients (Burnham and Anderson, 2002).

#### *Correlates of likelihood of exposure*

We also explored the relationship between the suite of extrinsic and intrinsic variables with the probability of AR residues being detected using a binomial generalised linear model with logit link function. The dependent variable in these models was whether the eagles were exposed (AR > LOD) or unexposed (AR concentrations < LOD). Models were again ranked by AICc. A logistic regression was also used to test for the individual relationship between year of death and probability of AR residues being detected in a sample. Model fit was evaluated using the likelihood ratio.

## Results

We analysed 50 eagle carcasses that were collected between 1996 and 2018. There was a bias towards samples collected after 2006, likely because sampling intensity increased ( $n = 37$ ). Forty-one were immature birds, and 22 were females. Data available for the sampled carcasses included location ( $n = 50$ ; Figure 3.1a), year the carcass was found ( $n = 50$ ) and the season the carcass was found ( $n = 44$ ). Assumed causes of death were recorded in 37 cases and were predominantly power line collisions ( $n = 27$ ) or road vehicle collisions ( $n = 6$ ; see Table B.2).

### *Individual and total AR burden*

AR residues were detected in 74% of wedge-tailed eagles included in the study (see Table 3.1). Residues of more than one AR compound were detected in 38% of the birds, with a maximum of three different compounds in 12%. The mean AR concentrations of birds with detected levels was 0.448 mg/kg ( $\pm$ SE 0.101) and the censored mean of the entire study sample was 0.333 mg/kg ( $\pm$ SE 0.080). The majority of AR residues were SGARs. Brodifacoum, flocoumafen and bromadiolone were the most predominant SGARs detected with 56%, 40% and 22% of birds showing detected levels respectively. FGARs were only detected in three individuals. Warfarin was detected in two birds and at very low levels ( $< 0.01$  mg/kg) and coumatetralyl in one bird (a bird that also had a probably fatal 1.33 mg/kg concentration of flocoumafen).

Table 3.1. Summary statistics of each AR investigated and the total liver AR concentrations. Censored summary statistics (bottom) and summary statistics for birds with detected AR levels are presented.

	Brodifacoum	Bromadiolone	Coumatetralyl	Difenacoum	Difethialone	Flocoumafen	Pindone	Warfarin	Total ARs
Reporting limit (mg/kg)	0.005	0.001	0.002	0.0025	0.01	0.0025	0.025	0.002	NA
Birds exposed (%)	56	22	2	0	0	40	0	4	74
Max (mg/kg)	2.198	0.867	0.054	0.000	0.000	1.329	0.000	0.006	2.276
Min (mg/kg)	0.012	0.008	0.054	0.000	0.000	0.005	0.000	0.004	0.000
Mean (mg/kg)	0.439	0.158	0.054	0.000	0.000	0.125	0.000	0.005	0.448
Median (mg/kg)	0.245	0.073	0.054	0.000	0.000	0.010	0.000	0.005	0.195
Standard error	0.097	0.076	0.000	0.000	0.000	0.079	0.000	0.001	0.101
Censored mean (mg/kg)	0.251	0.041	0.054	NA	NA	0.053	NA	0.004	0.333
Censored median (mg/kg)	0.034	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.059
Censored standard error	0.062	0.019	NA	NA	NA	0.033	NA	0.000	0.080

We recorded a potentially lethal total liver AR burden ( $> 0.1$  mg/kg; Newton *et al.*, 1999) in nearly half of the wedge-tailed eagles sampled (46%). Furthermore, in 16% of the eagles, concentrations were over 0.7 mg/kg, a threshold that is almost certainly lethal (Lohr, 2018; see Figure 3.2). Of the eight eagles with liver AR concentrations  $> 1$  mg/kg, four had unknown causes of death, three were listed as power line collisions and one as roadkill. It was not possible to categorise physiological signs of AR poisoning in the birds due to the damage and prolonged storage of carcasses.

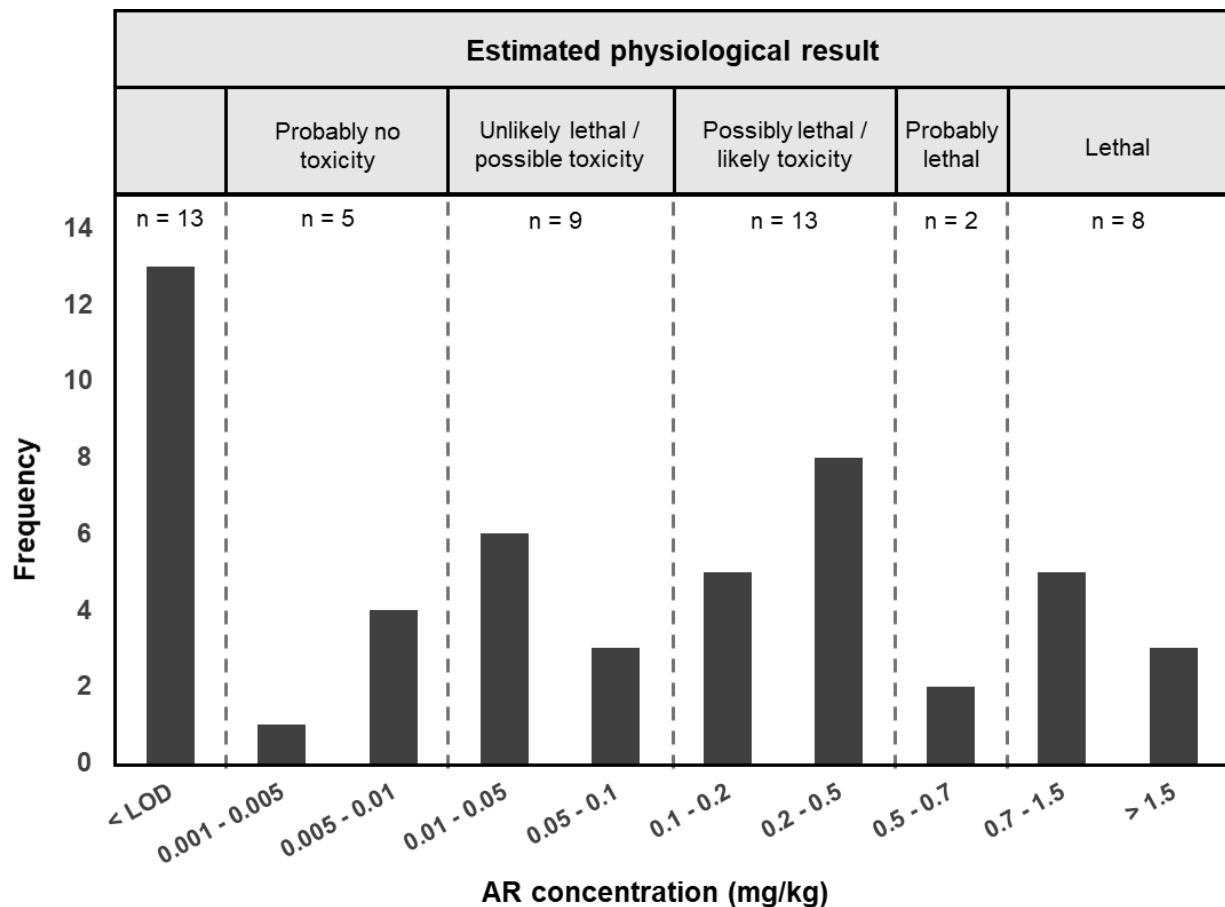


Figure 3.2. Number of eagles at each toxicity threshold proposed by Lohr (2018) and the estimated physiological result.

### *Individual intrinsic and seasonal correlates of AR burden*

There was no difference ( $\chi^2 = 0.6$ ,  $p = 0.448$ ; see Figure 3.3a) between mean AR concentrations of males (0.214 mg/kg  $\pm$ SE 0.088;  $n = 28$ , 21.4% < LOD) and of females (0.485 mg/kg  $\pm$ SE 0.140;  $n = 22$ , 31.8% < LOD). There was a tendency for higher liver AR concentrations in adult eagles (0.625 mg/kg  $\pm$ SE 0.243;  $n = 9$ , 22.2% < LOD) than in immatures (0.273 mg/kg  $\pm$ SE 0.080;  $n = 41$ , 26.8% < LOD), but the difference was marginally non-significant ( $\chi^2 = 2.9$ ,  $p = 0.09$ ; see Figure 3.3b). The majority of birds (4 of 6) with very high liver AR concentrations (> 1 mg/kg) were found in the winter months (see Figure 3.4), but mean liver AR concentrations did not differ between seasons ( $\chi^2 = 5.6$ ,  $df = 3$ ,  $p = 0.2$ ).

### *Correlates of degree of exposure*

The best performing censored regression models (< 2  $\Delta$ AICc) suggested that total AR concentrations were driven most strongly by the year the carcass was found, the amount of agricultural area and the human population density within the estimated age-specific home range (see Table B.3). The top model was 12.5 times more likely than the null model based on AICc weight. Modelled averaged coefficients of the top two performing models suggested that year of death, agricultural area and human population density were positively associated with total AR concentration (see Table 3.2 and Figure 3.5). Model coefficients estimated that a 10% increase in agricultural habitat proportion in the estimated home range would result in an increase in liver AR concentrations by a factor of 1.74. Likewise, each later year in the study was estimated to increase AR concentrations by a factor of 1.31. The relationship between total AR concentration and human population density suggested an increase in 100 habitants per km<sup>2</sup> would increase total AR by a factor of 4.49.

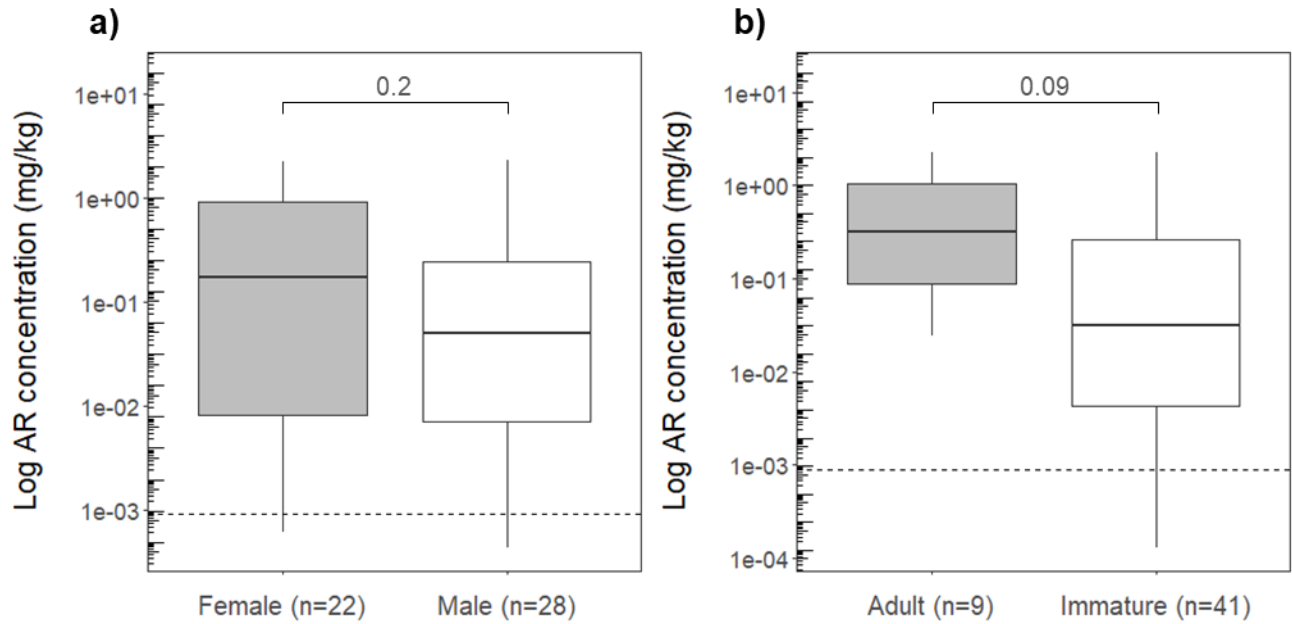


Figure 3.3. Censored box plots of mean hepatic AR concentrations between the sexes (a) and age classes (b). Dashed lines designate the LOQ and  $p$  values are presented.

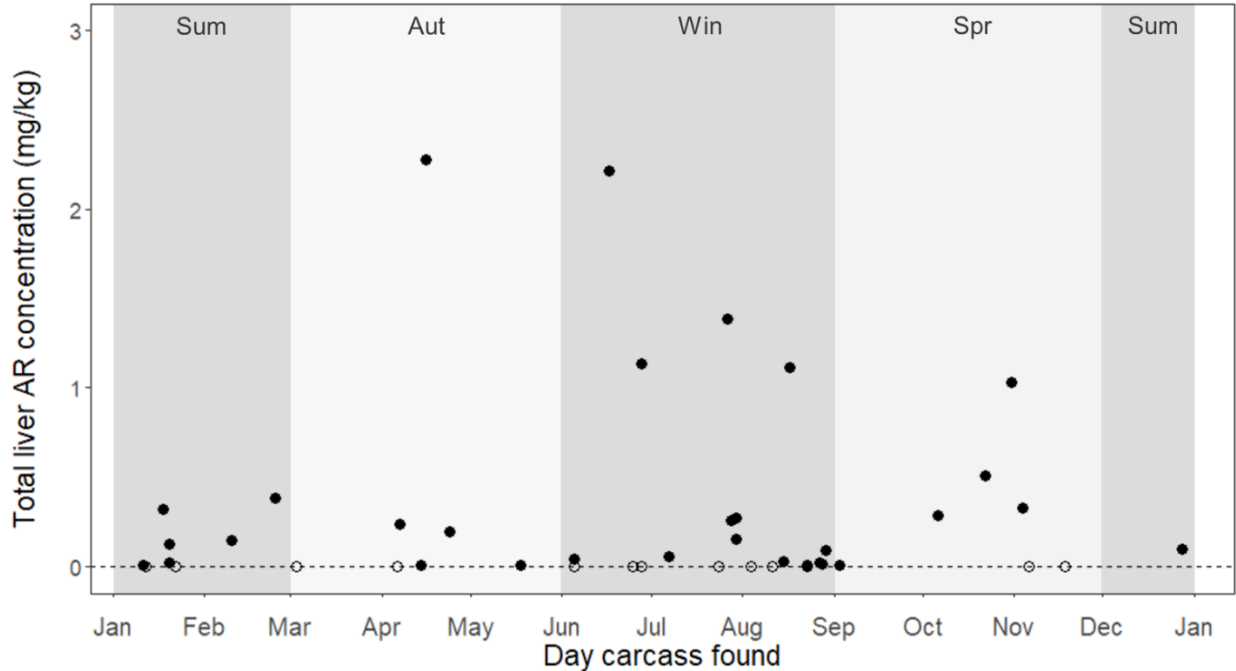


Figure 3.4. Scatterplot of the day of the year when study carcasses were found ( $n = 44$ ) and the respective total liver AR concentrations. Mortality is estimated to have occurred 0–3 days before carcass location based on carcass condition. The dashed line designates the LOQ. Solid points are samples with AR levels above the LOQ, hollow points are below the LOQ.

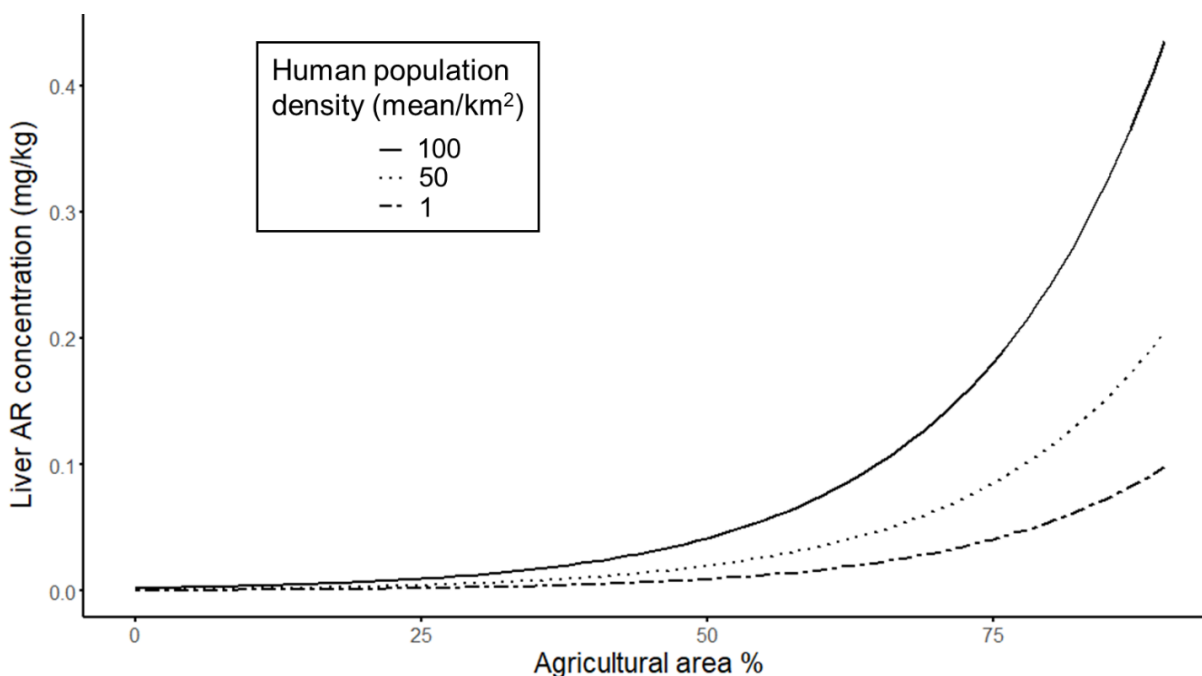


Figure 3.5. Predicted response of total liver AR concentrations in Tasmanian wedge-tailed eagles with respect to proportion of agriculture and mean human population density in the estimated home range. For this plot, year of carcass discovery is held at its mean. The three lines have set mean human habitats per km<sup>2</sup> at three levels. The plot shows that the predicted effect of agricultural land in an estimated eagle home range has a much lower effect on liver AR concentrations when there is a lower human population density. Note that this plot is not suggesting an interaction between these two variables; plotting on the log scale would result in parallel relationships (i.e. the effect is multiplicative).

Table 3.2. Averaged model coefficients describing the estimated effect of each variable on total liver AR concentrations (censored regression) and probability of ARs being detected in a sample (binomial probability).

Averaged Model	Parameter	Estimate	95% CI		z
			Lower	Upper	
AR concentration	Intercept	-10.061	-14.182	-5.941	4.786
	Agricultural area	0.556	0.105	1.007	2.414
	Human population density	1.502	0.059	2.944	2.040
	Year of death	0.271	0.087	0.455	2.884
Probability of exposure	Intercept	-3.063	-6.245	-0.120	1.886
	Agricultural area	0.379	0.005	0.752	1.988
	Year of death	0.197	0.036	0.357	2.404
	Human population density	0.557	-0.585	1.388	0.956

### *Correlates of likelihood of exposure*

The best performing binomial model to explain the probability of ARs being detected in an eagle included year the carcass was found and agricultural area. Including human population in the model resulted in equivalent model performance ( $< 2 \Delta AICc$ ; see Table B.4). The probability of ARs being detected increased with carcasses found more recently and with higher proportions of agricultural area in their estimated age-specific home range (see Table 3.2). The odds of AR levels being above the LOD were 1.13 times greater for each later year of the study period (Likelihood ratio  $\chi^2_7 = 4.09$ ,  $p = .043$ , see Figure 3.6).

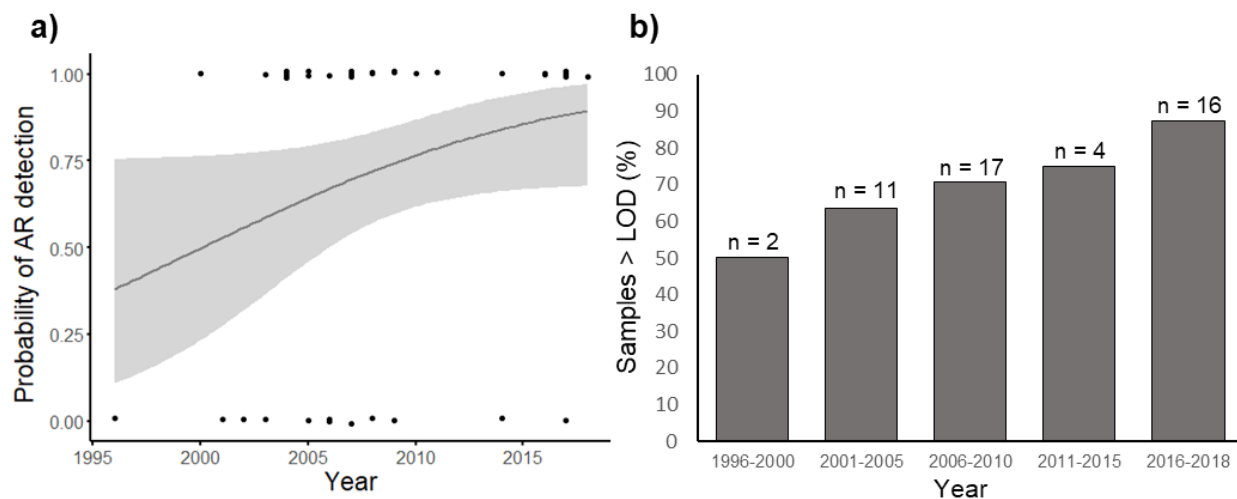


Figure 3.6. (a) Logistic plot of the effect of year of death on the probability of AR exposure. The shaded area represents the 95% confidence intervals of the predicted probabilities. (b) histogram of the proportion of samples where ARs were detected in each five-year period of the study.

## Discussion

The high frequency and levels of rodenticide exposure of Tasmanian wedge-tailed eagles, and their correlation to agricultural areas and human population density have several implications for our understanding of rodenticide exposure and the Tasmanian ecosystem. First, rodenticide exposure is high among these birds, suggesting not only heavy use of rodenticides but also that those rodenticides are frequently finding their way into top predators in the ecosystem. Furthermore, environmental rather than intrinsic factors (i.e. age, sex) influence the probability of exposure and total AR burden, and there is also evidence that the level of exposure has increased over the last 20 years. These findings suggest ARs pose a serious and previously unrecognised threat to this endangered population, and most likely to other predators in the region.

### *Individual AR exposure*

The high prevalence of SGARs detected in our study is consistent with research implicating SGARs as the predominant cause of non-target AR exposure in predators (Lohr, 2018; López-Perea *et al.*, 2015), due to the higher toxicity and longer persistence of these compounds. SGARs brodifacoum, bromadiolone and flocoumafen accounted for 99.6% of the total AR concentrations observed in the Tasmanian wedge-tailed eagle. Brodifacoum and bromadiolone are the predominant AR compounds identified in non-target predators in numerous ecosystems worldwide (Hosea, 2000; Koivisto *et al.*, 2016; Langford *et al.*, 2013; Ruiz-Suárez *et al.*, 2014; Sharp *et al.*, 2005). The extent of the flocoumafen contamination we detected is more surprising, representing one of the highest exposure rates documented (Koivisto *et al.*, 2016; López-Perea *et al.*, 2015; Sánchez-Barbudo *et al.*, 2012; see Table B.5). Flocoumafen is only

available through wholesale outlets in Tasmania, suggesting agricultural asset protection and professional pest controllers could be important sources of non-target AR exposure in Australia; aligning with conclusions from recent work on the southern boobook (*Ninox boobook*), an Australian predatory owl species (Lohr, 2018).

The low concentrations and proportion of birds in which FGARs were detected also corresponds with findings for many other species (Cypher *et al.*, 2014; Lohr, 2018; Murray, 2017). This was of particular interest in the case of the FGAR pindone, which was expected to be the AR most likely to cause exposure in wedge-tailed eagles, since it targets a common prey item for the species (European rabbit, *Oryctolagus cuniculus*), and wedge-tailed eagles show a high sensitivity to poisoning from this compound (Martin *et al.*, 1994). Our low rate and detection of FGARs could be due to the shorter half-life and lower toxicity of these compounds and, in the case of pindone, its more localised use in targeted control efforts relative to other ARs in Australia (Lohr, 2018). While this low rate of detection may suggest FGARs pose a lower risk of non-target exposure, their shorter half-life relative to SGARs may also impede their detection in studies using opportunistic sampling and prolonged tissue storage (Herring *et al.*, 2017; Rattner *et al.*, 2014). The use of blood sampling from live-caught individuals would provide a more accurate indication of exposure rates to these compounds.

### *Extent of total AR exposure*

Our use of opportunistically sampled individuals may have limitations when trying to quantify AR exposure in the wider Tasmanian wedge-tailed eagle population. Birds found dead or moribund are potentially predisposed towards AR exposure. For example, bird carcasses are more likely to be found in areas of higher human activity (Ward *et al.*, 2006), where rodenticide

use is more likely. Furthermore, several studies have suggested that sub-lethally exposed individuals may be more vulnerable to accidents (Albert *et al.*, 2010; Newton *et al.*, 1990; Stone *et al.*, 2003), such as collisions with cars and powerlines. Consequently, our sample may overestimate the rate of sub-lethal AR exposure in the population. Equally, opportunistic sampling of carcasses may underestimate the rate of fatal poisonings, due to birds dying in remote areas where they are unlikely to be discovered (e.g. at roost sites; Newton *et al.* 1990). Despite these limitations, AR exposure in predatory species is commonly monitored using these methods (e.g. Christensen *et al.*, 2012; Lohr, 2018; López-Perea *et al.*, 2015). Therefore, comparison to other studies of AR exposure in raptors, that have used equivalent sampling methods, provides context of the issue in the Tasmanian wedge-tailed eagle.

Both the total AR concentrations and the proportion of birds in which ARs were detected were high in the Tasmanian wedge-tailed eagle in comparison to other raptor species. Raptor studies showing comparable AR detection rates predominantly involve smaller species known to be at risk due to their dietary specialisation on rodents (Christensen *et al.*, 2012; López-Perea *et al.*, 2015; Walker *et al.*, 2011). Both the censored mean of all sampled (0.331 mg/kg) and the mean of those with detected burdens (0.448 mg/kg) indicate that Tasmanian wedge-tailed eagles have a substantially higher mean liver AR concentration than most other raptors studied, comparable only to the southern boobook (0.310 mg/kg; Lohr, 2018) and European red kite (*Milvus milvus*) (0.413–0.5 mg/kg; Christensen *et al.*, 2012; López-Perea *et al.*, 2018). Higher proportions of birds showing possibly lethal AR concentrations > 0.2 mg/kg have only been reported in eagle owls (Christensen *et al.*, 2012; López-Perea *et al.*, 2015), barn owls (*Tyto alba*) (López-Perea *et al.*, 2015) and red kites (Christensen *et al.*, 2012) in Europe, but these studies had notably smaller study samples (n = 3–19; see Table B.6).

Our finding of high exposure to ARs in the Tasmanian wedge-tailed eagle, a species not known to regularly prey upon synanthropic rodents, supports the suggestion that apex predators are vulnerable to SGAR residue bioaccumulation (López-Perea *et al.*, 2015; Riley *et al.*, 2007). However, this high level of exposure may also be driven by the improper use of these compounds and non-target AR vectors. The use of SGARs in Australia does not require a license, products can be easily purchased in large quantities and awareness of use guidelines may be low (Tosh *et al.*, 2011). If SGARs are being used improperly (e.g. placed in areas accessible by various species) numerous non-rodent species may consume the poisons and act as AR vectors to predators. Taken together with the potential of SGARs to move through multiple trophic levels, the improper use of ARs could therefore be causing widescale contamination of terrestrial food chains (Thomas *et al.*, 2011). If this is the case, then numerous other predatory species may be at risk in Tasmania, including the Tasmanian devil (*Sarcophilus harrisii*) and eastern quoll (*Dasyurus viverrinus*), which are both listed as endangered (IUCN, 2018).

### *Correlates of AR exposure*

We found little evidence of intrinsic or seasonal drivers of AR exposure in the Tasmanian wedge-tailed eagle. Our results suggest that AR exposure does not increase with age or vary between the sexes, suggesting dietary overlap between these demographic groups or exposure through various AR vectors. Total rodenticide burdens did not vary by season, suggesting seasonal changes in AR use or diet does not affect the risk of exposure. However, the lengthy persistence of ARs in liver tissue will impede the detection of seasonal effects. Analysis of blood would provide more conclusive results, as shown in bobcats (*Lynx rufus*), where seasonal variation was apparent in blood samples but not liver samples (Serieys *et al.*, 2015).

The positive association between hepatic AR concentrations and human population density in the estimated age-specific home range may indicate localised use around residences is having wider scale impacts. Numerous studies have linked AR residues in predators to human population density (López-Perea *et al.*, 2018, 2015) or urbanised area (Cypher *et al.*, 2014; Lohr, 2018; Serieys *et al.*, 2015). These relationships are unsurprising in study species known to use urban habitats. However, Tasmanian wedge-tailed eagles are not associated with such densely populated areas. Sparsely distributed residences have been shown to be a significant driver of non-target AR exposure (Nogeire *et al.*, 2015). Although human population growth has been relatively low in Tasmania for the past two decades, there has been an increase in the number of residences built in more rural and natural areas (Australian Bureau of Statistics, 2018). Such residences may introduce ARs into more natural areas. Furthermore, if ARs are passing through multiple trophic levels, they will spread more widely from the initial bait. The effects of these more remote developments may therefore have incommensurately greater impacts on predatory species than suggested by the landscape footprint.

The widespread use of ARs to protect agricultural assets is likely to explain the positive relationship between the total burden and probability of AR exposure in the eagles and the proportion of agricultural area in the estimated home range. Our study is one of only a few that have made a spatio-statistical link between agriculture and AR tissue residues in predators (Coeurdassier *et al.*, 2019; López-Perea *et al.*, 2018; Sainsbury *et al.*, 2018), and is the first to document this relationship in Australia. To reduce risk of non-target AR exposure in Australia SGARs are restricted to use within 2 m of buildings and no ARs are registered for use in cropping areas, crop perimeters or native vegetation (Australian Pesticides and Veterinary Medicines Authority, 2019). However, as with sparsely distributed residences, the localised use

around agricultural infrastructure may have wider scale impacts. Furthermore, the improper use of these compounds in agricultural areas may be having significant impacts, as the Tasmanian wedge-tailed eagle utilises these habitats (Pay unpublished data).

### *Recent increases in AR exposure*

The higher total AR burdens and probability of AR exposure of the birds that had died more recently could be due to either the increased exposure to ARs over time or the degradation of the compounds with prolonged storage. Although SGAR residues are stable within tissues over the short-term (24–72 hours; Gallocchio *et al.*, 2014; Jin and Chen, 2006), the effects of long-term -20°C freezer storage on tissue residues is less known, with studies documenting various rates of degradation (e.g. 6–41% over 0.5–3 years; P. Fisher unpublished data; Vindenes *et al.*, 2008). Despite this, patterns in the AR concentrations we detected are consistent with increased exposure over time. For residue degradation to cause the time-based increase in probability of AR exposure we detected, AR levels would need to decrease to below the LOD. The lowest AR concentration we recorded was 424% greater than the associated LOD. There would therefore need to be a substantial reduction in AR residues for an AR-exposed bird to be considered unexposed. Consequently, the increased AR concentrations in Tasmanian wedge-tailed eagles that had died more recently may be due to increases or changes in AR use in Tasmania throughout the study period. However, there is no information available on the volume of ARs used in Australia (Lohr and Davis, 2018), which impedes our quantification of the relationship between AR application and non-target AR exposure.

### *Conservation implications*

Our results suggest widespread exposure of wedge-tailed eagles to ARs, at levels that are lethal or that impair function. Although we could not investigate evidence of internal haemorrhaging, 46% of eagles tested had possibly lethal hepatic concentrations ( $> 0.1$  mg/kg; Lohr, 2018). Furthermore, 64% had levels that likely caused symptoms of toxicity ( $> 0.01$  mg/kg; Lohr, 2018), which may have caused reduced coordination and increased the risk of other causes of mortality (Murray, 2018). Collisions with anthropogenic structures and vehicles have been identified as threats to the Tasmanian wedge-tailed eagle (Bell and Mooney, 1998; Threatened Species Section, 2006) and there has been an increase in reported power line collisions and electrocutions over the last decade (TasNetworks, 2018). This increase in unnatural mortality can have significant impacts, as the loss of even small numbers of breeding adults and, to a lesser extent, dispersing juveniles can have big impacts on raptor population declines (Bekessy *et al.*, 2009).

The distribution of anthropogenic habitats within Tasmania may also result in a substantial proportion of the population being at risk of AR exposure. Agricultural land use and the human population is generally spread across the north, east and southeast of the island. The remote southwest is primarily a world heritage area, and is free of anthropogenic habitats associated with AR use (DPIPWE, 2015); however, wedge-tailed eagles are understood to occur at much lower densities in these regions due to dense forest subcanopies limiting prey availability (Threatened Species Section, 2006). With the spread of residences and agriculture throughout areas of higher wedge-tailed eagle population density, the large home ranges of the species, particularly in dispersing birds, are likely to encompass numerous sources of AR exposure.

SGARs are currently registered for domestic (non-commercial) use in Australia (Australian Pesticides and Veterinary Medicines Authority, 2019), despite increasing regulation and monitoring in other countries (USEPA, 2008). Increased legislative control of SGARs and removal from public retail may therefore be important steps to reducing the ecological impacts of SGAR use in Australia (Lohr and Davis, 2018). However, our findings of an association between agriculture and AR burdens in the Tasmanian wedge-tailed eagle, as well as widespread contamination of an AR not readily available for residential use (flocoumafen), suggests that commercial pest control may be an important cause of non-target AR exposure. Consequently, addressing mechanisms of spread from both commercial and non-commercial application of SGARs will be important to reducing AR burden of Tasmanian wedge-tailed eagle and other Australian wildlife.

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## Chapter 4

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Considering behavioural state when assessing habitat selection: a case study with the Tasmanian wedge-tailed eagle (*Aquila audax fleayi*)



Image D. James

## Abstract

Effective planning of species conservation management requires an understanding of habitat utilization. Animals may select different resources within the landscape depending on the behaviour they are undertaking. Incorporating behaviour into habitat selection analyses can therefore be important in guiding the management of threatened species. In this study, we use behaviour-specific habitat selection analyses to investigate the ecology of the endangered Tasmanian wedge-tailed eagle (*Aquila audax fleayi*) during the post-fledging dependence period. Using hidden Markov modelling, we characterised GPS tracking data from 24 eagles into three behavioural states; perching, short flights and long flights. We then used habitat selection ratios and habitat selection models to both explore how the eagles used resources and to predict where different behaviours are likely to occur in Tasmania. Eagles selected for areas less than 75 m from forest edges and with topographic slopes more than 15° during all behaviours, whereas open habitats were avoided. Long flights occurred more frequently over forests compared to other behaviours. Aspect and ridgelines did not appear to influence space use. Habitat selection models using distance to forest edge and slope performed well in predicting where eagles would perform short flights and long flights, emphasising the important role of these habitats in governing space use by the eagles. Our study provides the first detailed understanding of habitat use by the Tasmanian wedge-tailed eagle, which will be useful in the ongoing conservation management of the population. Notably, the habitat selection models for both flying behaviours provide guidance for efforts to reduce human-caused mortality. This study demonstrates the value of behaviour-specific habitat selection analyses for the conservation management of threatened species.

## Introduction

It is critical to understand what threatened species need from their environment for the planning of effective *in situ* conservation management. Resource selection analyses both describe and predict how animals select for different habitats, and have thus become important tools in balancing conservation with expanding human footprints (Johnson *et al.*, 2006; Manly *et al.*, 2002; Thurfjell *et al.*, 2014). These methods facilitate understanding of the extent of a species' distribution, the importance of different habitat resources, and prediction of where animals are likely to occur (Johnson *et al.*, 2004). The spatial delineation of resource selection can then be used to assess impacts of changes in land use (Sawyer *et al.*, 2006), guide the design and management of ecological reserves (Leroux *et al.*, 2007), target reintroduction sites (Klar *et al.*, 2008), and help identify and mitigate areas of human conflict, such as disturbance (Seip *et al.*, 2007) and sources of anthropogenically caused mortality (Meisingset *et al.*, 2014; Miller *et al.*, 2014; Reid *et al.*, 2015).

Habitat selection models do not traditionally take behaviour into account, which renders them susceptible to biased inferences of the spatial ecology of a species (Roever *et al.*, 2014). The way an animal selects for habitats in the landscape may vary substantially due to the distinct resource requirements of different behaviours. The resources required for foraging, resting and reproduction are likely to be disparate and potentially opposing. Habitat selection models that lack a behavioural component may, for example, fail to pick up on habitats that are highly important to infrequent behaviours; while opposing habitat requirements for different behaviours can effectively cancel each other out (Abrahms *et al.*, 2016; Bouyer *et al.*, 2015; Roever *et al.*, 2014). However, our ability to connect habitat selection to natural behaviour has been limited by

practical constraints in observing behaviour over long periods of time and without observer presence affecting behaviour (Iredale *et al.*, 2010; Mahoney and Young, 2017).

The increasing temporal resolution of modern GPS-tracking technologies and advances in statistical techniques are facilitating the detailed understanding of animal behaviour alongside habitat use. Frequent GPS relocations can identify explicit movement patterns, from which statistical techniques can be used to infer different behavioural states (Morales and Ellner, 2002; Patterson *et al.*, 2009). Furthermore, modern tracking technologies can collect additional data (e.g. altitude, depth, acceleration) that can be used to more accurately estimate behaviour (Duerr *et al.*, 2012; Mahoney and Young, 2017). Although care should be taken to not over-interpret the biological meaning (Patterson *et al.*, 2009), these movement states can be used in conjunction with resource selection analyses to provide a detailed ecological understanding of behaviours with increased conservation importance. For example, through behavioural space use modelling, Patterson *et al.*, (2016) clarified how mammals and birds use marine reserves for foraging, and Miller *et al.*, (2014) were able to identify where eagle flights are likely to coincide with a high risk of wind turbine collisions. Incorporating behaviour into resource selection analyses can therefore be particularly useful in guiding the management of threatened species.

The Tasmanian wedge-tailed eagle (*Aquila audax fleayi*) is an endangered subspecies endemic to the island of Tasmania (Commonwealth of Australia, 1999). With the loss of the thylacine (*Thylacinus cynocephalus*) and recent declines in populations of Tasmanian devils (*Sarcophilus harrisii*), the wedge-tailed eagle serves a particularly important ecological function as one of the few remaining top predators in Tasmanian ecosystems. However, the population is subject to a series of threats. Habitat loss is recognised as an issue due to the loss and fragmentation of native old-growth forests, which the eagles rely on for nesting (Mooney and

Holdsworth, 1991). Yet, there is a paucity of information on the habitats used away from nesting sites and during different life stages. The population also suffers from high rates of human-caused mortality, particularly power line and wind turbine collisions (Threatened Species Section, 2006). These impacts are expected to worsen with an increased focus on utilising wind energy in Tasmania and a growing need for electrical infrastructure to service expanding developments (Bell and Mooney, 1998).

In this study, we use behaviour-specific habitat selection to investigate the ecology of juvenile Tasmanian wedge-tailed eagles during the post-fledging dependence period (PFDP), which is thought to be a particularly vulnerable life stage. While no quantitative study on mortality rates for any life stage has been carried out for the species (Olsen, 2005), an estimate of 50% natural mortality in juveniles was derived from expert opinion (Bell and Mooney, 1998). Moreover, lack of experience makes young birds more vulnerable to unnatural mortality, with 86% of Tasmanian wedge-tailed eagles that are reported as electrocuted classified as immature (Tasmanian Museum and Art Gallery unpublished data). Our study had three overarching objectives; to: (1) describe the behaviour of juvenile wedge-tailed eagles during the PFDP; (2) investigate how these eagles use habitats for their different behaviours; and (3) explore if we could predict in which habitat types the different behaviours are likely to occur across Tasmania.

## Methods

### *Study area*

The study was conducted in Tasmania, an island state of Australia located 240 km south of mainland Australia. The island covers an area of 68,150 km<sup>2</sup> between latitudes -40°S and -43°S and longitudes 144°W and 149°W. Climatic conditions vary considerably across the island; coastal and low altitude regions have mean monthly temperatures ranging between 8°C and 25°C and experience 568.7 mm annual precipitation, whilst the central highlands has mean monthly temperatures ranging between -2°C and 18.6°C and experiences over 1000 mm of rainfall per year (Bureau of Meteorology, 2019). Wetter regions are characterised by wet sclerophyll eucalypt-dominated forests, temperate rainforests and button grass moorland; whereas dry eucalypt forest and native grasslands dominate in drier areas (DPIPWE, 2013). Areas of minimal land use, nature conservation and other protected areas account for 50.3% (34,280 km<sup>2</sup>) of the Tasmanian land area (DPIPWE, 2015). Anthropogenic landscapes, predominantly comprising grazing and modified pastures, occupy 27.7% (18,900 km<sup>2</sup>; DPIPWE, 2015), focused in the north and east of the state.

### *Eagle telemetry*

We fitted 24 Tasmanian wedge-tailed eagle nestlings from 21 different natal territories with GPS transmitters during the 2016–2017 breeding season ( $n = 8$ ) and the 2017–2018 breeding season ( $n = 16$ ). Tasmanian wedge-tailed eagles generally produce one nestling (Olsen, 2005), and at all nests used in this study only one nestling was present. At each of three study nests, a nestling was fitted with a GPS transmitter in both breeding seasons (Figure 4.1). We

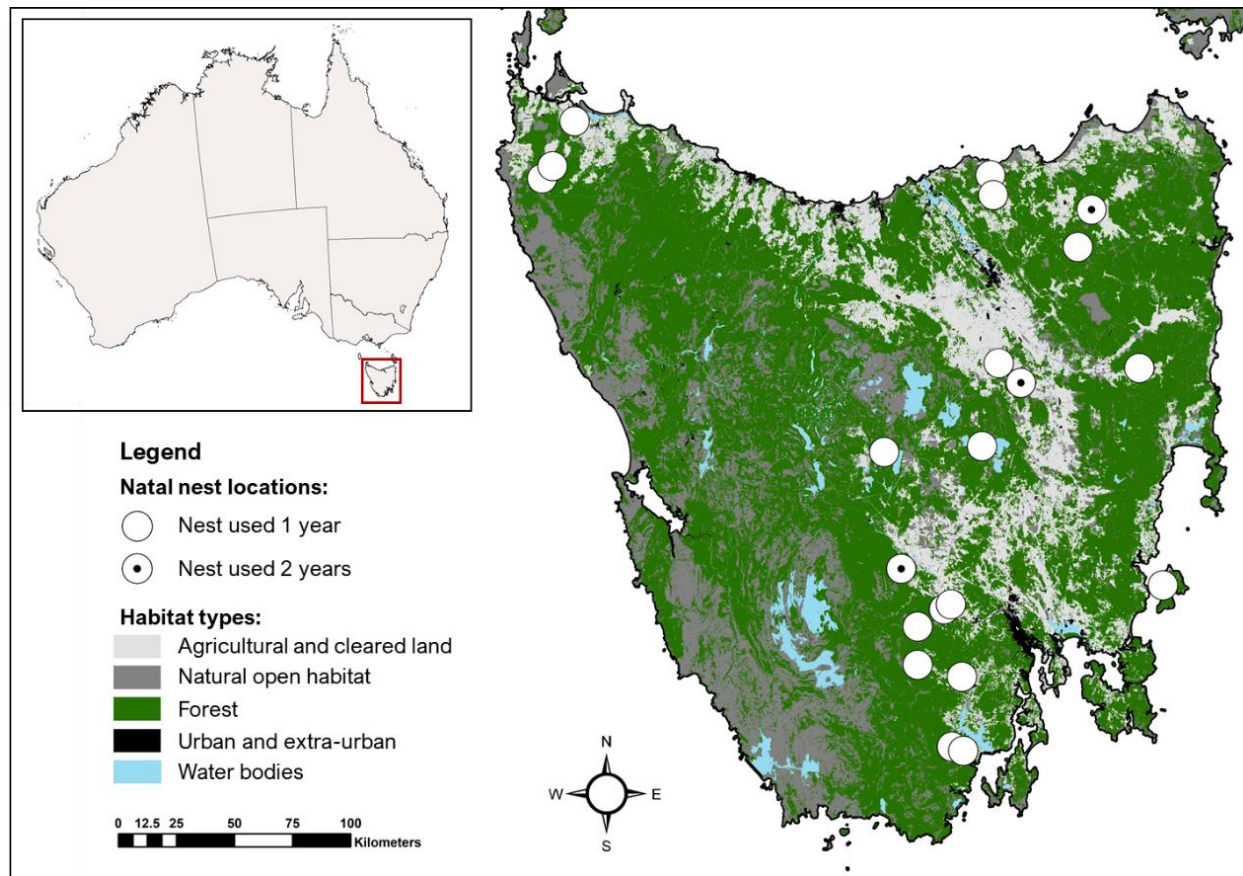


Figure 4.1. Study location in Tasmania (Australia) with the spatial distribution of study nest sites. Broad habitat categories are indicated by the shade of the land area.

selected target nests based on the presence of nestlings recorded during fixed-wing aerial nest surveys (see methods FPA, 2014). The age of nestlings, assessed on plumage development on the basis of expert opinion, was recorded during the surveys to guide timing of our visit to the nest sites. Individuals were tagged while still in the nest, at an estimated 65–75 days old (fledging age is ~77–90 days for the species; Olsen, 2005; Bell and Mooney, 1998). We collected standard morphological measurements from each nestling and determined sex using DNA extracted from a blood sample (see Appendix D.3). We fitted GPS-GSM solar-powered telemetry units (CTT-1000-BT3 Series; Cellular Tracking Technologies, Rio Grande, NJ, USA)

to each nestling using a 112 mm Teflon ribbon harness (Bally Ribbon Mills, Bally, PA). The transmitter weights (65 g) were always below the 3% of maximum body mass recommendation (Phillips *et al.*, 2003), as nestlings weighed 3.3–4.4 kg at the time of tagging. The telemetry units recorded GPS data every 15 minutes from one hour before sunrise to one hour after sunset. The data for each fix included the location in decimal degrees (circular error probability of  $< 2.5$  m; CTT, 2019), altitude ( $\pm 15$  m; CTT, 2019), speed and course over ground. The horizontal dilution of precision (HDOP) was also recorded for each fix, which provides a measure of the horizontal error in the GPS location based on the number and position of satellites used to obtain the GPS location. Data were sent over the GSM network once per day. If a connection to the GSM network was not possible due to inadequate signal, data were archived on the telemetry unit until the bird returned to coverage.

We filtered GPS data to improve the spatial accuracy of datapoints used in the analysis. All data with a HDOP  $> 4$  (9.7% of raw data) were filtered from the final data set. To estimate the total length of the PFDP for each eagle we needed to determine the fledging date (start of PFDP) and the initiation of natal dispersal (end of PFDP). Fledging was considered to have occurred when the GPS fixes were  $> 10$  m from the nest tree for four consecutive fixes, while the onset of natal dispersal was defined using ‘Method 7’ from Weston *et al.* (2013), using thresholds of estimated territory size for the subspecies (first day beyond 7 km from nest and not within 5 km for the following 10 days; Bell and Mooney, 1998). Five birds from the 2017–2018 season were still in the PFDP at the time of analysis, and thus were not included in the estimation of PFDP duration. For habitat selection analyses the first 50 days after fledging were excluded, as during this period the juvenile eagles were still associated with their natal nest and the home ranges were increasing over time (Pay unpublished data), making it difficult to distinguish

habitat selection in the wider natal territory. Two of the tracked eagles died during this 50-day period and were therefore not included in the habitat selection analyses. There were no other mortalities recorded for the GPS tracked eagles during the PFDP. One mortality occurred during the first year but after the PFDP had concluded for the individual.

### *Data analysis*

#### *Behavioural states*

Hidden Markov models (HMMs) applied to the distances and turning angles between consecutive GPS fixes have been used to discern behavioural states in various species (Patterson *et al.*, 2009). We used both two-state and three-state HMMs to investigate whether the juvenile wedge-tailed eagle data were best classified by two (i.e. ‘perching’ and ‘flying’) or three (i.e. ‘perching’, ‘short flights’ and ‘long flights’) behavioural states (R package ‘moveHMM’; Michelot *et al.*, 2016). We did not include turning angle in the models. This was because the highly mobile nature of the species and the confinement to the natal territory meant the 15-minute interval between GPS fixes was not able to capture differences in turning angles between behavioural states. We performed 60 model iterations to establish the optimal step length parameters to delineate the behavioural states. Each model was checked for convergence, and we identified the best performing model using AIC.

#### *Habitats used during different behaviours*

We used habitat selection ratios (Manly *et al.*, 2002) to compare the proportion of available habitats with the time spent in each habitat performing each behaviour. Our study was a Type III design, for cases where available habitat differs between individuals (Thomas and

Taylor, 1990). To estimate habitat availability for each eagle we used a 95% minimum convex polygon (MCP). MCP was chosen over other estimators of home range (e.g. local convex hulls), as we needed to measure potentially available habitat, rather than the actual utilisation distribution. A 100% MCP was not suitable as immature eagles perform exploratory flights away from the natal territory during the PFDP (Weston *et al.*, 2013); the impact of a single exploratory flight on the 100% MCP would result in erroneously large estimations of available habitat covering multiple wedge-tailed eagle territories. We therefore buffered each 95% MCP by 5% to estimate available habitat area and GPS fixes outside of the estimated available habitat area (i.e. exploratory flights) were excluded from the analysis (removing 2.4% of data).

We decided which habitat variables to investigate based on the ecology of the study species and published work on congeners. Various topographic variables have been identified as important in *Aquila* species (Balbontín, 2005; Braham *et al.*, 2015; Fielding *et al.*, 2019; LeBeau *et al.*, 2015; Tikkanen *et al.*, 2018); we therefore included slope, aspect, terrain ruggedness and distance to nearest ridgeline calculated from a 25 m<sup>2</sup> digital elevation model (DPIPWE, 2010). Additionally, we included distance to forest edge and general categorical land cover classifications (extracted from TasVeg 3.0; DPIPWE, 2013), as forest edges and land cover types are also significant for various predatory species, including raptors (Balbontín, 2005; Horikoshi *et al.*, 2017; Sandgren *et al.*, 2014). Since selection ratio analyses assume discrete habitat variables, we had to categorise continuous habitat variables into equal-ranged ordinal bins. We chose the number of data bins used for each variable based on the range of values and the available habitat area associated with each bin. This was important as the selection ratio can result in inaccurate spikes in selection values when the available habitat area within a bin is very

small (Basson *et al.*, 2012). The derivation of environmental variables and the corresponding number of data bins are detailed in Table C.1 and Table C.2.

We calculated habitat selection ratios following methods described by Manly *et al.* (2002) for each behavioural state (function ‘widesIII’ in ‘AdehabitatHS’; Calenge, 2006). The resulting selection ratio ( $w_i$ ) values identified which habitats bins were being selected for;  $w_i < 1$  indicate a habitat used proportionally less than its availability (i.e.  $w_i$  of 0.5 indicates a habitat type used half as often as expected),  $w_i > 1$  indicate a habitat used proportionally more than its availability (i.e. a  $w_i$  of 2 indicates a habitat type used twice as often as expected),  $w_i \approx 1$  indicates a habitat was used proportionally to its availability.

#### *Predicting where different behaviours are likely to occur*

We used a multivariate habitat selection value, both to understand how the eagles used combinations of habitat variables for different behaviours and to explore if this can effectively predict where eagle behaviours are likely to occur. The method involves quantifying the joint distributions of habitat variables across the available area, and comparing this to the joint distribution of these habitat variables used by the animals (Basson *et al.*, 2012; Eveson *et al.*, 2015; Patterson *et al.*, 2016). This technique was chosen over a generalised linear model (GLM) based resource selection analysis (e.g. Fortin *et al.*, 2005) because there is contention over the placement of pseudo-absences often required for a GLM approach (e.g. VanDerWal *et al.*, 2009). Furthermore, GPS fixes collected at very short intervals are vital for reducing bias in regression coefficients for highly vagile species (Zeller *et al.*, 2016). This issue is very relevant to our study as, even with relatively frequent fixes (15 minutes), the maximum distances that the eagles travelled between fixes were almost as far as the width of their natal territory (Table C.3).

We included habitat variables in the multivariate model that were identified as significant from the univariate analysis described above. Only ordinal variables can be included in the calculation, so categorical habitat variables were excluded. If habitat variables were correlated in their spatial distribution (Pearson's  $r > 0.8$ ; 'layerStats' function in R package 'raster'; Hijmans *et al.*, 2019), only the variable with the stronger univariate selection was retained. We created a raster stack of the habitat covariates and calculated the available (buffered 95% MCP) and used (extracted habitat values for each GPS fix) proportion of the combination of covariates for each eagle. We then used the frequency distributions of the habitat covariates in the available areas and the habitat covariates used by the eagles to calculate a selection value (SV; equation 5 in Patterson *et al.*, 2016). The resulting values are theoretically similar to the  $w_i$  habitat selection ratio (i.e.  $SV > 1$  indicating selection,  $SV < 1$  indicating avoidance; Manly *et al.*, 2002).

The SV scores can be used to construct a habitat selection map by taking the values for all relevant habitat variables at any given location and assigning that location the associated SV. Assessing the predictive ability of such maps is important to validate their use in conservation. To evaluate the predictive performance of the SV model, we used methods recommended by Johnson *et al.* (2006) modified for a leave-one-out cross-validation (LOOCV) framework. LOOCV was preferred over temporal or random  $k$ -fold partitioning as our aim was to assess the predictive ability of the model for areas and individuals across Tasmania. We carried out the SV estimation 22 times, with all GPS fixes for one individual left out for the modelling and used as the test data for the model validation. For each run of the LOOCV we calculated the SV for each 25 m<sup>2</sup> grid cell of available habitat for the test eagle. The SVs were then reclassified into SV bins. The number of SV bins was decided upon equal intervals between the minimum and maximum SVs, ensuring validation points and reliable amounts of available land area in each bin

(Boyce *et al.*, 2002). We then calculated the proportion of GPS fixes that were expected to fall within each SV bin based on the model (equation 4 and 5 in Johnson *et al.*, 2006) and compared this to the proportion of test data GPS fixes that fell within the corresponding bin. Model performance was assessed both for the overall averaged model and for each LOOCV eagle by comparing the expected and observed values using linear regression and  $\chi^2$  goodness-of-fit tests. A model that is predictive of habitat use would have a linear regression intercept approaching 0, a slope approaching 1 and different from 0, a high  $R^2$  value ( $>0.8$ ) and a non-significant  $\chi^2$  goodness-of-fit (Johnson *et al.*, 2006). To explore spatial variability in model performance we grouped LOOCV eagles grouped into six regions (Figure C.1) and calculated the regional mean expected vs. observed regression  $R^2$ . Finally, we used separate  $\chi^2$  tests to assess the individual performance of each SV bin, where  $\chi^2$  residuals  $\leq -2$  or  $\geq 2$  (Agresti, 2002) identified eagles that used an SV bin more or less than expected.

## Results

Our data consisted of 231,478 GPS positions from 22 juvenile birds, with the mean number of fixes per eagle of 10,522 (range 5,299–22,373). The median 15-minute step length was 51.8 m (11.5–95.8 m), with the maximum step length by an individual ranging from 2,456–7,078 m (Table C.3). The mean daily distance travelled by the juvenile birds was 12.7 km (range 3.6–27.7 km). The mean duration of the PFDP was 299.2 days (range 147–575; Table C.3). There was little variation in the duration of the PFDP between the two breeding seasons studied (2016–2017 season mean = 309 days; 2017–2018 mean = 294.6 days).

The mean available habitat area (buffered 95% MCP) was 34.4 km<sup>2</sup> (range 10.4–94.5 km<sup>2</sup>; Table C.3). The minimum distance between the natal nest location and the border of the corresponding available habitat area ranged 0.2–3.9 km, whereas the maximum distance ranged 3.3–6.8 km (see Figure C.2).

### *Behavioural states*

HMMs assigning three behavioural states outperformed all two-state models. The best performing three-state model used the following step length parameters to estimate the three behaviours; a ‘perching’ state characterised by short step lengths within the error of the GPS unit (mean step length 10 ±SD 8 m), a ‘short flight’ state characterised by medium step lengths (mean step length 166 ±SD 96 m), and a ‘long flight’ state characterised by long step lengths (795 ±SD 414 m; Table C.4 and Figure C.3).

In total the eagles spent 41.8% of their time in the perching behavioural state, 39.3% of their time in short flights and 18.9% of their time in long flights (Table C.4). During the early ( $\leq$

2 hours after sunrise) and late hours ( $\leq 2$  hours before sunset), birds spent the highest proportion of their time perching (61.1%). During the middle of the day (11am–2pm) long flights were the commonest behaviour (34.2%; Figure 4.2). Perching and short flights occurred at low altitudes (perching = mean  $20.6 \pm \text{SD } 5.3$ ; short flights = mean  $22.8 \pm \text{SD } 5.9$ ), whilst long flights generally occurred at higher altitudes (mean  $63.5 \pm \text{SD } 23.6$  m; Table C.4).

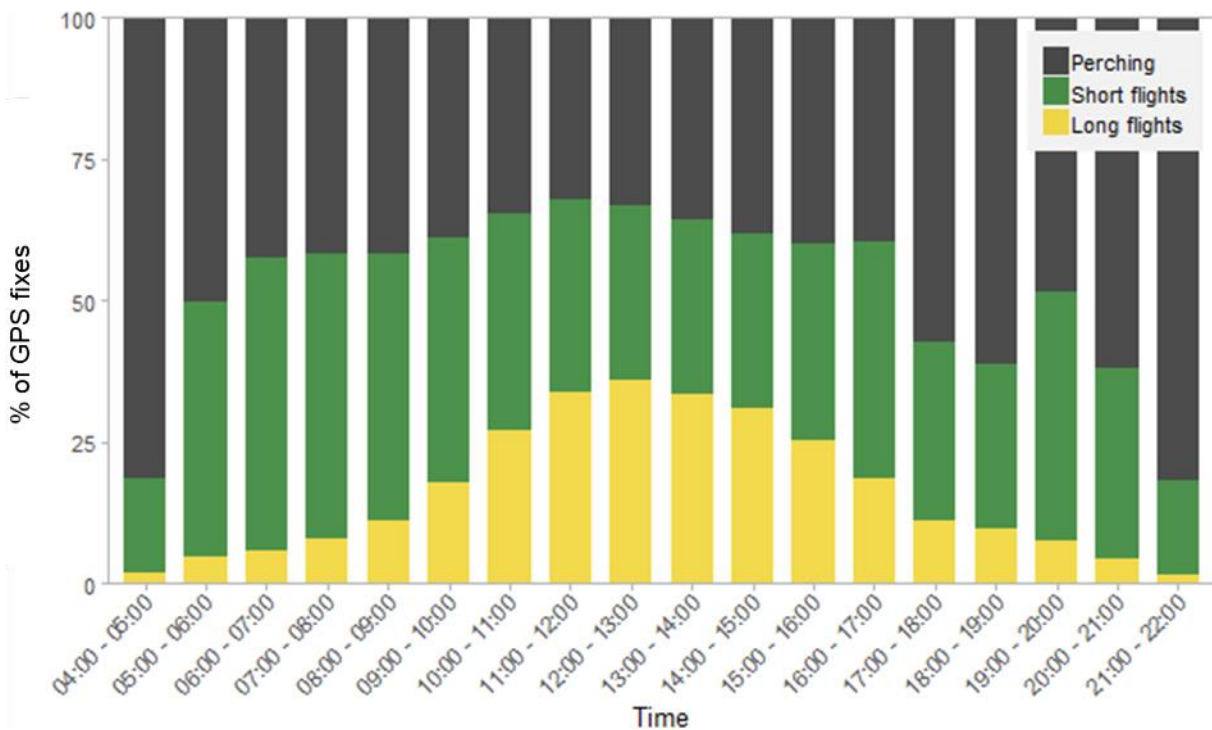


Figure 4.2. Proportion of time spent in each behavioural state at different hours of the day for 22 juvenile Tasmanian wedge-tailed eagles (range 97–525 days). Perching was the commonest behaviour early and late in the day, whereas the eagles spent proportionally most of their time flying short or long distances during the middle of the day.

### *Habitats used during different behaviours*

There was evidence of selection for habitats based on the distance to forest edge during perching ( $\chi^2 = 998.66$ ,  $df = 120$ ,  $p < 0.001$ ), short flights ( $\chi^2 = 948.49$ ,  $df = 120$ ,  $p < 0.001$ ) and long flights ( $\chi^2 = 511.7$ ,  $df = 120$ ,  $p < 0.001$ ). In all three behavioural states eagles used areas < 75 m from the forest edge more than the relative availability in the landscape ( $wi = 1\text{--}3.8$ ), and every bird selected for habitats within 25 m of the forest edge ( $wi = 2.1\text{--}3.8$ ). Long flights generally occurred over areas within the forest proportional to their availability ( $wi \approx 1$ ), whilst during perching and short flights the eagles selected against this habitat ( $wi = 0.8\text{--}0.4$ ; Figure 4.3a).

Selection for habitats based on the slope of the land was also apparent in all three behavioural states (Perching:  $\chi^2 = 258.54$ ,  $df = 99$ ,  $p < 0.001$ ; Short flights:  $\chi^2 = 222.04$ ,  $df = 99$ ,  $p < 0.001$ ; Long flights:  $\chi^2 = 327.05$ ,  $df = 99$ ,  $p < 0.001$ ). Land with steeper slopes ( $>15^\circ$ ) was used more than expected ( $wi > 1$ ) in all three behavioural states, however eagles selected more strongly for these areas when performing long flights ( $wi = 1.7\text{--}2.4$ ). Land with a slope  $< 15^\circ$  was either underused or used in proportion to its availability regardless of the behaviour being performed (Figure 4.3b).

There was no evidence of an overall selection for habitat based on distance to ridgeline in any of the behavioural states (Perching:  $\chi^2 = 210.45$ ,  $df = 219$ ,  $p = 0.590$ ; Short flights:  $\chi^2 = 168.52$ ,  $df = 219$ ,  $p = 0.995$ ; Long flights:  $\chi^2 = 205.27$ ,  $df = 219$ ,  $p = 0.671$ ). There was considerable variability in how eagles used habitats in relation to ridgeline (Figure 4.3c). However, the eagles used areas within 100 m of the nearest ridgeline more than expected during long flights ( $wi = 1.3\text{--}1.4$ ).

The juvenile eagles showed no habitat selection based on aspect (Perching:  $\chi^2 = 238.18$ ,  $df = 153$ ,  $p = 0.180$ ; Short flights:  $\chi^2 = 336.31$ ,  $df = 153$ ,  $p = 0.307$ ; Long flights:  $\chi^2 = 304.84$ ,  $df = 153$ ,  $p = 0.783$ ). All aspect categories were used proportionally to their availability in all three behavioural states ( $wi \approx 1$ ; Figure 4.4a).

The eagles showed overall selection for habitats based on the ruggedness of the terrain when perching ( $\chi^2 = 244.42$ ,  $df = 146$ ,  $p < 0.001$ ), performing short flights ( $\chi^2 = 206.29$ ,  $df = 146$ ,  $p < 0.001$ ) and long flights ( $\chi^2 = 349.21$ ,  $df = 146$ ,  $p < 0.001$ ). The more rugged the terrain, the more the birds utilised a habitat type in all three behavioural states, with a slightly stronger selection for more rugged terrain during long flights (Figure 4.4b).

Exploring habitat utilisation based on the habitat type categories showed evidence of selection in all three behavioural states (Perching:  $\chi^2 = 1050.53$ ,  $df = 172$ ,  $p < 0.001$ ; Short flights:  $\chi^2 = 989.73$ ,  $df = 172$ ,  $p < 0.001$ ; Long flights:  $\chi^2 = 562.39$ ,  $df = 172$ ,  $p < 0.001$ ). Forest edges were used proportionally more than their availability in all behavioural states ( $wi = 1.4$ – $3.1$ ), except the edges of plantation forests that were used in proportion to their availability ( $wi = 0.9$ – $1.4$ ). During long flights, the eagles used dry eucalypt forest and wet eucalypt forest in proportion to their availability ( $wi = 1.1$ ), whereas when perching, or during short flights, the eagles selected against these areas ( $wi = 0.7$ – $0.8$ ; Figure 4.4c). Open habitats (native grassland, cleared land and agriculture) rainforests, plantation forests and non-eucalypt forests were also used proportionally less than their availability in all behavioural states ( $wi = 0.2$ – $0.7$ ).

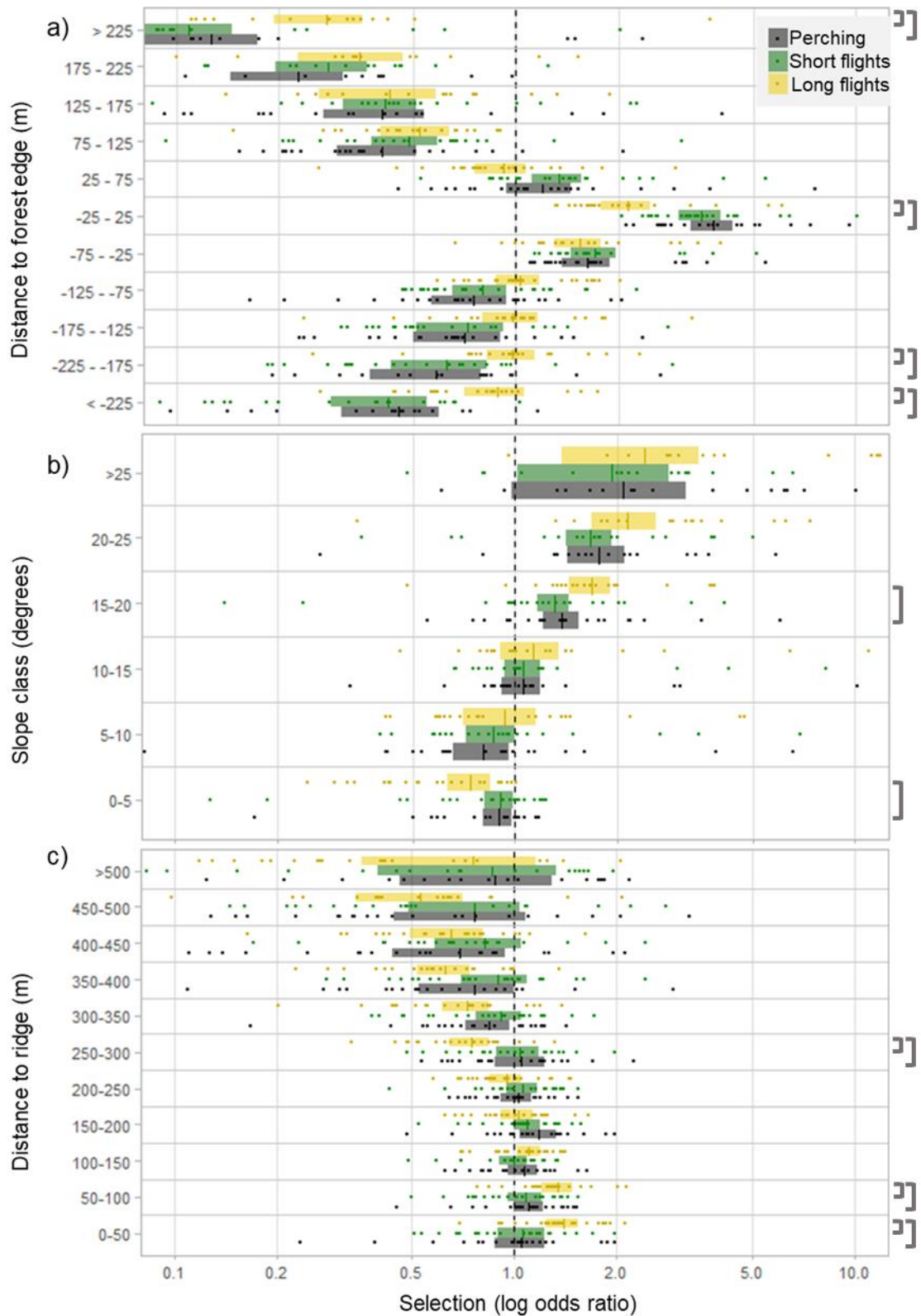


Figure 4.3. Plots showing the habitat selection ratios for different ordinal categories of distance to forest edge (a), slope (b) and distance to ridgeline (c). The selection ratio ( $w_i$ ) for each behaviour is indicated with 95% CI shown by shaded bars.  $w_i$  values above one (dashed line) indicate proportionally more time was spent in this habitat relative to its availability, while  $w_i$  values below one indicate that less time was spent there relative to its availability.  $w_i$  values for each individual eagle are plotted as points. Brackets on the right of the plot indicate where habitat selection differed significantly between behavioural states ( $p < 0.05$ ). In plot a) negative distances to forest edge values represent areas within the forest, whereas positive values relate to areas outside the forest (e.g. open habitats).

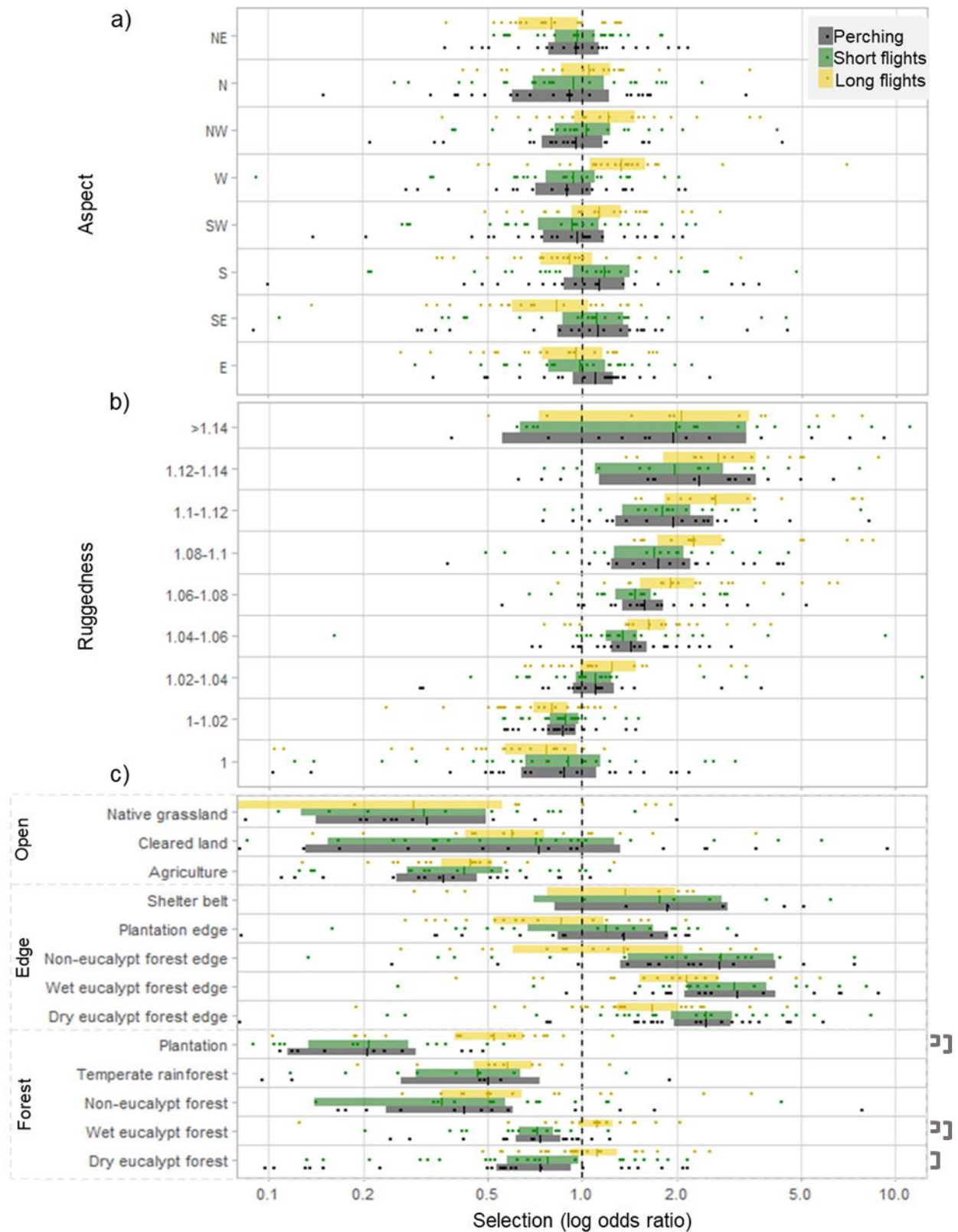


Figure 4.4. Plots showing the habitat selection ratios for different categories of aspect (a), ruggedness (b) and land cover type (c). The selection ratio ( $w_i$ ) for each behaviour is indicated with 95% CI shown by shaded bars.  $w_i$  values above one (dashed line) indicate that proportionally more time was spent in this habitat relative to its availability, while  $w_i$  values below one indicate that less time was spent there relative to its availability.  $w_i$  values for each individual eagle are plotted as points. Brackets on the right of the plot indicate where habitat selection differed significantly between behavioural states ( $p < 0.05$ ).

*Predicting where different behaviours are likely to occur*

On the basis of their significance in the univariate habitat selection ratio analyses, distance to forest edge, slope and ruggedness qualified for inclusion in the predictive models. Ruggedness and slope were correlated (Pearson's  $r = 0.91$ ); the eagles showed slightly weaker overall selection for ruggedness in the univariate selection analysis, and so this parameter was excluded from the final model. The models assigned SVs to each combination of distance to forest edge and slope. SVs for the perching state ranged from 0–7.24 across all the combinations of slope and distance to forest edge in the analysis, whilst SVs for short flights were in the range 0–6.12, and long flights were 0–6.72.

The juvenile eagles tended to spend more time in steep habitats near the forest edge, with some variation between long flights and other behaviours. Specifically, they were more likely to perch and take short flights in areas close to the forest edge and with steeper slopes ( $SV > 3$ ). Areas  $< 25$  m from the forest edge and with slopes  $> 25^\circ$  were the most utilised habitats during these behaviours ( $SV = 6.12$ ). Steep habitats ( $> 25^\circ$ ) further within the forest were also selected for when perching or taking short flights, whereas steep habitats  $> 75$  m outside the forest were used proportionally less than their availability in the landscape (Figure 4.5). During long flights, the eagles similarly selected for steep areas close to the forest edge, although they also selected for a broader range of areas within the forest (Figure 4.5).

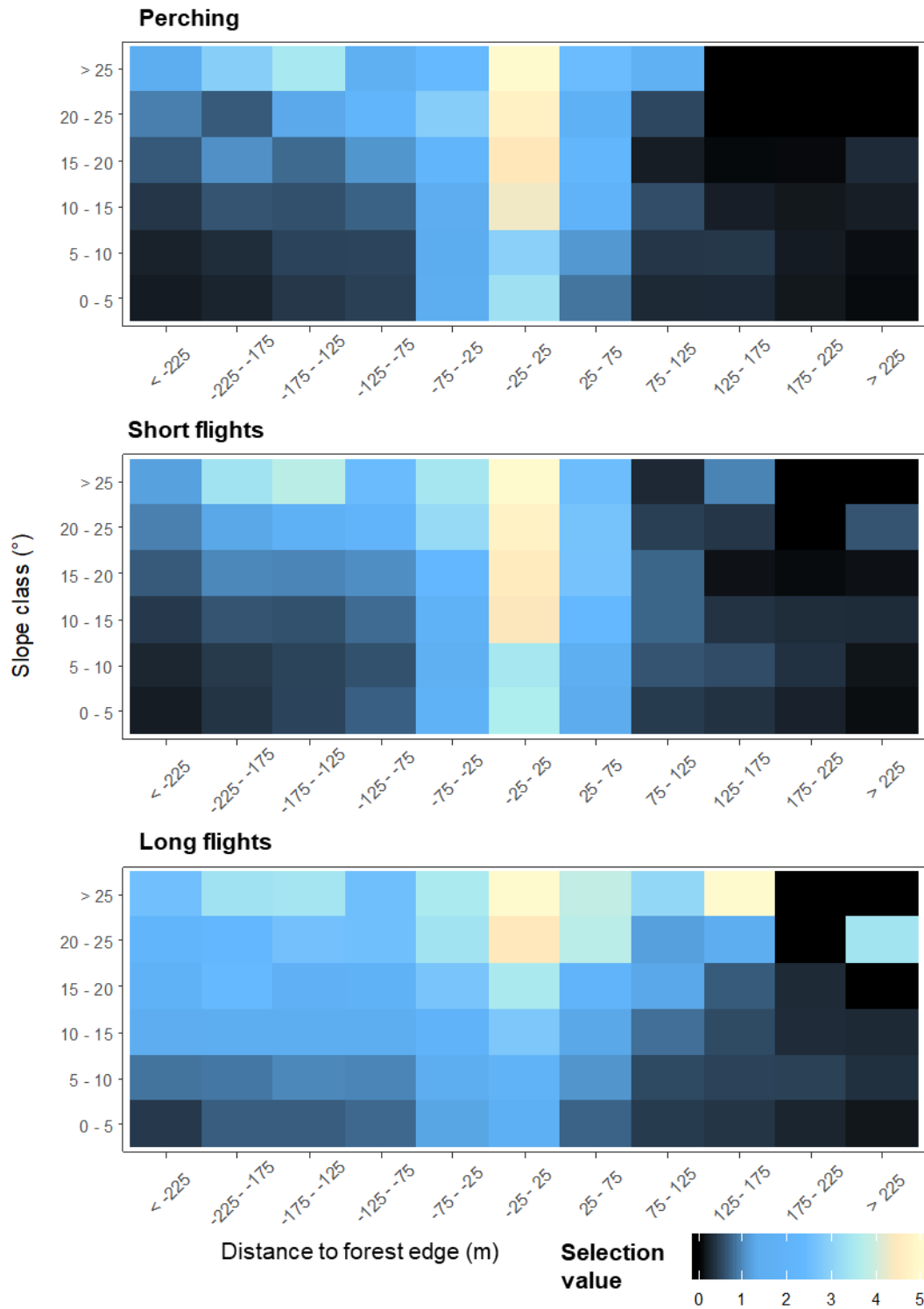


Figure 4.5. Bivariate habitat selection surfaces for juvenile Tasmanian wedge-tailed eagles in terms of the slope of the terrain and the distance to nearest forest edge (negative forest edge values are inside the forest; positive values are outside the forest). Each surface represents a different behavioural state. The colour scale specifies the selection values (SVs) for the corresponding combinations of habitat types. Selection values < 1 indicate habitats used proportionally less than their availability in the landscape.

### *Model performance – Perching*

The perching model indicated strong selection for specific habitats, although its predictive power appears to be limited. The model showed that habitats utilised proportionally less than availability ( $SV < 1$ ) for this behaviour covered 64.0% of the available land area, with 20.8% of perching GPS records in these habitats. By contrast, 11.2% of perching data occurred in areas assigned SVs  $> 4$ , yet these habitats covered only 2.0% of the available land area (Table 4.1). In terms of the used vs. expected regression, the overall averaged predictive performance of the perching model appeared reasonable ( $b_0 = -0.011$ ,  $b_1 = 1.067$ ,  $R^2 = 0.903$ ; Figure 4.6). However, the goodness-of-fit test indicated poor model performance ( $\chi^2$ :  $p = 0.049$ ). Furthermore, when applied to the individual LOOCV eagles, the model only performed well at predicting where perching behaviours were likely to occur in four birds (regression:  $b_0 \approx 0$ ,  $b_1 \approx 1$ ,  $R^2 > 0.8$ ;  $\chi^2$ :  $p > 0.05$ ; Table 4.2 and Table C.5). Assessment of each SV bin underlined the variability in the performance of the perching model between individuals, with only three eagles using all six SV bins at the predicted proportion ( $\chi^2$  residuals  $< |2|$ ; Figure C.7).

Table 4.1. Percentage of used and available habitat for each selection value (SV) bin, used to assess performance for the perching, short flight and long flight models. Bin 1 represents areas with the lowest SV (i.e. areas expected to be selected against), whereas Bin 6 represents areas with the highest SV (i.e. areas selected very strongly for) “% Available” indicates the proportion of the land area within the natal territories that was assigned to each SV bin. “% Used” is the proportion of GPS fixes within each SV bin.

SV bin	Perching		Short flights		Long flights	
	% Available	% Used	% Available	% Used	% Available	% Used
Bin 1	63.98	20.82	65.87	23.19	59.92	28.13
Bin 2	23.56	32.30	21.40	32.32	18.68	23.50
Bin 3	3.46	8.70	3.36	8.14	8.22	14.62
Bin 4	7.02	26.95	7.60	27.25	9.85	21.50
Bin 5	1.62	8.81	0.89	4.27	2.98	10.22
Bin 6	0.36	2.43	0.89	4.83	0.36	2.02

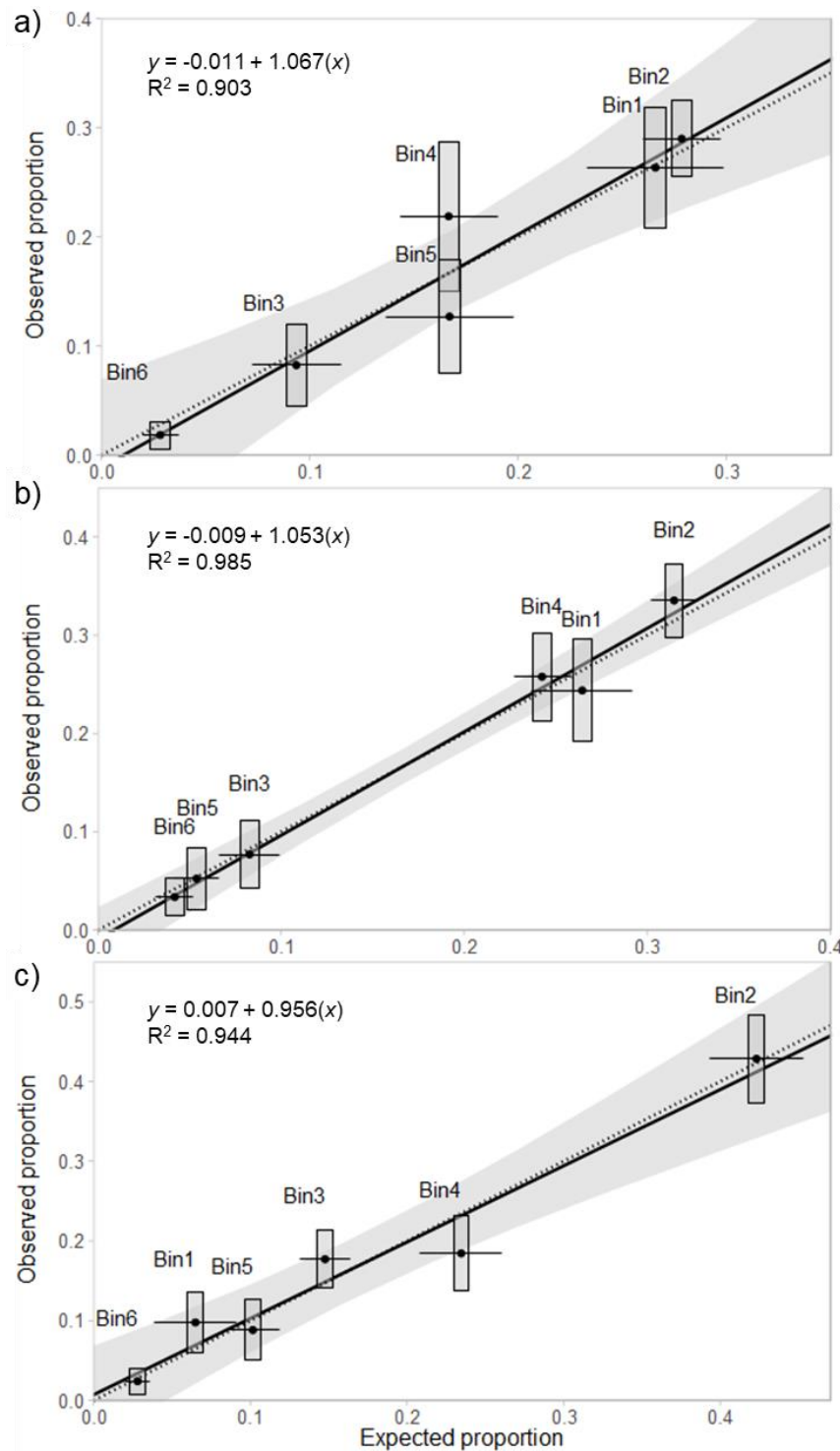


Figure 4.6. Expected vs. observed regressions assessing model performance for the perching (a), short flights (b) and long flights (c) models. The observed proportion is the proportion of telemetry observations in the six SV bins for each of the 22 LOOCV test birds. The expected proportion is the proportion of LOOCV GPS fixes that were expected to fall within each SV bin based on the model prediction. The averaged performance of each bin is plotted, with the vertical error bars designating the observed proportion 95% CI and the horizontal lines the 95% CI of the expected proportion. The fitted regression of the LOOCV performance of each bin is shown as the solid dark line, with the shaded grey area denoting the 95% CI. A model 100% accurate in predicting habitat use would have a regression line with a small CI that sat along the dashed line.

Table 4.2. Perching, short flight and long flight model performance for each of the LOOCV eagles as assessed by expected vs. observed regressions and  $\chi^2$  goodness-of-fit tests. The  $R^2$  value from each regression is presented with indicators of poor model performance based on the corresponding  $b_0$  and  $b_1$  indicated by superscript (a, b, c).  $\chi^2$  values from each goodness-of-fit test are shown with significance indicated (d).  $\chi^2$  tests could not be computed due to an absence of SV bin 6 available habitat area in the natal territory for three eagles (\*). Cell shade summarises the LOOCV predictive performance of each model according to both expected vs. observed regression and  $\chi^2$  goodness-of-fit test (dark grey = poor performance, light grey = moderate performance, white = good performance). The region of Tasmania where each natal territory was located is also presented.

Eagle	Perching		Short flights		Long flights		Region
	$R^2$	$\chi^2$	$R^2$	$\chi^2$	$R^2$	$\chi^2$	
Ernie	0.559 <sup>b</sup>	17.985 <sup>d</sup>	0.92	12.792	0.971	21.479 <sup>d</sup>	East
Wanda	0.885	11.499 <sup>d</sup>	0.881	34.212 <sup>d</sup>	0.934	10.788	East
Enid	0.865	19.312 <sup>d</sup>	0.973	28.742	0.969	9.441	Highlands
Winifred	0.961	6.033	0.98	6.961	0.971	19.209 <sup>d</sup>	Highlands
Erin	0.395 <sup>b</sup>	39.411 <sup>d</sup>	0.250 <sup>b</sup>	234.292 <sup>d</sup>	0.813	10.159	Midlands
Ethan	0.854	*	0.691	*	0.988	*	Midlands
Woldja	0.763	13.163 <sup>d</sup>	0.747	55.175 <sup>d</sup>	0.989	2.27	Midlands
Edward	0.782	16.755 <sup>d</sup>	0.97	10.253	0.996	1.656	North East
Eli	0.759	5.082	0.945	5.696	0.929	11.762 <sup>d</sup>	North East
Emilio	0.663	18.141 <sup>d</sup>	0.978	5.136	0.988 <sup>c</sup>	8.556	North East
Walden	0.623	49.850 <sup>d</sup>	0.806	37.886 <sup>d</sup>	0.994 <sup>c</sup>	0.644	North East
Eggbert	0.373 <sup>b</sup>	*	0.510 <sup>b</sup>	113.782 <sup>d</sup>	0.913	*	North West
Ellen	0.866	11.266 <sup>d</sup>	0.836	35.518 <sup>d</sup>	0.898	32.945 <sup>d</sup>	North West
Erika	0.558 <sup>b</sup>	43.491 <sup>d</sup>	0.164 <sup>b</sup>	232.419 <sup>d</sup>	0.757	21.956 <sup>d</sup>	North West
Emma	0.786	24.292 <sup>d</sup>	0.948	26.064	0.973	2.841	South
Engelbert	0.847	15.948 <sup>d</sup>	0.903	23.785	0.941	9.994	South
Ethel	0.903	9.865	0.962	8.296	0.978	2.632	South
Eva	0.544 <sup>b</sup>	7.56	0.479 <sup>b</sup>	24.728	0.91	8.094	South
Ezio	0.858	3.308	0.477 <sup>b</sup>	20.388	0.695	6.804	South
Willow	0.726 <sup>c</sup>	13.719 <sup>d</sup>	0.503 <sup>b</sup>	55.913 <sup>d</sup>	0.955	6.667	South
Wollowra	0.729	*	0.773	*	0.821	12.107 <sup>d</sup>	South
Wyatt	0.836	11.019	0.933	7.838	0.92	11.843 <sup>d</sup>	South
All animals	0.903	11.086 <sup>d</sup>	0.985	4.405	0.944	6.338	

<sup>a</sup>  $b_0$  significantly different from 0 ( $p < 0.05$ )

<sup>b</sup>  $b_1$  not significantly different from 0 ( $p \geq 0.05$ )

<sup>c</sup>  $b_1$  significantly different from 1 ( $p < 0.05$ )

<sup>d</sup>  $\chi^2$  significantly different from expected ( $p < 0.05$ )

### *Model performance – Short flights*

The short flight model again found strong habitat selection, with variable predictive power depending on region. The model indicated that habitats utilised proportionally less than availability ( $SV < 1$ ) covered 65.8% of the available land area, with 23.2% of short flight GPS records in these habitats. Areas that eagles were predicted to strongly select for when performing short flights ( $SV \geq 4$ ) covered only 1.8% of the available land area, yet 9.0% of short flights occurred in these areas (Table 4.1). Model performance assessment suggested the overall averaged short flight model performed well (used vs. expected regression:  $b_0 = -0.009$ ,  $b_1 = 1.053$ ,  $R^2 = 0.985$ ;  $\chi^2$ :  $p = 0.493$ ; Figure 4.6). The LOOCV assessment of model performance showed some variation in the predictive ability of the model for each individual. The model performed well at predicting the areas of short flights for 10 birds (Regression:  $b_0 \approx 0$ ,  $b_1 \approx 1$ ,  $R^2 > 0.8$ ;  $\chi^2$ :  $p > 0.05$ ; Table 4.2 and Table C.6) and moderately for five birds (regression:  $R^2 < 0.8$  or  $\chi^2$ :  $p < 0.05$ ). Model performance was poor in seven birds (regression:  $b_1 \neq 1$ ,  $R^2 < 0.8$ ;  $\chi^2$ :  $p < 0.05$ ), which were characterised by available habitats in their natal territories predominated by slopes  $< 15^\circ$  (89.9–99.9%). Accordingly, the predicted performance of the short flight model was weakest for flatter landscapes in the Midlands ( $\mu R^2 = 0.562$ ) and North West ( $\mu R^2 = 0.503$ ). Regionally, the predictive performance was best for the Highlands ( $\mu R^2 = 0.970$ ), North East ( $\mu R^2 = 0.925$ ) and East ( $\mu R^2 = 0.901$ ). Assessment of the individual performance of each SV bin showed bin 1 (SVs 0–1) performed least well, with eight birds having  $\chi^2$  residuals  $> |2|$  (Figure C.8).

*Model performance – Long flights*

The long flight model also indicated clear selection for certain habitats, with good predictive power. Habitats utilised proportionally less than availability ( $SV < 1$ ) for this behaviour covered 59.9% of the available land area with 28.13% of long flights occurring in these habitats. In contrast, 12.2% of long flight data occurred in areas assigned SVs  $> 4$ , yet these habitats covered only 3.3% of the available land area (Table 4.1). Averaged LOOCV performance indicated the model predicted effectively where long flights would occur (used vs. expected regression:  $b_0 = -0.007$ ,  $b_1 = 0.956$ ,  $R^2 = 0.944$ ;  $\chi^2$ :  $p = 0.275$ ; Figure 4.6). The LOOCV showed some variation in the predictive ability of the model for each individual. The model performed well for 10 of the birds according to both the linear regression for that individual ( $b_0 \approx 0$ ,  $b_1 \approx 1$ ,  $R^2 > 0.8$ ) and  $\chi^2$  goodness-of-fit test ( $p > 0.05$ ). The predictive performance was moderate for 10 birds ( $R^2 < 0.8$  or  $\chi^2$ :  $p < 0.05$ ) and poor for two birds (regression:  $b_1 \neq 1$ ,  $R^2 < 0.8$ ;  $\chi^2$ :  $p < 0.05$ ; Table 4.2 and Table C.7). The long flight model performed well for cross validated eagles in all regions ( $\mu R^2 > 0.8$ ). Assessment of each SV bin emphasised the performance of the model, with 11 eagles using all six SV bins as predicted ( $\chi^2$  residuals  $< |2|$ ; Figure C.9).

Examples of the perching, short flights and long flight habitat selection models are shown in Figure 4.7 (and Figure C.10 and Figure C.11). The Tasmania-wide models are shown in Figure C.12.

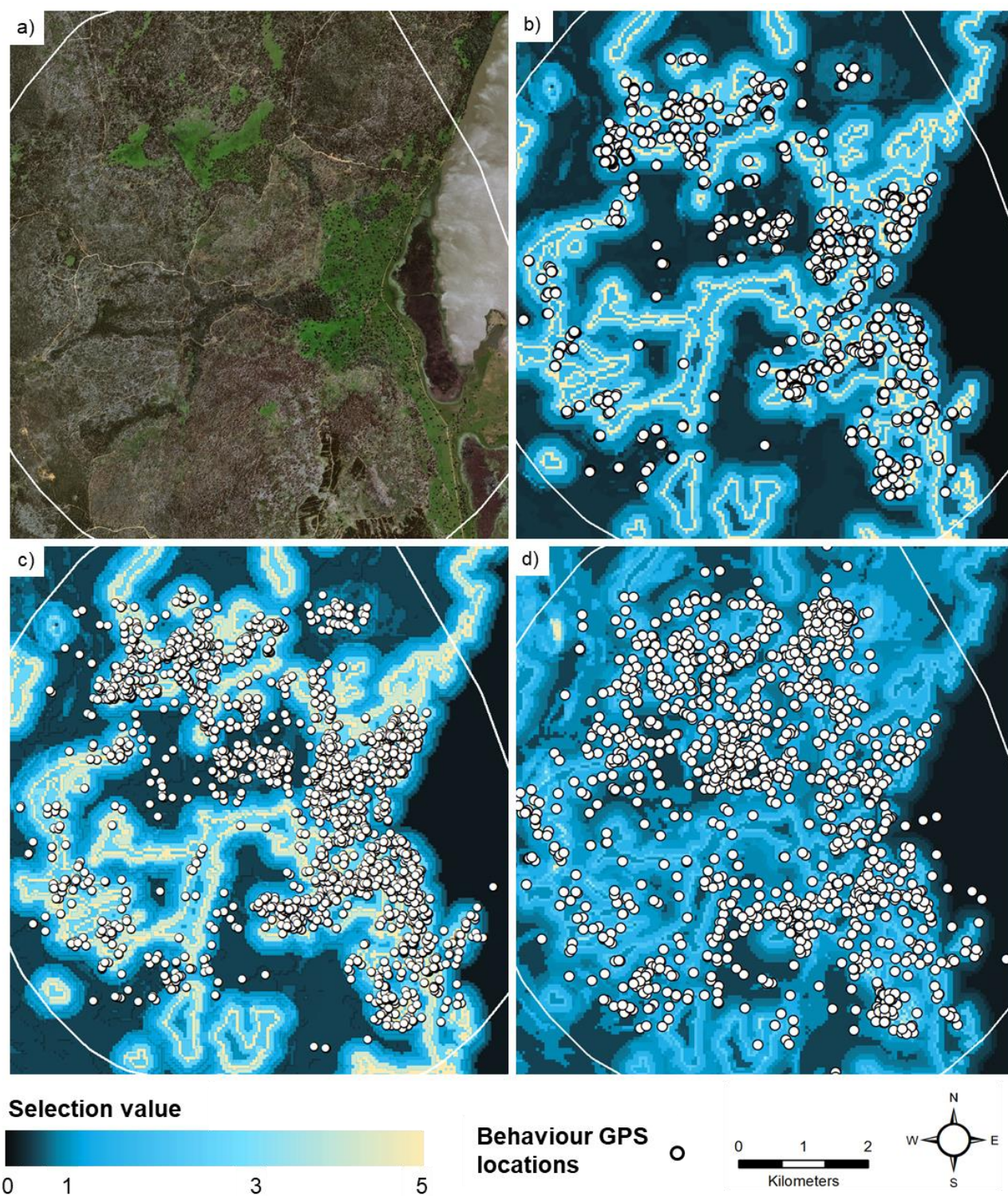


Figure 4.7. Example mapped predictions of a LOOCV test juvenile Tasmanian wedge-tailed eagle (Wanda). a) Satellite image of the available habitat area (buffered 95% MCP shown by white border). b) Modelled perching habitat use with all perching GPS locations shown as white dots. c) Modelled short flight habitat use with all short flight GPS locations shown as white dots. d) Modelled long flight habitat use with all long flights GPS locations shown as white dots.

## Discussion

This study provides the first quantitative assessment of habitat selection by the wedge-tailed eagle of any age, anywhere in Australia, and does so according to three distinctive behaviour types. During the PFDP, juvenile Tasmanian wedge-tailed eagles selected for habitats based on the steepness of the terrain and the distance to forest edge, and behaviour-specific habitat selection models indicated these two criteria could effectively predict the habitats in which different flying behaviours would occur. These findings have important implications for our understanding of the ecology of the species and for the management of this endangered population in the face of increasing land use changes in Tasmania.

### *Behaviour during the PFDP*

The PFDP ranged from 147–575 days in length, which was longer than previously estimated for the species based on field observation (90–180 days; Allott *et al.*, 2006; Bell and Mooney, 1998; Debus *et al.*, 2007; Olsen, 2005) and a GPS-tracked juvenile (121 days; Hatton *et al.*, 2015). The PFDP was also longer than documented in non-migratory congeners (9–251 days; Ferrer, 1992; Murphy *et al.*, 2017; Soutullo *et al.*, 2006; Weston *et al.*, 2013). This extended period of dependence may have consequences for subsequent breeding attempts by the adults, as the PFDP overlapped the phenology of incubation in almost all of the juveniles we tracked (19/22). Furthermore, seven juveniles (or 32%) remained in their natal territory over a year after fledging. Although nest activity surveys showed that at least five of our study nests were used in the subsequent breeding season (Forest Practices Authority unpublished data), further work is required to confirm how the presence of juveniles affects breeding activity when they remain in the natal territory throughout the next season.

Our study showed three distinct behavioural states: perching, short flights and long flights, which we recognise will involve different ethological processes. Perching will likely comprise behaviours such as resting, prey handling and foraging, as wedge-tailed eagles commonly perch hunt from vantage points (Olsen, 2005). Short flights and long flights could be indicative of hunting or transitory movements (Olsen, 2005). Long flights occurred at substantially higher altitudes than short flights and were the most common behaviour in the middle of the day. Thermal updrafts are strongest in the middle of the day, suggesting that the eagles are utilising thermal lift during long flights (Murgatroyd *et al.*, 2018; Poessel *et al.*, 2018a). Short flights are likely to involve flapping-gliding flight, since they are shorter in duration and occur at much lower altitudes (Sapir *et al.*, 2011).

### *Habitats used during different behaviours*

Juvenile eagles show strong selection for forest edges when perching and performing short flights, probably due to the high habitat suitability for foraging in these ecotones. Forest edges are primary foraging habitats for many predatory species, including other *Aquila* spp. (Balbontín, 2005; Sandgren *et al.*, 2014), as they provide expansive views, access to spatially open habitats conducive to hunting and often have higher densities of prey species. However, this conclusion assumes that juvenile eagles hunt independently before starting dispersal. Although some raptors are known to hunt whilst in the PFDP (Muiz-López *et al.*, 2012; O'Toole *et al.*, 1999), others remain reliant on parental provisioning until dispersal (Newton, 1979; Wood *et al.*, 2007). Therefore, the selection for forest edges may also be driven by an association with the space use of their parents, or the more open areas being easier to navigate as flight ability develops.

The avoidance of open habitats during all behaviours contrasts with findings in congeners, where grasslands, pastures and clear-cuts have been identified as important foraging areas in both juveniles and adults (Meyburg and Scheller, 2004; Sandgren *et al.*, 2014; Singh *et al.*, 2016; Soutullo *et al.*, 2008). However, these studies do not typically consider distance to forest edge, which impedes ecological comparison. For example, our results indicate wedge-tailed eagles did select for open habitats, but only within 75 m of the nearest forest edge.

The lower biodiversity and dense vegetation cover associated with plantation monocultures are believed to make plantation forests inimical to *Aquila* spp. (Watson and Whitfield, 2002), and may be driving the strong avoidance we detected during perching and short flights. Our analysis may, however, miss differences in selection between plantation types, as more open and diverse plantations (e.g. of *Eucalyptus nitens* and *Eucalyptus globulus*) were grouped with dense *Pinus radiata* monocultures.

The juvenile eagles' observed selection for steeper slopes and more rugged terrain while performing flying behaviours is in line with observations of congeners and soaring species (Katzner *et al.*, 2012; Poessel *et al.*, 2018a; Tikkanen *et al.*, 2018). Steeper topography and more variable terrain facilitate orographic winds, which can then be exploited during flight for the vertical lift they provide (Duerr *et al.*, 2012; Fielding *et al.*, 2019; Murgatroyd *et al.*, 2018; Newton, 2008). As such, our findings of a stronger selection for steep slopes and avoidance of flatter areas during long flights suggest that orographic winds are important to this behaviour. We also found that more rugged and steeper terrains are used proportionally more than their availability for perching. These selections may be driven by similar motivations to that of forest edge, with these areas providing views over the surrounding landscape.

*Predicting where different behaviours are likely to occur*

The wedge-tailed eagle habitat selection models varied in predictive performance with the behaviour being modelled. The model for perching performed least well, with poor cross-validated performance in all regions of Tasmania. Although the eagles generally selected steep areas at the forest edge for perching, paddock trees are also important perching habitats for the species. However, these isolated trees occur at too fine a scale for inclusion in the model, which may explain the limited ability of the model to accurately predict perching areas. The models using distance to forest edge and topographic slope predicted the location of flying behaviours with more accuracy, emphasising the importance of these habitats for flight. The long flight model had high predictive accuracy across all regions, whereas the short flight model performed well except in the North West and Midlands. In these flatter landscapes the eagles selected more strongly for forest edges (Figure C.13), suggesting models localised to these regions would more effectively predict where short flights occur.

Although short flight and long flight models performed well, there are important provisos to their application. First, the models are specific to the habitats used by juvenile wedge-tailed eagles during the PFDP; thus, the predictive ability of the model is localised to areas that support a breeding territory. The models should therefore be used in conjunction with state-wide estimations of nesting habitat suitability (Forest Practices Authority, 2013) or known nest sites (DPIPWE, 2016). Second, nests in our study were often situated at the border of the natal home range, with utilised areas ranging 3.3–6.8 km from the nest itself. We advise a conservative approach of using the model to predict pre-dispersal juvenile habitat use within a 6.8 km buffer of a nest, excepting where more detailed information about the associated territory boundaries is available. Third, the absence of tracking data from juveniles living in the regions of expansive

buttongrass (*Gymnoschoenus sphaerocephalus*) moorlands and forests with dense understory characteristic of the West and South West of Tasmania (DPIPWE, 2013) limits model application in these regions.

### *Conservation applications*

Our behaviour-specific habitat selection analyses have provided valuable new insights to guide the conservation management of the Tasmanian wedge-tailed eagle. For juveniles at least, which avoided large open areas, it is likely to be important to retain patches of standing forest in areas that have been harvested or cleared for forestry and agriculture (Lindenmayer *et al.*, 2012). Furthermore, given the eagles' avoidance of plantation forests, they may benefit from plantation designs that incorporate patches of native vegetation (Hobbs *et al.*, 2003). The habitat selection models for flying behaviours can also be used to guide efforts to reduce risks from sources of human-caused mortality. Short flights occurred at low altitudes (mean  $22.8 \pm \text{SD } 5.9$  m) that overlap the range of power line heights in the state (4–50 m; TasNetworks pers. comm.); therefore, the model can be helpful in prioritising high risk areas for mitigation (e.g. flight diverters; Bernardino *et al.*, 2018) and positioning of future infrastructure. Long flights occurred at higher altitudes (mean  $63.5 \pm \text{SD } 23.6$  m) associated with a risk of wind turbine collision (blade spans of current turbines in Tasmania range from approximately 30 to 125 m over ground; Hydro-Electric Corporation, 2019). With Tasmania's landscape being increasingly exploited for wind energy projects, the long flight model will similarly help in assessing environmental impacts.

Our study provides insights into one life stage of the Tasmanian wedge-tailed eagle, but similar investigations remain to be carried out into the habitat selection of dispersing sub-adults

and resident adults, to fully understand the population's ecological requirements. An animal's resource requirements are likely to change with its age (Kamler and Gipson, 2000; Pagen *et al.*, 2000). Breeding Tasmanian wedge-tailed eagles are sensitive to disturbance and typically require large areas of predominantly old growth forest to nest (Forest Practices Authority, 2013; Mooney and Holdsworth, 1991); this is a habitat type that we observed to be generally avoided by juveniles.

## ***Conclusion***

This study provides a demonstration of the value of behaviour specific habitat selection analyses for the conservation management of a highly vagile species. Using hidden Markov modelling and a novel multivariate habitat selection ratio approach, we were able to describe which habitats Tasmanian wedge-tailed eagles used for perching, short flights and long flights. We were then able to model where these behaviours were likely to occur across the landscape, providing valuable guidance for efforts to reduce human-caused mortality. It is important to ensure that such models are biologically meaningful and have predictive abilities to prevent management decisions being based on incorrect inferences. The LOOCV approach allowed us to break down predictive capacities of our models by individual, region and overall, thus ensuring that only high-performing models are passed on to conservation managers. Our case study demonstrates the utility of this approach for conservation management.

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## Chapter 5

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Morphometric sex identification of nestling and free-flying Tasmanian  
wedge-tailed eagles (*Aquila audax fleayi*)



Image T. Katzner

## Abstract

The endangered Tasmanian wedge-tailed eagle (*Aquila audax fleayi*) is the focus of increasing research to help guide conservation management of the population. A tool for accurate and efficient identification of the sex of individuals would be a valuable aid to research. However, plumages are monomorphic between the sexes, making sex identification difficult without molecular analysis. Our aim was to assess whether Tasmanian wedge-tailed eagles of different age classes could be sexed accurately using morphological measurements taken when the bird is in the hand. We took measurements of 25 live late-stage eagle nestlings and 108 carcasses of free-flying birds found opportunistically throughout Tasmania. Sex was confirmed via PCR assay. Free-flying birds were larger than nestlings in all measurements, excluding tarsus breadth. Therefore, age-specific statistical tools should be used to evaluate sex. For both nestlings and free-flying birds, females were significantly larger than males, but overlap between the sexes prevented accurate sex identification using single measurements. We used stepwise linear discriminant function analyses to select morphometric measurements necessary for accurate sex identification. Free-flying birds could be sexed with 97.6% accuracy using a combination of forearm, tarsus width and hallux length. Late-stage nestlings could be sexed with 95.4% accuracy using hallux width, hallux breadth and tarsus breadth. The equations provide a valuable research tool for studies of behaviour and causes of mortality of the species.

## Introduction

Accurate and efficient sex identification is important to ecological research (Dunn *et al.*, 2001) and can be paramount to clarifying the nature of conservation threats faced by a population (Clutton-Brock, 1985; Vanstreels *et al.*, 2013). Physiological and behavioural differences between the sexes can make one sex more susceptible to environmental changes and anthropogenic threats, potentially resulting in increased impacts to the population due to sex-bias. For example, large raptors of the genus *Aquila* raise fewer female chicks in years with lower prey availability (Väli, 2004), and female Spanish imperial eagles (*Aquila adalberti*) are more vulnerable to collisions and electrocutions from power lines than males (Ferrer and Hiraldo, 1992).

Techniques for sexing individuals based on sex-specific behaviours (e.g. vocalisations, copulation position) or physiological characteristics are effective and widespread in ornithological research (Ballintijn and Cate, 1997; Harmata and Montopoli, 2013). When behaviour cannot be assessed, molecular methods are increasingly used for accurate and reliable identification of sex (Donohue and Dufty, 2006; Griffiths *et al.*, 1996). However, the use of molecular sexing techniques can be limited due to the invasiveness and training required to collect DNA samples, as well as the financial costs associated with their processing and analysis (Hartman *et al.*, 2016). Furthermore, they do not allow rapid, field-based assessments of the sex of individuals, limiting their value in ecological studies.

Many raptor taxa have sexually monomorphic plumages, making visual identification of sex difficult. That said, raptor species often exhibit sexual size dimorphism, with females being larger than males. Although these size differences offer a non-invasive way of identifying sex,

there is often overlap of single measurements between the sexes, complicating morphometric sex identification. Combinations of measurements are more effective as they can often reliably identify the sex of an individual. As such, the use of discriminant function analyses (DFA) is predominant in the literature for sex identification of eagles (Balbontin *et al.*, 2001; Ferrer and De Le Court, 1992; García *et al.*, 2013; Shephard *et al.*, 2004; Smith *et al.*, 2016) and other raptors (Dykstra *et al.*, 2012; Xirouchakis and Poulakakis, 2008; Zuberogoitia *et al.*, 2011). DFAs are useful because they assess the discriminating power of each morphological measurement and provide an equation that effectively discriminates between the sexes. The equation is calibrated and its effectiveness tested using individuals of known sex (usually identified through molecular techniques; Dechaume-Moncharmont *et al.*, 2011).

Separate DFAs often need to be formulated for difference age classes due to changes in morphology as an individual ages (Dykstra *et al.*, 2012; McPherson *et al.*, 2017). Sex of some raptor species cannot be discriminated morphologically until after they have fledged the nest (López-López *et al.*, 2011). Other species change aspects of their morphology when they transition to adult plumage (Bortolotti, 1984a). Likewise, soft tissue measurements (e.g. feathers) are known to vary as a raptor ages (Dykstra *et al.*, 2012; Pitzer *et al.*, 2008) and some hard tissue measurements (e.g. talons, bill) can change due to continued growth and wear (Bortolotti, 1984a; Donohue and Dufty, 2006). In contrast, skeletal measurements are not thought to change after leaving the nest (Bortolotti, 1984b); theoretically making these measurements more reliable in discriminating the sexes of multiple age classes.

Accurate and efficient sex identification can provide a valuable research and management tool for endangered populations. The Tasmanian wedge-tailed eagle (*Aquila audax fleayi*) is geographically isolated and exhibits morphological (e.g. larger body size) and behavioural (e.g.

smaller brood size and higher sensitivity to anthropogenic disturbance) trait divergence from mainland and New Guinean wedge-tailed eagle populations (*Aquila audax audax*) (Bell and Mooney, 1998; Marchant and Higgins, 1993; Olsen, 2005). As such, it is considered a distinct subspecies (Condon and Amadon, 1954; but see Burridge *et al.* 2013). The population is listed as endangered (Commonwealth of Australia, 1999; State Government of Tasmania, 1995), with conservation concern principally based upon low rates of breeding success, habitat disturbance and high rates of unnatural mortality (Bekessy *et al.*, 2009; Bell and Mooney, 1998; Threatened Species Section, 2006). The demographic consequences of each of these stressors is likely influenced by the sex of individuals and the population sex ratio. However, there is currently no validated morphological method to sex Tasmanian wedge-tailed eagles.

The aim of our study was to assess whether Tasmanian wedge-tailed eagles of different age classes could be sexed accurately using a DFA of morphological measurements. We focused on two objectives, (1) identify if there are morphological differences between three different age classes of birds sampled (nestling, free-flying immatures, free-flying adults), and (2) for each morphologically different group, identify the morphological differences between sexes and use DFAs to create simple tools that can be used in the field to distinguish between the sexes.

## Methods

### *Sample collection*

Eagles in this study came from two sources. First, eagle carcasses collected over the past 15 years from throughout Tasmania were placed in freezer storage by the Department of Primary Industries, Parks, Water and the Environment (DPIPWE, Threatened Species Section, Hobart, Tasmania) and the Tasmanian Museum and Art Gallery (TMAG, Collection and Research Facility, Rosny, Tasmania). Second, we captured nestlings by hand prior to fledging as part of a study on other aspects of the ecology of these birds (Figure D.1.1).

We measured eagle carcasses within 48 hours of discovery or within 24 hrs of thawing. All eagle carcasses were fully defrosted before measuring to ensure accuracy. Carcasses included in the study were from free-flying birds, which we broadly categorised into two age classes (adult or immature) based on plumage (see Appendix A.1). Live-caught eagle measurements were taken from nestlings at approximately 14 days prior to fledging (65–75 days of age).

We collected standard morphological measurements from all live and dead birds (see Harmata and Montopoli, 2013; Wink, 2007). The measurements collected from dead birds included head length, head width, bill length, bill width, bill depth, exposed culmen length, tarsus length, tarsus width, tarsus breadth, hallux length, hallux width, hallux breadth, forearm (ulna) length, wing chord and tail length (see Table D.2.1). To reduce handling time, for nestlings we only collected a subset of nine measurements (head length, bill length, exposed culmen, tarsus length, tarsus width, tarsus breadth, hallux length, hallux width and hallux breadth). Measurements were taken by the same person (J.P.) with a mechanical calliper and

wing rule. We recorded most measurements to the nearest 0.1 mm; forearm, wing chord and tail were to the nearest 1 mm.

### *Molecular sexing*

We collected a  $\approx 1 \text{ cm}^3$  piece of liver or thigh muscle tissue from each eagle carcass and a  $\approx 0.05 \text{ ml}$  blood sample from each nestling. All samples were stored in 0.5 ml of lysis buffer until DNA extraction. We extracted DNA using a Qiagen DNEasy Blood and Tissue Kit (Qiagen Inc.), following the manufacturer's protocol. Sex was then determined via a PCR assay (see Appendix D.3.). We used primers developed for the wedge-tailed eagle by Wadley (2009) to co-amplify the sex-linked CHD-W and CHD-Z genes. Molecular sexing for 52 of the carcasses was previously carried out by Nankervis (2010) using the same methods. We included these data in our study with a subset of samples ( $n = 7$ ) re-analysed to confirm analogy of the genetic sex results.

### *Data analysis*

We conducted all statistical analyses in R 3.4.3 (R Core Team, 2016). Morphometric data were tested for normality (Shapiro-Wilk test) and homogeneity of variance (Fligner-Killeen test; R package *stats*; R Core Team, 2016). Tests for multivariate normality (Shapiro-Wilk Multivariate Normality Test; R package *mvnrm*; Genz *et al.*, 2018) and homogeneity of variance (Box's M statistic; R package *biotools*; da Silva, 2017) were used to check that the data met assumptions for multivariate analyses. Multivariate outliers were identified by measuring robust Mahalanobis distances (R package *mvoutlier*; Gschwandtner, 2005) and the leverage of

the outliers was assessed by removal from the dataset and rerunning analyses. Prolonged freezer storage may cause shrinkage of morphological features (Smith *et al.*, 2016); accordingly, we tested for the relationship between each morphometric measurement and time in storage using linear least-squares regressions.

### *Morphological differences between age classes*

We used multivariate analysis of variance (MANOVA) to evaluate differences in morphometric measurements between age classes and sexes. First, we assessed whether morphometrics were different between nestlings or free-flying birds. Only the nine measurements taken on all nestling and free-flying birds were used in this analysis. We also used a MANOVA of all data from free-flying birds to investigate whether immatures and adults differed morphometrically, and thus whether we needed to evaluate sexual dimorphism separately among different age birds. To verify age-related differences were not driven by disparity in the numbers of each sex within each age group, we performed one-way MANOVAs to assess morphological differences between the age classes separately for each sex.

### *Morphological differences and distinguishing between sexes*

For each morphologically different age group we carried out a one-way analysis of variance (ANOVA) on each morphometric to explore the degree of sexual dimorphism. The percentage of dimorphism in each measurement was calculated as  $100 * [(mean \text{ ♂} / mean \text{ ♀}) - 1]$  (López-López *et al.*, 2011). We identified cut-off values between the sexes for each individual morphometric and the respective accuracy using linear DFAs.

We used a linear DFA (R package *MASS*; Ripley *et al.*, 2018) to discriminate the sexes using morphometric measurements. Any highly correlated measurements were removed from the DFA (Pearson's  $r > 0.8$ ), as these can lead to unstable parameter estimates (Dechaume-Moncharmont *et al.*, 2011). We used stepwise DFA (R package *klaR*; Roever *et al.*, 2018) to select the minimum number of morphometric measurements necessary to identify sex accurately. Wilks'  $\lambda$  and Uschi's classification performance were used to identify the morphometric measurements most diagnostic of sex. The significance level for measurement term entry or removal from the model was 0.1.

All DFA models were validated using the leave-one-out (or jackknife) procedure. Leave-one-out was chosen over sample splitting and re-substitution because the latter two methods result in higher variance (Dechaume-Moncharmont *et al.*, 2011). Confidence intervals are rarely reported with DFAs, despite being vital to understanding the robustness of the models. Therefore, we calculated confidence intervals by bootstrapping leave-one-out models (Dechaume-Moncharmont *et al.*, 2011). Linear classification equations were extracted from each model so that they could be used easily in future field studies. We used a logistic regression to denote the thresholds where discriminant scores had a  $>75\%$  probability of correctly classifying sex and compared the discriminant function accuracy within and outside of this range (following methods described in Hartman *et al.*, 2016).

## Results

We obtained morphometric data for 133 Tasmanian wedge-tailed eagles, 108 of which were free-flying birds and 25 of which were live late-stage nestlings. Sex was genetically identified for 100 free-flying birds (61 female and 39 male) and all nestlings (15 female and 10 male). Sex identification was the same for the 7 animals sexed by Nankervis (2010) and here. Mahalanobis distance indicated 11 outliers within the data, but their removal did not improve model performance or diagnostics and they were therefore kept in further analyses. Tarsus width and exposed culmen of free-flying birds had leptokurtic distributions and were transformed for normality using heavy tail Lambert W x F distributions (R package *LambertW*; Georg, 2016). We transformed analogous measurements of nestlings for statistical comparison. Multivariate variance was homoscedastic (Box's  $M$  test,  $\chi^2 = 51.5$ ,  $df = 45$ ,  $p = 0.24$ ), as was the variance of each measurement (Fligner-Killeen test  $p > 0.05$ ).

### *Morphological differences between age classes*

There was a significant effect of fledging status (nestling vs. free-flying) on morphology (MANOVA, Wilks'  $\lambda = 0.226$ ,  $F_{9,113} = 43.03$ ,  $P < 0.001$ ). Free-flying birds were larger in all measurements (ANOVA,  $P < 0.001$ ), excluding tarsus breadth (ANOVA,  $F_{1,121} = 0.58$ ,  $P = 0.45$ ; see Figure 5.1). Consequently, we treated nestlings and free-flying birds separately in all further analyses. Of the free-flying birds, 26 were adults and 74 were immature. Multivariate analysis of the effect of age class (immature and adult) on morphometric measurements (MANOVA, Wilks'  $\lambda = 0.77$ ,  $F_{15,82} = 1.63$ ,  $P = 0.08$ ) and the interaction between age and sex (MANOVA, Wilks'  $\lambda = 0.849$ ,  $F_{15,82} = 0.97$ ,  $P = 0.49$ ) were not significant. Adult birds had a significantly longer wing

chord and tail than immatures (ANOVA,  $P < 0.05$ ; see Figure 5.2); accordingly, we did not include these two measurements in the DFA for free-flying birds. All other measurements showed no significant difference between the two age classes (ANOVA,  $P > 0.05$ ). Therefore, immatures and adults (i.e., all free-flying birds) were combined for further analyses.

### *Morphological classification of sex of free-flying birds*

Morphometrics differed between the sexes of free-flying birds (MANOVA, Wilks'  $\lambda = 0.133$ ,  $F_{15,84} = 36.55$ ,  $p < 0.001$ ). Females were significantly larger in all morphometric measurements (ANOVA,  $p > 0.001$ ; see Table 5.1); however, in every measurement there was overlap (i.e. largest measurement for male birds were larger than the smallest measurement for females). The measurements showing the greatest percentage of sexual dimorphism were all hallux measurements, tarsus width and forearm ( $> 8.5\%$ ). Bill and head measurements were generally less sexually dimorphic (3–7.6%; see Table 5.1).

All free-flying data met the DFA assumptions of univariate and multivariate homogeneity of variance. We identified 14 multivariate outliers in free-flying data. Removal of these outliers improved normality of the data and modestly improved the performance of the models. These outliers were therefore excluded from the DFA of free-flying birds. Exposed culmen was correlated with both bill length and head length (Pearson's  $r > 0.8$ ; Figure D.5.1). Furthermore, exposed culmen was the only measurement that had a significant relationship with time spent in freezer storage (see Figures D.4.1–3). However, although this relationship held for males ( $r^2 = 0.37$ ,  $p = 0.006$ ), it was absent for females ( $r^2 = 0.13$ ,  $p = 0.13$ ). For these reasons exposed culmen was removed from the DFA.

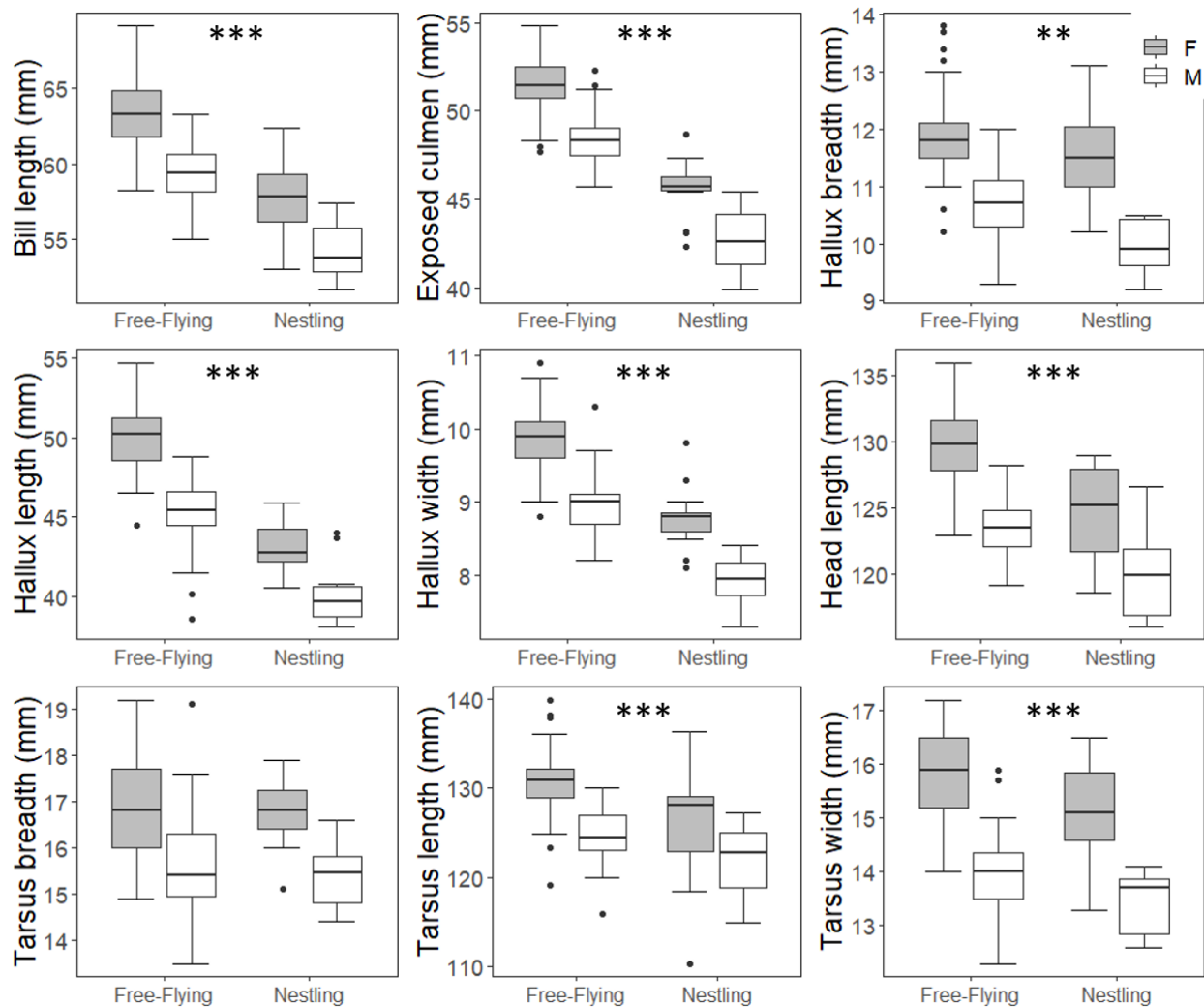


Figure 5.1. Box plots showing the differences in each morphometric measurement between free-flying and nestling eagles. Confirmed genetic sex within each age category is denoted by the box shade. The lower and upper hinges correspond to the first and third quartiles. Whiskers show the sample minimum and maximum, extended to a maximum and minimum value  $1.5 \times$  the inter-quartile range respectively. Data outside of this range are plotted individually. Free-flying birds were larger than the nestlings in all measurements excluding tarsus breadth. The significance in size difference between the age categories is indicated by asterisks (ANOVA:  $p < 0.001 = \text{***}$ ,  $p < 0.01 = \text{**}$ ).

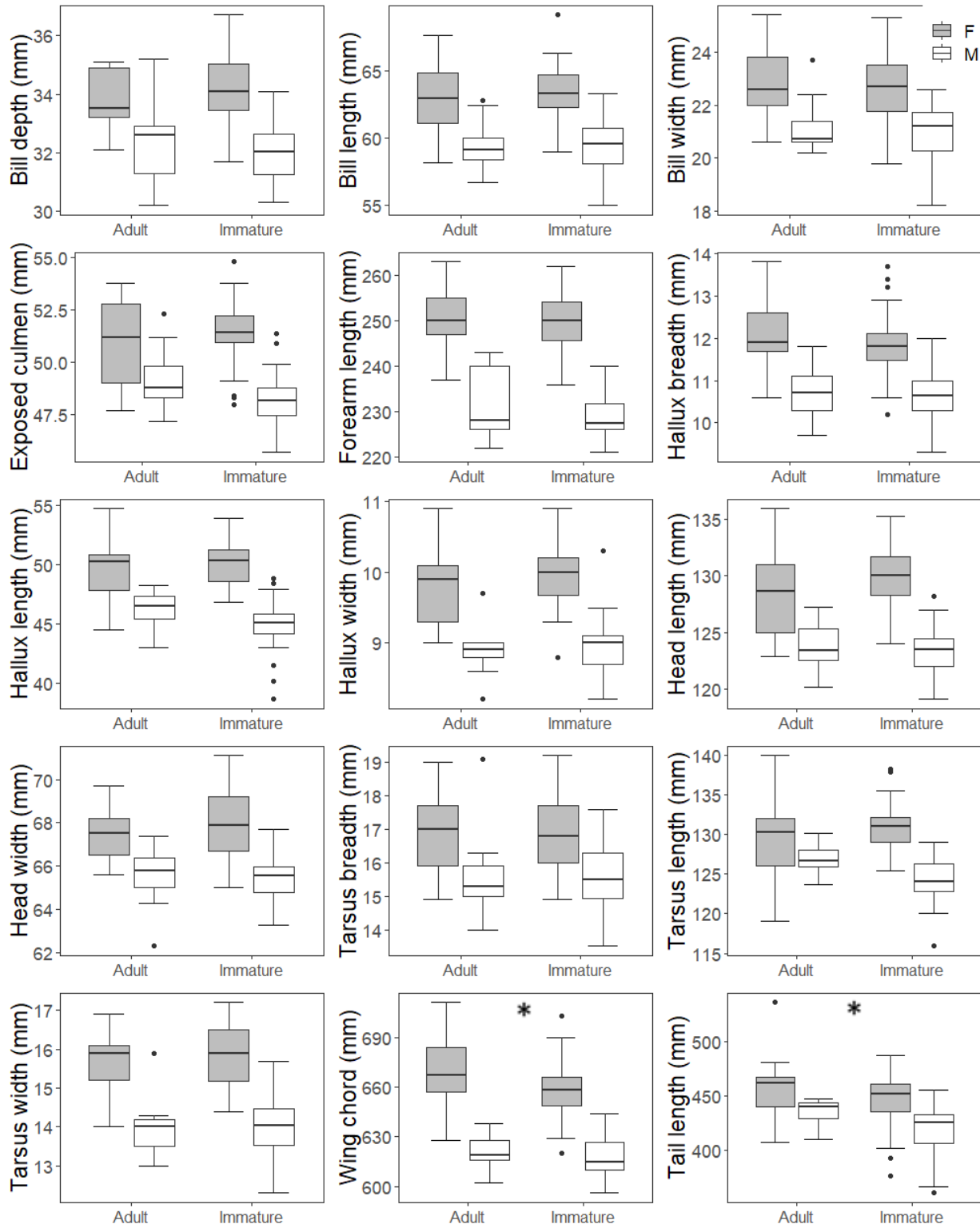


Figure 5.2. Box plots showing the differences in morphometrics between adult and immature birds. Confirmed genetic sex is denoted by the box shade. Only tail length and wing chord measurements show a difference between the two age classes. The significance in size difference between the age categories is indicated by asterisks (ANOVA:  $p < 0.05 = *$ ).

Stepwise DFA using Wilks'  $\lambda$  retained forearm, tarsus width and hallux length. This model had 97% accuracy, misclassifying 3.3% ( $n = 2$ ) of females and 2.6% ( $n = 1$ ) of males (Wilks'  $\lambda = 0.167$ ,  $F_{3,96} = 159.84$ ,  $p < 0.001$ ). Leave-one-out cross-validation resulted in 97.6% accuracy (CI: 94–100%). The linear classification equation from this model was: -

$$\text{Equation 1: Discriminant score} = (0.755 * \text{tarsus width}) + (0.121 * \text{forearm}) + (0.147 * \text{hallux length}) - 47.206$$

*Discriminant scores*  $> 0$  indicate female and  $< 0$  male, and scores between -0.25 and 0.25 have less than 75% probability of being classified as the correct sex (see Figure 5.2a). The scores of the three misclassified eagles were between -0.25 and 0.25. Outside of this range the model was 100% accurate in discriminating the sexes.

DFA of only forearm and tarsus width was selected as the best model based on stepwise selection using Uschi's classification performance. This model also had 97% accuracy (Wilks'  $\lambda = 0.179$ ,  $F_{2,97} = 221.84$ ,  $p < 0.001$ ; see Figure 5.3), but cross-validated performance was slightly improved at 97.9% (CI: 94.9–100%). The linear classification equation from this model was: -

$$\text{Equation 2: Discriminant score} = (0.853 * \text{tarsus width}) + (0.136 * \text{forearm}) - 45.322$$

For this equation the  $< 75\%$  correct sex allocation probability range (*Discriminant score* = -0.23–0.23) was slightly reduced over Equation 1. Only two eagles in this study fell in this range. However, outside of this range the function misclassified one female as male (see Figure 5.2b).

Table 5.1. Summary statistics for free-flying Tasmanian wedge-tailed eagle morphometrics. Mean, range and standard deviation (SD) are shown as well as *F* and *p* values for one-way ANOVAs between the sexes for each morphometric. DFA data includes the estimated discriminatory accuracy (leave-one-out cross validated value) of each morphometric and cut off values for which smaller measurements are likely male and larger female. Dim% is the percentage of sexual dimorphism calculated as  $100 * [(\text{♂ mean} / \text{♀ mean}) - 1]$  (López-López *et al.*, 2011).

Morphometric	Female (n = 61)			Male (n = 39)			Dim%	ANOVA		DFA	
	Range	Mean	SD	Range	Mean	SD		<i>F</i>	<i>p</i>	Accuracy (CI)	Cut-off (mm)
Bill depth	31.7–36.7	34.11	1.15	30.2–35.2	32.05	1.05	6.04	81.46	<0.001***	85% (76-92%)	32.8
Bill length	58.2–69.1	63.24	2.28	55–63.3	59.39	1.96	6.08	75.41	<0.001***	82% (74-89%)	60.7
Bill width	19.8–25.4	22.75	1.32	18.2–23.7	21.03	1.06	7.56	46.62	<0.001***	76% (65-84%)	21.4
Exposed culmen	47.7–54.8	51.38	1.69	45.7–52.3	48.42	1.42	5.75	82.21	<0.001***	83% (74-90%)	49.6
Forearm length	236–263	250.16	6.85	221–243	229.38	5.52	8.31	253.34	<0.001***	91% (84-96%)	239
Hallux breadth	10.2–13.8	11.87	0.72	9.3–12	10.73	0.67	9.55	61.47	<0.001***	83% (73-91%)	11.1
Hallux length	44.5–54.7	49.87	1.95	38.6–48.8	45.20	2.14	9.36	126.19	<0.001***	86% (79-93%)	47.1
Hallux width	8.8–10.9	9.92	0.44	8.2–10.3	8.96	0.38	9.73	126.07	<0.001***	89% (83-95%)	9.4
Head length	122.9–135.9	129.65	3.03	119.1–128.2	123.51	2.20	4.73	118.98	<0.001***	85% (77-93%)	126
Head width	65–71.1	67.79	1.48	62.3–67.7	65.46	1.12	3.44	70.42	<0.001***	82% (74-90%)	66.3
Tail length	376–536	448.89	25.97	360–455	421.23	22.13	6.16	30.18	<0.001***	72% (58-83%)	425
Tarsus breadth	14.9–19.2	16.91	1.17	13.5–19.1	15.58	1.06	7.89	33.30	<0.001***	73% (64-82%)	15.8
Tarsus length	119.1–139.9	130.66	3.71	115.9–130.1	124.84	2.91	4.45	68.86	<0.001***	79% (71-87%)	126.8
Tarsus width	14–17.2	15.81	0.83	12.3–15.9	14.05	0.71	11.16	120.37	<0.001***	87% (80-93%)	14.8
Wing chord	620–711	661.34	18.47	596–644	618.87	12.52	6.42	159.14	<0.001***	91% (84-96%)	637

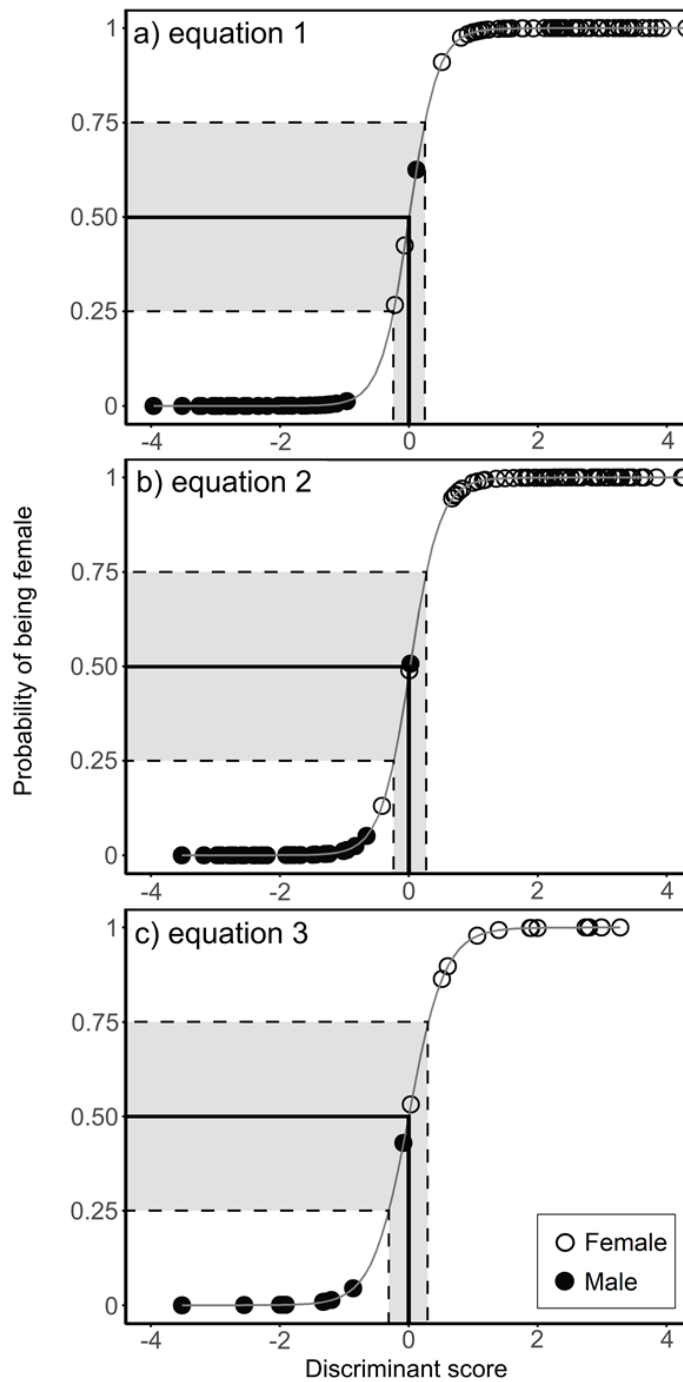


Figure 5.2. Plots representing the probability of being female according to the resulting discriminant scores from: a) Equation 1 using tarsus width, forearm length and hallux length to discriminate the sexes of free-flying birds. b) Equation 2 using only tarsus width and forearm length to discriminate the sexes of free-flying birds. c) Equation 3 using hallux width, hallux breadth and tarsus breadth to discriminate the sexes of nestlings. The solid line signifies the cut-off point where the discriminant score is 0 and the probability of being male or female is 50%. The shaded area denotes the range in discriminant scores where there is less than 75% probability of correct sex assignment (see Hartman *et al.*, 2016). Confirmed genetic sex is indicated by the symbols.

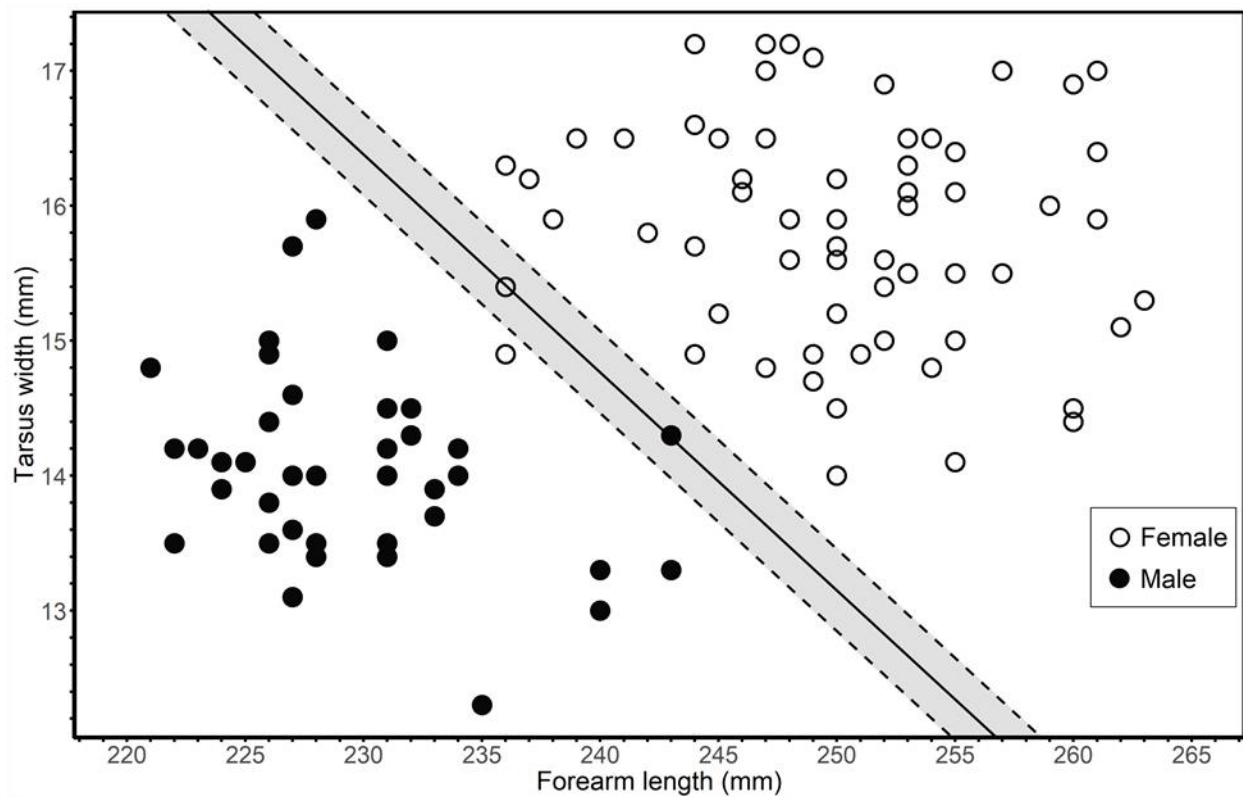


Figure 5.3. Discriminant function for free-flying birds using forearm length and tarsus width. Individuals above the solid line are classified as female and below the solid line as male. Confirmed genetic sex is indicated by the symbols. The shaded area inside the dashed lines defines where the discriminant function had a less than a 75% probability of correct sex allocation.

Forearm length was the best individual predictors of sex in free-flying wedge-tailed eagles, correctly classifying the sex of 91% of birds (Wilks'  $\lambda = 0.279$ ,  $F_{1,98} = 253.24$ ,  $p < 0.001$ ). Cross-validation also correctly classified 91% (CI: 84–96%). Hallux width was the next best performing single measurement, with 89% accuracy (Wilks'  $\lambda = 0.437$ ,  $F_{1,98} = 126.07$ ,  $p < 0.001$ ) and 89% (CI: 83–95%) cross validated accuracy. Tarsus breadth, bill width and tarsus length were the least effective at discriminating between the sex of the eagles with 73%, 76% and 89% accuracy respectively (Wilks'  $\lambda > 0.5$ ; see Table 5.1).

### *Morphological classification of sex of nestlings*

Nestling morphometrics differed significantly between the sexes (MANOVA, Wilks'  $\lambda = 0.193$ ,  $F_{9,15} = 7$ ,  $p < 0.001$ ). Females were significantly larger in all morphometric measurements (ANOVA,  $p > 0.001$ ) excluding tarsus length (ANOVA,  $F_{1,23} = 3.14$ ,  $p = 0.09$ ). Once again there was overlap in all measurements. Hallux breadth, tarsus width and hallux width showed the greatest degree of sexual dimorphism ( $> 8.5\%$ ; see Table 5.2). Data from nestlings met assumptions of normality, univariate homogeneity of variance (Fligner-Killeen test  $p > 0.05$ ) and multivariate homogeneity of variance (Box's  $M$  test,  $\chi^2 = 47$ ,  $df = 45$ ,  $p = 0.39$ ). Exposed culmen was correlated with head length, hallux length and bill length (Pearson's  $r > 0.8$ ; Figure D.6.2) and was not included in DFAs.

Stepwise DFA using Wilks'  $\lambda$  retained hallux width, hallux breadth and tarsus breadth. This model classified the sex of nestlings with 100% accuracy (Wilks  $\lambda = 0.232$ :  $F_{3,21} = 23.22$ ,  $p < 0.001$ ; see Figure 5.4). Leave-one-out cross-validation resulted in 95.4% accuracy (CI: 84–100%). The resulting linear classification equation from this model was: -

$$\text{Equation 3: Discriminant score} = (1.935 * \text{hallux width}) + (0.605 * \text{hallux breadth}) + (0.747 * \text{tarsus breadth}) - 34.597$$

*Discriminant scores* between -0.31 and 0.31 have less than 75% probability of being assigned the correct sex (see Figure 5.2c). Two nestlings fell within this range, but their sex was assigned correctly. Uschi's classification performance selected for hallux width and tarsus breadth. However, this model performed worse than the equation including three morphometric measurements (96% accuracy; Wilks  $\lambda = 0.432$ :  $F_{2,22} = 30.2$ ,  $p < 0.001$ ).

DFA's applied to single morphometric measurements showed that hallux breadth, hallux width and tarsus breadth were the best individual predictors of the sex of nestlings, correctly classifying sex with 87–89% accuracy. However, confidence intervals were large (60–100%). Head and bill measurements were less effective at discriminating the sexes than all other measurements excluding tarsus length (see Table 5.2).

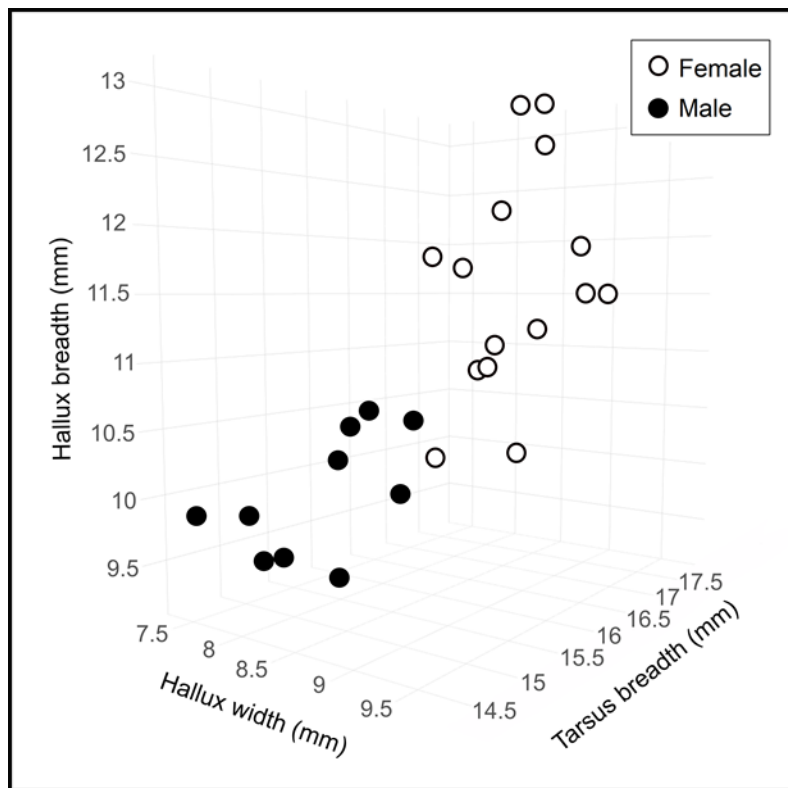


Figure 5.4. Morphological distribution of male and female nestling Tasmanian wedge-tailed eagles in respect to morphological measurements used in Equation 3 (hallux width, tarsus breadth and hallux breadth). Confirmed genetic sex is indicated by the symbols.

Table 5.2. Summary statistics for late-stage nestling Tasmanian wedge-tailed eagle morphometrics. Mean, range and standard deviation (SD) are shown as well as  $F$  and  $p$  values for one-way ANOVAs between the sexes for each morphometric. DFA data includes the estimated discriminatory accuracy (leave-one-out cross validated value) of each morphometric and cut off values for which smaller measurements are likely male and larger female. Dim% is the percentage of sexual dimorphism calculated as  $100 * [(\text{♂ mean} / \text{♀ mean}) - 1]$  (López-López *et al.*, 2011).

Morphometric	Female (n = 15)			Male (n = 10)			Dim%	ANOVA		DFA	
	Range	Mean	SD	Range	Mean	SD		$F$	$p$	Accuracy (CI)	Cut-off (mm)
Bill length	53–62.4	57.93	2.61	51.7–57.4	54.29	2.05	6.28	13.71	<0.01 **	78% (58-94%)	55.5
Exposed culmen	42.3–48.7	45.59	1.66	39.9–45.4	42.65	1.90	6.44	16.75	<0.001 ***	75% (54-92%)	43.7
Hallux breadth	10.2–13.1	11.61	0.88	9.2–10.5	9.95	0.35	14.32	29.78	<0.001 ***	89% (69-100%)	10.7
Hallux length	40.5–45.9	43.22	1.53	38.1–44	40.19	2.11	7.01	17.40	<0.001 ***	86% (60-100%)	41.3
Hallux width	8.1–9.8	8.77	0.41	7.3–8.4	7.93	0.32	9.61	30.20	<0.001 ***	87% (62-100%)	8.28
Head length	118.5–128.9	124.46	3.78	116–126.6	120.06	3.52	3.54	8.59	<0.01 **	70% (52-88%)	120
Tarsus breadth	15.1–17.9	16.75	0.71	14.4–16.6	15.40	0.71	8.08	21.98	<0.001 ***	84% (67-97%)	15.9
Tarsus length	110.4–136.3	125.76	6.14	114.9–127.3	121.82	4.14	3.13	3.14	0.0897	63% (40-83%)	120.8
Tarsus width	13.3–16.5	15.12	0.90	12.6–14.1	13.42	0.60	11.24	27.09	<0.001 ***	87% (60-100%)	14.1

## Discussion

Both free-flying and nestling Tasmanian wedge-tailed eagles show sexual size dimorphism. However, morphological differences between nestling and free-flying birds required creation of separate discriminant functions for these two age categories. The sex of free-flying immatures and adults could be discriminated effectively using tarsus width, forearm and hallux length (Equation 1). Tarsus width and forearm alone resulted in only a slight reduction in discriminant performance (Equation 2). For nestlings, hallux width, hallux breadth and tarsus breadth allowed accurate classification of sex (Equation 3). Furthermore, because the tools we created for both age classes are based in discriminant scores, they also identify cases where sex identification may be in doubt.

### *Morphological differences between age classes*

Morphometric measurements of late-stage nestling Tasmanian Wedge-Tailed Eagles were smaller than those of free-flying birds, suggesting that the younger birds were still growing. Morphological studies of raptor species have determined that late-stage nestlings are usually yet to reach full development of their bills (Bortolotti 1984a, Donohue and Dufty 2006), hallux claws (Bortolotti 1984b) and feathers (Dykstra *et al.* 2012, McPherson *et al.* 2017). The smaller tarsus measurements in our study also indicate that the late-stage nestlings were continuing to undergo skeletal growth, which is unexpected at this age (Bortolotti 1984b). We took nestling measurements at 65–75 days of age, when, if, as reported, they fledge at 77–90 days (Bell and Mooney, 1998; Olsen, 2005), skeletal features should be close to full growth. However, GPS data from the nestlings included in our study indicated fledging age was 77–105 days (mean 90

days; Pay unpublished data) and were thus potentially earlier in development than expected. This may explain why we observed these age-related differences in morphology.

Free-flying immature and adult Tasmanian wedge-tailed eagles were morphologically comparable, except in feather measurements. Both wing chord and tail length were larger in adult birds. This is not unexpected, as feather measurements often vary in raptor species as immatures transition to adult plumage (Bortolotti, 1984c; Pitzer *et al.*, 2008). Furthermore, wedge-tailed eagles have especially dramatic age-related differences in tail structure, with adult females in particular developing long central rectrices with age (Olsen, 2005). That said, differing moult stages and feather damage are also likely to explain the increased variation in these measurements that we observed. As a consequence, caution should be taken when using these measurements to identify sex.

### *Morphological sex identification*

We found tarsus, forearm and hallux measurements allowed accurate classification of sex in the Tasmanian wedge-tailed eagle. These results are supported by findings of other *Aquila* species, where forearm, tarsus and/or hallux measurements are included in DFAs to identify sex in various age classes (Ferrer and De Le Court, 1992; García *et al.*, 2013; Harmata and Montopoli, 2013).

We have presented two similarly effective equations for accurate sex identification of free-flying Tasmanian wedge-tailed eagles and a separate equation for sex identification in late-stage nestlings. However, it is important to note that Equation 2 misclassified one female as male outside of the < 75% probability of correct sex attribution range. Therefore, there is slightly less

confidence in values outside of this range and caution should be taken in interpreting external values close to the range limits. However, use of two morphometric measurements to identify sex allows clear graphical representation of the discriminant function, which can be used to quickly ID sex in the field (i.e. Figure 5.3 could be easily added to data collection sheets). When time permits, Equation 1 should be used for sex identification, with the caveat that birds with discriminant scores close to zero should be prioritized for genetic sex identification.

We recorded a greater overlap in morphometric measurements between the sexes in nestlings, which may be driven by age differences amongst the nestlings measured. We aged nestlings using two aerial nest surveys during the breeding season and at the nest site before they were included in the study. Although these represent our best efforts to accurately estimate age, we approximated that chicks included in the study potentially differed up to 10 days in age (65–75 days of age). This age difference may mean there is greater variability in the morphometric measurements we recorded for late-stage nestlings, even though limited skeletal growth is expected to occur during this period (Bortolotti, 1984b). Aging of nestlings without observation of hatching can be subjective as growth rates can be affected by hatching date (Masterov, 2000) and the quantity of food provisioned (Bortolotti, 1989). Consequently, these nuances in aging nestlings need to be considered carefully when applying Equation 3 to late-stage Tasmanian wedge-tailed eagle nestlings.

The discriminant functions we have reported have a slightly lower accuracy than equivalent functions developed for golden eagles (*Aquila chrysaetos*) (100%; Harmata and Montopoli, 2013) and Bonelli's eagles (*Aquila fasciata*) (100%; García *et al.*, 2013). This finding is consistent with the suggestion that wedge-tailed eagles show less sexual dimorphism than other *Aquila* species (Olsen, 2005). Additionally, the degree of sexual dimorphism of

analogous tarsus, head and bill measurements were less for wedge-tailed eagles than for congeners such as golden eagles (0.3–5.6% more dimorphic; Harmata and Montopoli, 2013), Bonelli's eagles (1.9–12.8% more dimorphic; García *et al.*, 2013) and Spanish imperial eagles (4.5–21.3% more dimorphic; Ferrer and De Le Court, 1992). However, forearm and hallux measurements were slightly more dimorphic (+0.1–2.4%) for Tasmanian wedge-tailed eagles than for congeners (Ferrer and De Le Court, 1992; García *et al.*, 2013; Harmata and Montopoli, 2013). This is likely why these measurements were so important for differentiating sex of birds of this species.

### *Geographical range of applicability*

Our study provides further evidence for a larger body size of Tasmanian wedge-tailed eagles, since all comparable morphometric measurements were larger than the mainland subspecies (Brooker, 1996; Nankervis, 2010). This difference is substantial, with many average measurements for mainland females being smaller than those for Tasmanian males. For instance, reported average hallux lengths of mainland females, at 42.9 mm (Nankervis, 2010) and bill width, at 20 mm (Brooker, 1996), are shorter than the respective 45.2 mm and 21 mm lengths found for males in this study. The larger size of the Tasmanian wedge-tailed eagle relative to mainland populations likely necessitates the recalibration of the discriminant functions before they can be applied to birds outside of Tasmania (Smith *et al.*, 2016). Further work should also investigate Bergmann's Ecogeographical Rule of Clinal Variation (Ashton, 2002) in mainland wedge-tailed eagles to clarify the range of applicability of any future sexing criteria developed.

## ***Conclusion***

We demonstrate that discriminant functions can be used to sex Tasmanian wedge-tailed eagles with confidence. Non-invasive sex identification of live birds will help clarify behavioural differences between the sexes. Furthermore, sex identification of eagle carcasses is an important aspect of understanding conservation threats (Ferrer and Hiraldo, 1992). However, the nature of the injury and scavenging of the carcass can limit morphometric measurements available. The single morphometric cut-off values and associated accuracy we have included in this study provide an additional tool in identifying the sex of incomplete eagle remains.

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## Chapter 6

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### General discussion



Image J. Pay

The Tasmanian wedge-tailed eagle is a top predator that has a critical role in Tasmanian ecosystems, but is currently listed as endangered (Commonwealth of Australia, 1999; State Government of Tasmania, 1995). The threatening processes that contribute to its conservation status are not well understood. The overarching aim of my research was to address knowledge gaps on the ecology and threats faced by Tasmanian wedge-tailed eagles in order to inform conservation management. Eagle management in Tasmania currently focuses on nest sites and protecting breeding eagles from anthropogenic sources of disturbance (FPA, 2014; Threatened Species Section, 2006). My research has shown that this approach, while important, is limited in scope and does not address a number of additional threatening processes. This study has also underscored priority remaining knowledge gaps to be addressed for optimal conservation management outcomes.

I showed that two environmental contaminants, hitherto almost unrecognised as potential threats in Australia, are likely to adversely impact survival rates within the population. The tissue lead concentrations I detected in both adult and immature birds were at a level and prevalence comparable to other raptor studies that have identified lead exposure as a major conservation threat (Berny *et al.*, 2015; Rodriguez-Ramos Fernandez *et al.*, 2011). I also found one of the highest rates and levels of anticoagulant rodenticide (AR) exposure recorded in any raptor species worldwide, with the more persistent second-generation ARs (SGARs) posing a particular issue. Furthermore, my results suggest that both agricultural and residential AR use are contributing to the exposure in the Tasmanian wedge-tailed eagle. ARs had not been considered as a threat to the Tasmania wedge-tailed eagle, which is not known to regularly feed on synanthropic rodents (Debus *et al.*, 2007; Olsen *et al.*, 2010). Since neither of these sources of toxicity had been recognised as a conservation priority for the Tasmanian wedge-tailed eagle,

there is currently no management in place to address these threats. Furthermore, these contaminants may be an overlooked threatening process for other raptors and predators in Tasmanian and wider Australia (Hampton *et al.*, 2017; Lohr and Davis, 2018).

I also carried out the first detailed research on the behaviour of juvenile Tasmanian wedge-tailed eagles, thereby identifying other areas where management needs improvement. Using GPS-tracking, I found that juvenile eagles stayed substantially longer in their natal territory than expected (147–575 days compared to previous estimates of 90–180 days; Allott *et al.*, 2006; Bell & Mooney, 1998; Debus *et al.*, 2007; Hatton *et al.*, 2015; Olsen, 2005), and remained strongly associated with their nest for extended periods after fledging (24–58 days). Current regulations to reduce disturbance at eagle nests focus on the breeding season, only up until fledging (FPA, 2014). Given this prolonged period of dependence around the nest, these regulations may also need to address disturbance near nests after the chicks have fledged. I also identified key habitat associations of juvenile Tasmanian wedge-tailed eagles, demonstrating the importance of forest edge habitats for multiple behaviours and a general avoidance of open areas and large patches of silviculture plantation. The impacts of land-use changes on juvenile birds have not previously been considered; my work can now be applied to guide assessments.

In addition to the contributions my research has made to identifying new threats and management considerations, I have also provided tools for more efficiently assessing impacts. Habitat selection models I created performed well in predicting where juvenile wedge-tailed eagles, prior to dispersal, are likely to fly in Tasmania. These models can be used to identify high risk areas for power line and wind turbine collisions, which can be prioritised for mitigation (e.g. flight diverters; Bernardino *et al.*, 2018) and used to assess the environmental impacts of future infrastructure. Furthermore, the approach I developed to identify the sex of birds using

morphological measurements represents an easy, cost-effective method to obtain sex-specific information on the impacts of different threats. The equations also identify cases where morphological sex identification may be in doubt, and genetic sex identification of an individual should be prioritised.

Taken together, these findings have several management implications for Tasmanian wedge-tailed eagle conservation moving forward. Recommended management actions and their corresponding justification are summarised in Table 6.1.

### *Identifying future conservation management priorities*

Modelling future population changes under different scenarios will be important to the successful planning of the conservation management of the Tasmanian wedge-tailed eagle moving forward. My findings provide information that can be used to inform an updated Population Viability Analysis (PVA) for the Tasmanian wedge-tailed eagle.

PVAs provide a technique for modelling population trajectories under different management scenarios, generating quantitative predictions on population changes over time (Boyce, 1992; Burgman *et al.*, 2000). These models incorporate demographic and environmental variables to predict how populations will respond to environmental change (e.g. habitat loss or unnatural increases in mortality; Carrete *et al.*, 2009; Heinsohn *et al.*, 2015) and quantify the risk of extinction (Boyce, 1992). Although the accuracy of the quantitative PVA results may be limited (Fantle-Lepczyk *et al.*, 2018), sensitivity analyses can identify the most significant threatening processes (Boyce, 1992). This makes PVAs particularly valuable in evaluating the effectiveness of different management options in aiding the conservation of a population or

Table 6.1. Summary of threats addressed in the work presented in this thesis and the recommended management actions based on the findings.

Threat to address	Management action	Justification
Lead exposure	Promote lead-free alternatives to ammunition  Restrict sale and use of lead-based bullets <sup>1</sup>	<b>Chapter 2</b> - Toxic levels of lead found to be prevalent in free-flying birds and elevated in some nestlings
Second generation anticoagulant rodenticide (SGAR) exposure	Increase legislative control of SGARs <sup>2</sup>  Remove SGARs from public retail  Improve user awareness and consider review of correct usage practices (residential and agricultural)	<b>Chapter 3</b> - High levels and rates of exposure to SGARs in the population  Exposure positively associated with agricultural area and human population density  High exposure to a SGAR primarily available through agricultural suppliers (flocoumafen)
Loss of important habitats for juvenile eagles during the post-fledging dependence period	Promote retention forestry and retention of forest patches in agricultural areas <sup>3</sup>  Promote the incorporation of patches of native vegetation in plantations for silviculture	<b>Chapter 4</b> - Avoidance of open areas and large patches of silviculture plantation exhibited by juvenile Tasmanian wedge-tailed eagles  Strong selection for the edges of native forests exhibited by juvenile Tasmanian wedge-tailed eagles
Electrocutions and power line collisions  Wind turbine strikes	Require designers and regulators to incorporate habitat selection models when designing location <sup>4</sup> and impact mitigation <sup>5</sup> of powerline and wind turbine infrastructure	<b>Chapter 4</b> - Spatial models created performed well in predicting where post-fledging dependent juvenile eagles carry out short (lower altitude) and long (higher altitude) flights in Tasmania
Demographic consequences of causes of unnatural mortality	Establish a standard protocol for all injured and dead eagles to have their sex identified using morphological techniques  Prioritise eagles with morphological discriminant scores close to zero for genetic sex ID	<b>Chapter 5</b> - Discriminant function equations identified the sex of individuals with 97.6% accuracy  Equations include thresholds where discriminant scores have a less than 75% probability of correctly classifying sex

1 Kelly, T.R., Bloom, P.H., Torres, S.G., Hernandez, Y.Z., Poppenga, R.H., Boyce, W.M., & Johnson, C.K. (2011) Impact of the California lead ammunition ban on reducing lead exposure in golden eagles and turkey vultures. *PLoS ONE* **6**, e17656.

2 United States Environment Protection Agency (2017) Restrictions on Rodenticide Products [WWW Document]. URL <https://www.epa.gov/rodenticides/restrictions-rodenticide-products> (accessed 3.14.18).

3 Lindenmayer, D.B., Franklin, J.F., Löhms, A., Baker, S.C., Bauhus, J., Beese, W., Brodie, A., Kiehl, B., Kouki, J., Pastur, G.M., Messier, C., Neyland, M., Palik, B., Sverdrup-Thygeson, A., Volney, J., Wayne, A., & Gustafsson, L. (2012) A major shift to the retention approach for forestry can help resolve some global forest sustainability issues. *Conservation Letters* **5**, 421–431.

4 Miller, T., Brooks, R., Lanzone, D., Cooper, J., O'Malley, K., Maisonneuve, C., Tremblay, J., Duerr, A., & Katzner, T. (2014) Assessing risk to birds from industrial wind energy development via paired resource selection models. *Conservation Biology* **28**, 745–755.

5 Bernardino, J., Bevanger, K., Barrientos, R., Dwyer, J.F., Marques, A.T., Martins, R.C., Shaw, J.M., Silva, J.P., & Moreira, F. (2018) Bird collisions with power lines: State of the art and priority areas for research. *Biological Conservation* **222**, 1–13.

species, which can then be used to prioritise the use of limited conservation resources (Akçakaya and Sjögren-Gulve, 2000). As such, PVAs have been used to develop management strategies for numerous species (Ebenhard, 2000; Heinsohn *et al.*, 2004).

One published PVA currently exists for the Tasmanian wedge-tailed eagle (Bekessy *et al.*, 2009), but it only applies to a small part of Tasmania and very few life history data were available at the time. This PVA predicted a population decline over the next 160 years if rates of mortality and disturbance to breeding eagles continued at the levels modelled. However, the model is confined to the Bass District, an area incorporating approximately 17% of the entire state, which limits inferences on changes at the population level. Furthermore, the majority of life history data included in the model was based on expert opinion and extrapolated data from conspecifics. Although valuable, such data may not be detailed enough for a PVA to accurately estimate population trends in different scenarios (Morris *et al.*, 2002). Finally, the PVA did not incorporate sex differences between parameters, which could significantly affect its predictive ability. For example, females in raptor species can be more susceptible to power line collisions (Dwyer and Mannan, 2007; Ferrer and Hiraldo, 1992). These limitations and the conservation status of the Tasmanian wedge-tailed call for an updated PVA at a state-wide scale.

My findings provide a range of information on the threats and life-history of Tasmanian wedge-tailed eagles which was not incorporated in the previous PVA. My morphological sex determination technique, combined with improved protocols to identify cause of death, will help identify any sex biases in mortality. Additionally, I have identified that lead and anticoagulant rodenticides are threats which should be included in future population models. The GPS tracking of juvenile birds has also identified an extended period of dependency and information on first-year survival rates, which includes some key differences from the values used in the current

PVA. For example, the first-year survival rate of 87.5% was much higher than the previous estimates of 50–60% (Bekessy *et al.*, 2009; Bell and Mooney, 1998). The incorporation of this life history information into updated models will be critical to accurate quantitative predictions of population change.

### *Future research needs*

As well as providing valuable info to guide the conservation of Tasmanian wedge-tailed eagles, my research has highlighted significant remaining knowledge gaps. My study has improved understanding of juvenile behaviour and life-history traits, but similar information on dispersing eagles is still needed. In non-migratory raptor populations, distances travelled by immature birds during dispersal are much greater than those that they travel once they have entered the breeding population (Serrano, 2018). This makes natal dispersal a particularly important life-stage to study, as it is the primary driver of gene flow in such populations (Weston *et al.*, 2018). The GPS-tracked Tasmanian wedge-tailed eagles described in Chapter 4 will continue to generate movement data for at least three years (Cellular Tracking Technologies pers. comm.), providing insight into the duration and distance of natal dispersal and related mortality rates. Similar research on adults will be key to clarifying life-history details and potential threats once birds have entered the breeding population.

In providing evidence of lead and AR exposure as threats, my study has identified a need for accurate estimates of the extent of this exposure and the population impacts, to guide management and contribute to the updated PVA. For lead, this could be achieved through assessment of lead levels in blood from a random sample of live-caught birds. My work relied on

an opportunistic sample of eagles found dead or moribund, which can underestimate the rate of fatal poisonings due to birds dying in remote areas where they are unlikely to be discovered. Equally, such opportunistic samples can overestimate sub-lethal poisonings, as sick birds may be more likely to collide with anthropogenic structures (Kelly and Kelly, 2005) and die in areas where they are likely to be found. However, a similar approach is less feasible for ARs, due to their short persistence in blood plasma (Van Den Brink *et al.*, 2018). The presence of other environmental contaminants in the Tasmanian landscape, such as organochlorine pesticides (OCPs) and polybrominated biphenyl ether flame retardants (PBDEs) may also warrant more research, especially as they have been documented in other predators, including Tasmanian devils (*Sarcophilus harrisii*; Vetter *et al.*, 2008).

Another question of key concern is the effectiveness and efficiency of current nest management regulations at reducing the impacts of anthropogenic disturbance. Population recovery plans have identified nest disturbance and the associated low level of breeding success as the major factor limiting the size and density of the population (Bell and Mooney, 1998; Threatened Species Section, 2006). Current management recommendations were developed after an initial study into the effects of disturbance (Mooney and Holdsworth, 1991), and have evolved over time to now involve nest searching in areas to be harvested or impacted, surveying nests for breeding activity, establishment of 10 ha nest reserves and strict restrictions on human activities within 500 m/1 km line-of-sight of nests with breeding eagles (FPA, 2014). These management prescriptions involve considerable costs for numerous industries, particularly forestry.

Nesting success research needs to focus on the behavioural response of the eagles to different sources of disturbance. Previous work has suggested current restrictions are effective in preventing detrimental disturbance to breeding eagles (Forest Practices Authority, 2013; Mooney

and Taylor, 1996). However, these studies used breeding success as the measure of disturbance, rather than assessing fine-scale behavioural responses. O'Sullivan (2014), attempted to assess the behavioural response to disturbance, but the observation technique used caused high rates of nest abandonment (10 of 12 monitored nests resulted in a nest failure). Although these nest abandonments confirmed that the eagles are very sensitive to disturbance during the breeding season, they did not provide an assessment of the efficacy of current regulations.

In work not presented in this thesis, I endeavoured to address this knowledge gap and identified logistical constraints that must be addressed in order to answer this question in the future. With increasing pressure to reduce the current regulations, my goal was to experimentally assess the impacts of a novel disturbance introduced at the minimum allowable distance (500 m) from nests with breeding eagles. However, measuring the behavioural response of the eagles proved challenging without causing additional and potentially severe disturbance. For many raptors, such studies can be carried out by observers positioned far from the nest with telescopes (Arroyo and Razin, 2006; Cosgrove *et al.*, 2017; Grubb *et al.*, 2010); this is not an option for Tasmanian wedge-tailed eagles, since they typically nest in sheltered areas surrounded by closed forest. I therefore explored various camera technologies, but the isolated locations of study nests and the inability to service camera set-ups without causing disturbance limited the frequency of camera footage and thus the detail of behaviours recorded.

A final area of important future research is an ongoing population monitoring program to clarify the vulnerability of the population and validate the efficacy of current or introduced management prescriptions. The last population estimate for the subspecies, of between 1000 and 1500 birds, was proposed in 2006 (Threatened Species Section, 2006). This was based on an approximation of 426 territories in the state and estimates of territory occupancy, productivity

and mortality. Notably, the estimate assumes 50% mortality of juveniles, which is much higher than the 12.5% I found in my research. Further, many changes have occurred since this time which may have affected reproduction and survival rates. According to my own findings, exposure to rodenticides has increased markedly. Additionally, nest protection regulations have been strictly maintained, food availability is likely to have increased with the decline in Tasmanian devils, and there have been increases in numbers of wind turbines across Tasmania. Continued GPS tracking to verify mortality of different age classes and tracking of adults to verify territory size estimates will be important for a renewed population estimate. Furthermore, long-term monitoring of population trends will help determine whether the population is growing, declining or stable. The recently established “Where? Where? Wedgie!” citizen science program (C. Hawkins, in prep) is currently developing an approach to monitor population trends and to potentially contribute to population estimates. These combined efforts will continue the research efforts begun here, and further enhance our understanding of the conservation ecology of the Tasmanian wedge-tailed eagle.

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## Appendix A

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### Supplementary materials to Chapter 2



Image D. James

### *A.1. Age categories of free-flying birds*

Eagle carcasses were categorised into two age classes (immature and adult) based on plumage differences. Immatures are estimated to include all juveniles to approximately 5th year birds (Ridpath and Brooker, 1986). Plumage characteristics used to identify immature eagles were a pale nape, broad dorsal wing bars (more than quarter of wing width; Debus, 2012), barring apparent on remiges and rectrices, pale tail coverts and light brown elements to back feathers (Debus, 2012; Olsen, 2005; Ridpath and Brooker, 1986 see Figure A.1.1). Birds categorised as adults had darker plumages (particularly on back, chest, nape and neck), narrower dorsal wing bars (less than quarter of wing width; Debus, 2012), brown tail coverts and a pale base to flight feathers (Debus, 2012; Ridpath and Brooker, 1986; see Figure A.1.2).

A more accurate method to determine age using a moult chart (e.g. Bloom & Clark, 2001) has not been developed for the species. In lieu of a moult chart not being available we had to use the plumage characteristics described above. However, there are some important considerations with this aging method. First, immature plumage can remain into the year that eagles become mature and population stability (i.e. levels of unnatural mortality and the number of non-territorial, non-breeding adults) can influence the age at which birds breed (Bell and Mooney, 1998; J. Wiersma pers. comm). Second, without a moult chart it is too subjective to separate pre-dispersal juveniles from other immatures. Therefore, we used the two age classes described, representing birds definitely of breeding age (adults) and all younger birds (immatures).



Figure A.1.1. Example images of birds classified as immature showing the different plumage characteristics; a) dorsal image showing paler brown across back and pale nape, b) ventral image showing pale elements on contour feathers, c) dorsal wing showing wing bar and barring on remiges, d) ventral wing showing barring on remiges, e) ventral tail showing barring on rectrices and pale tail coverts, f) dorsal tail showing pale tail coverts and barring on rectrices.



Figure A.1.2. Example images of birds classified as adult showing the different plumage characteristics; a) dorsal image showing dark contour feathers across back, b) ventral image showing dark contour feathers across body, c) dorsal wing showing narrow wing bar, d) ventral wing showing pale base to flight feathers, e) ventral tail and f) dorsal tail showing brown tail coverts and lack of barring on rectrices.

## References

- Bell, P., & Mooney, N., (1998). *Wedge-tailed Eagle Recovery Plan 1998-2003*. Department of Primary Industries, Parks, Water and Environment, Hobart.
- Bloom, P.H., & Clark, W.S., (2001). Molt and sequence of plumages of golden eagles and a technique for in-hand ageing. *North American Bird Bander* **26**, 97–116.
- Debus, S., (2012). *Birds of prey of Australia*. CSIRO Publishing, Clayton South.
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- Ridpath, M.G., & Brooker, M.G., (1986). Age, movements and the management of the wedge-tailed eagle, *aquila audax*, in arid Western Australia. *Wildlife Research* **13**, 245–260.

### A.2. Correlation between liver and femur lead

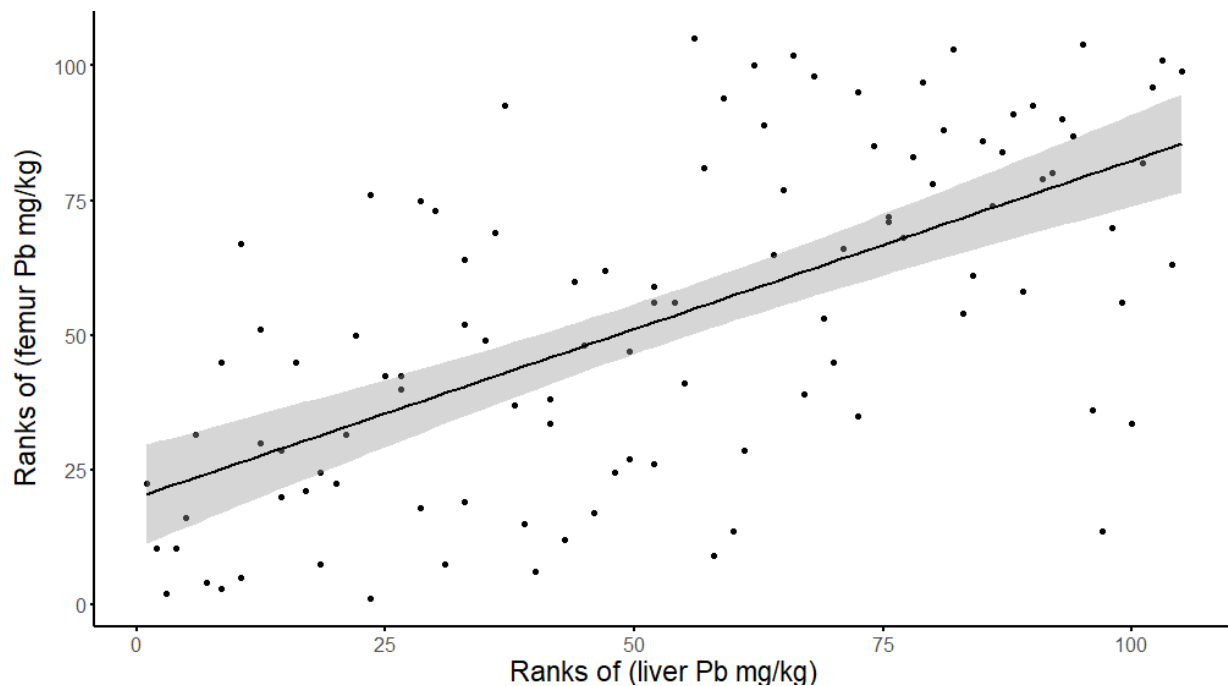


Figure A.2.1. Association between ranked Pb concentrations in liver and femur samples from eagles where both sample types were collected ( $n = 105$ ). The 95% CI is indicated by the shaded area.

### A.3. Seasonal patterns in lead exposure

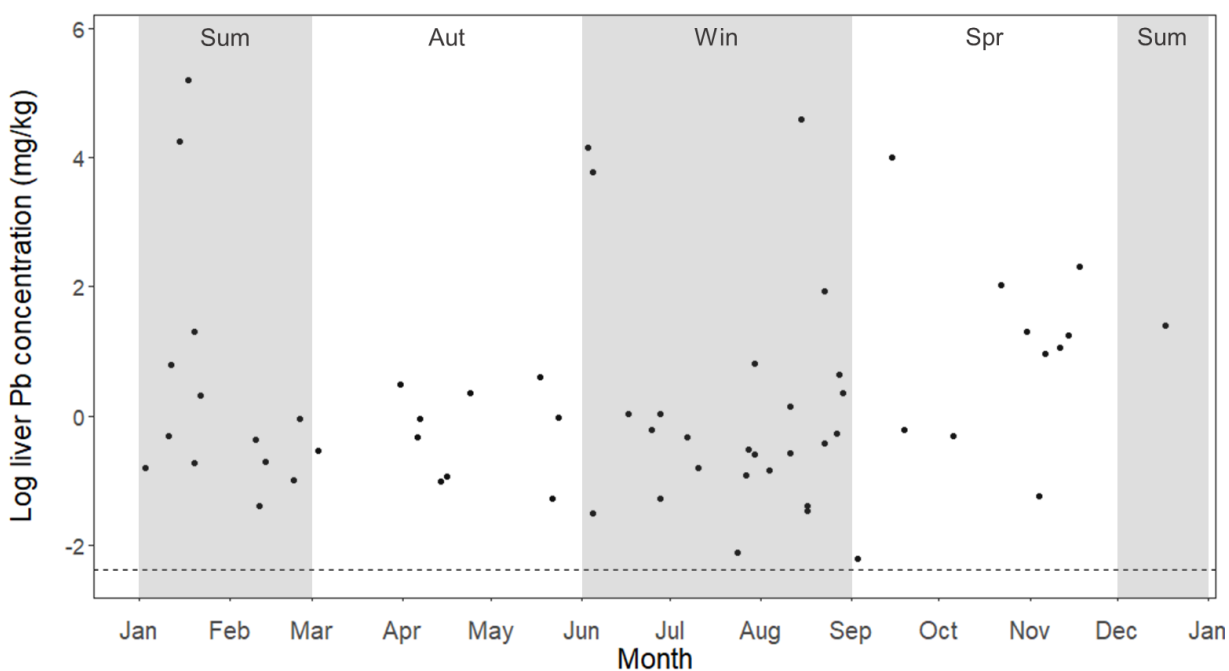


Figure A.3.1. Liver lead concentrations of each eagle and the day of the year the carcass was discovered ( $n = 61$ ). Shaded areas differentiate the seasons.

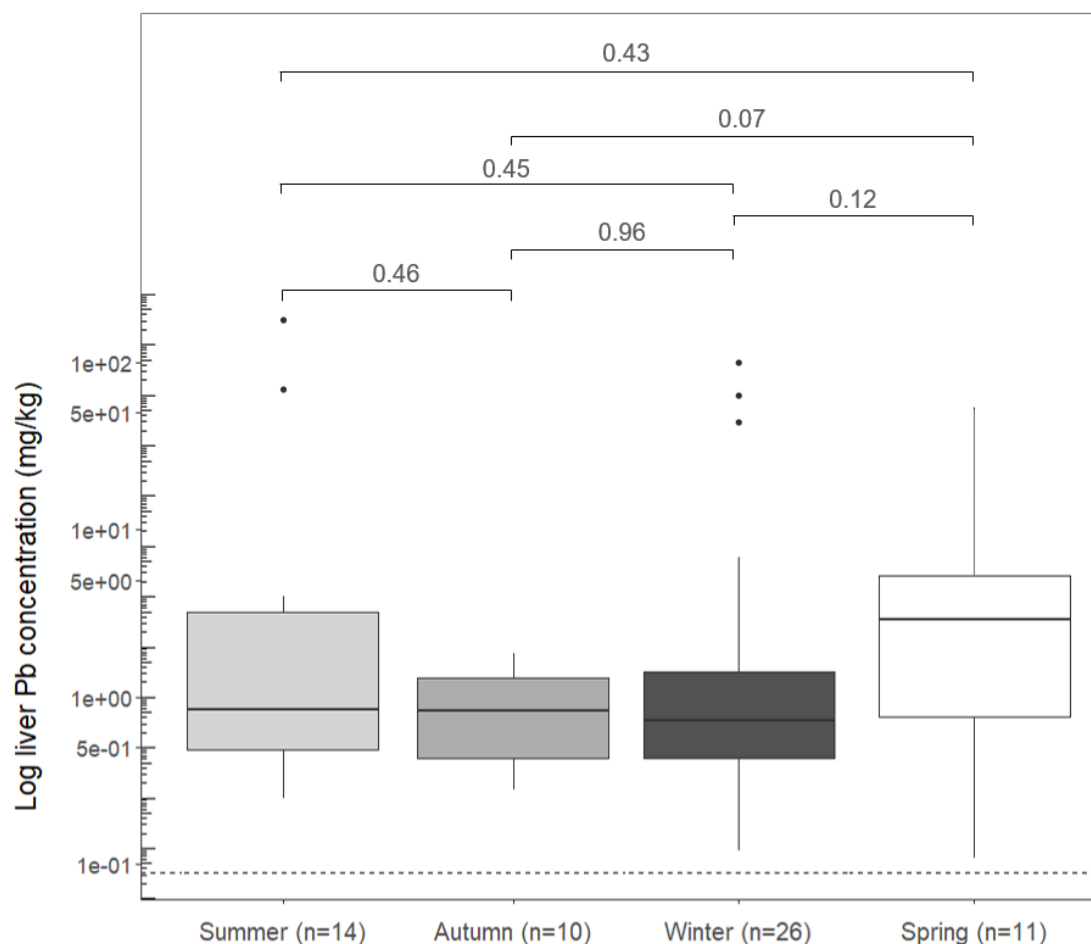


Figure A.3.2. Censored boxplots of liver pb concentrations in the season the eagles died. Whiskers are extended to maximum values within 150% of the interquartile range, values beyond this are plotted individually as outliers. The significance ( $p$  value) of pairwise comparisons between seasons are shown top.

#### A.4. Isotopic differences between exposure categories

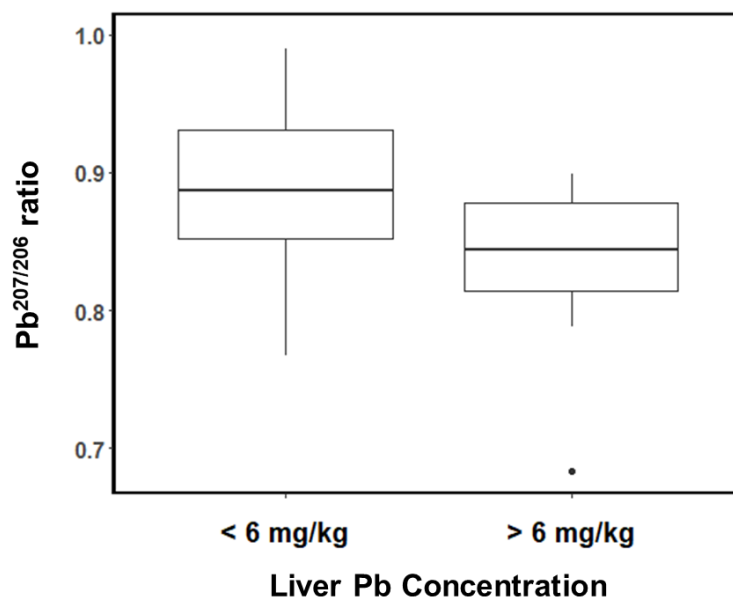


Figure A.4.1. Box plot of the differences in lead<sup>207/206</sup> isotope ratios between birds with background (< 6 mg/kg) and birds with elevated (> 6 mg/kg) liver lead levels. Whiskers are extended to maximum values within 150% of the interquartile range, values beyond this are plotted individually as outliers.

## Appendix B

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### Supplementary materials to Chapter 3



Image D. James

Table B.1. Limits of detection (LOD), limits of quantification (LOQ) and average recovery rates of LC-MS calculated from chicken livers spiked with three working solutions of each AR standard.

Compound	LOD (mg/kg)	LOQ (mg/kg)	Average recovery % (RSD)
Brodifacoum	0.0025	0.0050	100.7 (4.5)
Bromadiolone	0.0005	0.0010	106.3 (5.9)
Coumatetralyl	0.0010	0.0020	101.8 (2.9)
Difenacoum	0.0013	0.0025	94.4 (4.6)
Difethialone	0.0050	0.0100	101.3 (4.3)
Flocoumafen	0.0013	0.0025	96.9 (7.1)
Pindone	0.0125	0.0250	66.4 (6.0)
Warfarin	0.0010	0.0020	106.2 (6.2)

Table B.2. The assumed cause of death recorded for each individual at the time of carcass collection. The total number of samples in each cause of death category as well as the mean summed AR burden are presented.

Assumed cause of death	N	Mean AR
Power line collision or electrocution	27	0.254
Road vehicle collision	6	0.349
Suspected shot	2	< LOD
Starvation	1	0.271
Lead poisoning	1	0.318
Unknown	13	0.542

Table B.3. Comparison of models included in the selection set to determine variables that influence the concentrations of ARs found in Tasmanian wedge-tailed eagle livers. Models are ranked by AICc.

<b>Model Variables</b>	<b>df</b>	<b>AICc</b>	<b><math>\Delta</math>AICc</b>	<b>AICc weight</b>
Year of death + Agricultural area + Human population density	5	77.183	0	0.449
Year of death + Agricultural area	4	78.726	1.543	0.208
Full model	7	80.280	3.097	0.095
Year of death	3	80.955	3.772	0.068
Year of death + Human population density	4	81.354	4.170	0.056
Null model	2	82.240	5.057	0.036
Age	3	82.981	5.798	0.025
Agricultural area	3	83.217	6.033	0.022
Human population density	3	83.402	6.219	0.020
Agricultural area + Human population density	4	84.100	6.890	0.014
Sex	3	84.478	7.295	0.012

Table B.4. Comparison of models included in the selection set to determine variables that influence the probability of AR detection in Tasmanian wedge-tailed eagle livers. Models are ranked by AICc.

<b>Model Variables</b>	<b>df</b>	<b>AICc</b>	<b><math>\Delta</math>AICc</b>	<b>AIC weight</b>
Year of death + Agricultural area	3	55.099	0	0.423
Year of death + Agricultural area + Human population density	4	56.292	1.194	0.233
Year of death	2	57.471	2.373	0.129
Null model	1	59.389	4.290	0.049
Year of death + Human population density	3	59.477	4.378	0.047
Agricultural area	2	60.369	5.270	0.030
Sex	2	60.873	5.775	0.024
Full model	6	61.258	6.160	0.019
Age	2	61.477	6.379	0.017
Human population density	2	61.498	6.399	0.017
Agricultural area + Human population density	3	62.506	7.407	0.010

Table B.5. Published estimations of flocoumafen exposure in raptor species. The species/studies are ordered by the percent of individuals with flocoumafen residues detected. The present study is highlighted in bold. The only work showing higher flocoumafen exposure rates for a raptor species have very low sample sizes.

Study	Species	Location	Individuals (n)	Flocoumafen exposed (%)
Sanchez-Barbudo <i>et al.</i> , 2012	Short-toed snake eagle	Spain	1	100
Koivisto <i>et al.</i> , 2016	Goshawk	Finland	2	50
<b>Present study</b>	<b>Tasmanian wedge-tailed eagle</b>	<b>Tasmania</b>	<b>50</b>	<b>40</b>
Sanchez-Barbudo <i>et al.</i> , 2012	Black kite	Spain	5	40
Lopez-Perea <i>et al.</i> , 2015	Eagle owl	Spain	14	28.6
Sanchez-Barbudo <i>et al.</i> , 2012	Eagle owl	Spain	7	28.6
Christensen <i>et al.</i> , 2012	Kestrel	Denmark	66	27.3
Herring <i>et al.</i> , 2017*	Golden eagles	US and Europe	48	27
Lopez-Perea <i>et al.</i> , 2015	Tawny owl	Spain	27	25.7
Sanchez-Barbudo <i>et al.</i> , 2012	Red kite	Spain	8	25
Koivisto <i>et al.</i> , 2016	Eagle owl	Finland	12	25
Christensen <i>et al.</i> , 2012	Barn owl	Denmark	80	19.9
Koivisto <i>et al.</i> , 2016	Tawny owl	Finland	13	15
Langford <i>et al.</i> , 2013	Eagle owl	Norway	8	12.5
Langford <i>et al.</i> , 2013	Golden eagles	Norway	16	12.5
Sanchez-Barbudo <i>et al.</i> , 2012	Spanish imperial eagle	Spain	8	12.5
Christensen <i>et al.</i> , 2012	Tawny owl	Denmark	44	11.4
Langford <i>et al.</i> , 2013	Total raptors	Norway	30	10
Lopez-Perea <i>et al.</i> , 2015	Total raptors	Spain	344	8.7
Christensen <i>et al.</i> , 2012	Long eared owl	Denmark	38	7.9
Lohr, 2018	Boobook	Western Australia	73	2.7

\*This is a review of the golden eagle literature including 48 birds in total.

Table B.6. Summary table of the AR raptor literature modified from Lohr (2018). For each species/study the location of the study, the number of individuals sampled, the percentage of samples with ARs detected, the percentage of samples above two thresholds, the mean total AR exposure and the method to calculate the mean are presented. The present study is highlighted in bold.

Species	Location	n	% ARs detected	% >0.1 mg/kg	% >0.2 mg/kg	Mean exposure (mg/kg)	Mean method	Study
<b>Tasmanian wedge-tailed eagle</b>	<b>Australia</b>	<b>50</b>	<b>74</b>	<b>54</b>	<b>46</b>	<b>0.448</b>	<b>Exposed</b>	<b>Present study</b>
Barn owl	Denmark	80	94	37.4	13.7	0.1141	Unclassified	Christensen <i>et al.</i> , 2012
Buzzard	Denmark	141	94	20.6	5.7	0.0745	Unclassified	Christensen <i>et al.</i> , 2012
Eagle owl	Denmark	10	100	70	70	0.1931	Unclassified	Christensen <i>et al.</i> , 2012
Kestrel	Denmark	66	89	27.2	13.6	0.099	Unclassified	Christensen <i>et al.</i> , 2012
Little owl	Denmark	9	100	33.3	22.2	0.1186	Unclassified	Christensen <i>et al.</i> , 2012
Long-eared owl	Denmark	38	95	0	0	0.0194	Unclassified	Christensen <i>et al.</i> , 2012
Marsh harrier	Denmark	3	100	0	0	0.0123	Unclassified	Christensen <i>et al.</i> , 2012
Red Kite	Denmark	3	100	0	66.7	0.413	Unclassified	Christensen <i>et al.</i> , 2012
Rough-legged buzzard	Denmark	31	84	12.9	0	0.0408	Unclassified	Christensen <i>et al.</i> , 2012
Short-eared owl	Denmark	5	100	0	0	0.015	Unclassified	Christensen <i>et al.</i> , 2012
Tawny owl	Denmark	44	93	20.5	9.1	0.0784	Unclassified	Christensen <i>et al.</i> , 2012
Barn owl	Scotland	63	34.9		17.5	0.076	Exposed	Hughes <i>et al.</i> , 2013
Buzzard	Scotland	479	44.3		2.1	0.047	Exposed	Hughes <i>et al.</i> , 2013
Eurasian sparrowhawk	Scotland	37	54.1		2.7	0.06	Exposed	Hughes <i>et al.</i> , 2013
Kestrel	Scotland	22	40.9		9.1	0.173	Exposed	Hughes <i>et al.</i> , 2013
Peregrine falcon	Scotland	24	29.2		0	0.017	Exposed	Hughes <i>et al.</i> , 2013
Red Kite	Scotland	114	69.3		17.5	0.155	Exposed	Hughes <i>et al.</i> , 2013
Tawny owl	Scotland	34	38.2		2.9	0.047	Exposed	Hughes <i>et al.</i> , 2013
Eagle owl	Finland	12					NA	Koivisto <i>et al.</i> , 2016
Eurasian sparrowhawk	Finland	1					NA	Koivisto <i>et al.</i> , 2016
Northern goshawk	Finland	2					NA	Koivisto <i>et al.</i> , 2016
Hen harrier	Finland	1					NA	Koivisto <i>et al.</i> , 2016
Tawny owl	Finland	13					NA	Koivisto <i>et al.</i> , 2016
White-tailed sea eagle	Finland	1					NA	Koivisto <i>et al.</i> , 2016
Eagle owl	Norway	8	62.5	37.5	12.5	0.087	All	Langford <i>et al.</i> , 2013
Golden eagle	Norway	16	73.3	25	6.3	0.051	All	Langford <i>et al.</i> , 2013
Gryfalcon	Norway	1	0	0	0	0	All	Langford <i>et al.</i> , 2013
Osprey	Norway	3	0	0	0	0	All	Langford <i>et al.</i> , 2013
Peregrine falcon	Norway	2	0	0	0	0	All	Langford <i>et al.</i> , 2013
Southern boobook	Australia	73	72.6	50.7	35.6	0.31	All	Lohr, 2018
Barn owl	Spain	19	84.2		57.9	0.2337	Geometric	López-Perea <i>et al.</i> , 2015
Barn owl	Spain	22	54.5		13.6	0.1178	Geometric	López-Perea <i>et al.</i> , 2015
Common buzzard	Spain	56	64.3		26.8	0.1253	Geometric	López-Perea <i>et al.</i> , 2015
Eagle owl	Spain	14	100		64.3	0.2896	Geometric	López-Perea <i>et al.</i> , 2015
Little owl	Spain	7	71.4		28.6	0.1972	Geometric	López-Perea <i>et al.</i> , 2015
Long-eared owl	Spain	12	58.3		0	0.0111	Geometric	López-Perea <i>et al.</i> , 2015
Scops owl	Spain	7	14.3		0	0.1584	Geometric	López-Perea <i>et al.</i> , 2015

Species	Location	n	% ARs detected	% >0.1 mg/kg	% >0.2 mg/kg	Mean exposure (mg/kg)	Mean method	Study
Scops owl	Spain	26	57.7		0	0.0134	Geometric	López-Perea <i>et al.</i> , 2015
Tawny owl	Spain	27	77.8		29.6	0.0952	Geometric	López-Perea <i>et al.</i> , 2015
Barn owl	Spain	5	60			0.053	Exposed	López-Perea <i>et al.</i> , 2018
Bearded vulture	Spain	9	22			0.019	Exposed	López-Perea <i>et al.</i> , 2018
Black kite	Spain	6	33			0.079	Exposed	López-Perea <i>et al.</i> , 2018
Bonelli's eagle	Spain	1	0			0	Exposed	López-Perea <i>et al.</i> , 2018
Common buzzard	Spain	6	50			0.214	Exposed	López-Perea <i>et al.</i> , 2018
Eagle owl	Spain	8	63			0.279	Exposed	López-Perea <i>et al.</i> , 2018
Egyptian vulture	Spain	3	67			0.133	Exposed	López-Perea <i>et al.</i> , 2018
Eurasian griffon	Spain	42	19			0.021	Exposed	López-Perea <i>et al.</i> , 2018
Golden eagle	Spain	5	0			0	Exposed	López-Perea <i>et al.</i> , 2018
Lesser kestrel	Spain	7	0			0	Exposed	López-Perea <i>et al.</i> , 2018
Little owl	Spain	1	100			0.056	Exposed	López-Perea <i>et al.</i> , 2018
Long-eared owl	Spain	3	67			0.052	Exposed	López-Perea <i>et al.</i> , 2018
Marsh harrier	Spain	6	100			0.266	Exposed	López-Perea <i>et al.</i> , 2018
Red Kite	Spain	13	77			0.5	Exposed	López-Perea <i>et al.</i> , 2018
Short-toed snake eagle	Spain	2	50			0.022	Exposed	López-Perea <i>et al.</i> , 2018
Barred owl	USA	24	88				NA	Murray, 2017
Eastern screech-owl	USA	16	100				NA	Murray, 2017
Great Horned owl	USA	17	100				NA	Murray, 2017
Red-tailed hawk	USA	37	97				NA	Murray, 2017
Barbary Falcon	Spain	16	31.2			0.0915	Unclassified	Ruiz-Suárez <i>et al.</i> , 2014
Barn owl	Spain	21	76.2			0.1344	Unclassified	Ruiz-Suárez <i>et al.</i> , 2014
Common buzzard	Spain	9	26.3			0.0368	Unclassified	Ruiz-Suárez <i>et al.</i> , 2014
Eurasian sparrowhawk	Spain	14	85.7			0.0577	Unclassified	Ruiz-Suárez <i>et al.</i> , 2014
Kestrel	Spain	21	66.6			0.219	Unclassified	Ruiz-Suárez <i>et al.</i> , 2014
Long-eared owl	Spain	23	73.9			0.1322	Unclassified	Ruiz-Suárez <i>et al.</i> , 2014
Various raptor species	Spain	104	63.5	34.8			NA	Ruiz-Suárez <i>et al.</i> , 2014
Short-toed snake eagle	Spain	1	100	100	100	0.21	Unclassified	Sanchez-Barbudo <i>et al.</i> , 2012
Barn owl	UK	100	94	16			NA	Shore <i>et al.</i> , 2016
Great Horned owl	USA	22	82	36	9	0.07	Geometric	Stansley <i>et al.</i> , 2014
Red-tailed hawk	USA	105	81	47	25	0.117	Geometric	Stansley <i>et al.</i> , 2014
Bald eagle	Canada	4	25				NA	Thomas <i>et al.</i> , 2011
Great Horned owl	Canada	123				0.016	Unclassified	Thomas <i>et al.</i> , 2011
Red-tailed hawk	Canada	58				0.005	Unclassified	Thomas <i>et al.</i> , 2011
Tawny owl	UK	172	19.2	12.2	5.8	0.125	Unclassified	Walker <i>et al.</i> , 2008
Barn owl	UK	58	84	17.2			NA	Walker <i>et al.</i> , 2011
Kestrel	UK	20	100				NA	Walker <i>et al.</i> , 2011
Red Kite	UK	18	94				NA	Walker <i>et al.</i> , 2011

## Appendix C

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### Supplementary materials to Chapter 4



Image T. Katzner

Table C.1. Habitat categories included in the analysis. The corresponding data type, number of data bins and details of the source and calculation are provided.

Habitat category	Data type	Data bins	Source and calculation
Distance to forest edge	Continuous - distance (m) from the nearest forest edge. Areas outside of the forest receive positive values and areas within the forest receive negative values.	11	Calculated from TasVeg 3.0 (DPIPWE, 2013) updated using recent satellite imagery (June 2018 – February 2019) of each natal territory. All areas classified as forest types in TasVeg 3.0 (see Table C.2) were grouped. The interface between forest habitats and all non-forest habitats were considered as forest edge.
Distance to ridgeline	Continuous - distance (m) from the nearest ridgeline.	11	Calculated from Tasmania 25 metre Digital Elevation Model (DPIPWE, 2010) using ArcGIS “Map Algebra” and “Hydrology” tools.
Topographic ruggedness	Continuous - ranging 0–6.5. Higher values indicating higher topographic variation.	9	Calculated from Tasmania 25 metre Digital Elevation Model (DPIPWE, 2010) using “DEM Surface Tools” (Jenness, 2013) for ArcGIS. The ruggedness value of each cell is the 3-dimensional surface area of each cell (calculated from surrounding eight cells) divided by the flat area of the cell (625 m <sup>2</sup> ).
Aspect	Categorical - 16 cardinal directions	16	Calculated from Tasmania 25 metre Digital Elevation Model (DPIPWE, 2010) using ArcGIS “Aspect: 3D analyst” tool.
Habitat type	Categorical - 21 habitat types.	13	Modified habitat categories from TasVeg 3.0 (DPIPWE, 2013) updated using recent satellite imagery (June 2018 – February 2019) of each natal territory. Each ‘forest’ habitat type was further categorised into an associated ‘forest edge’ habitat 50m either side of the interface with an ‘open’ habitat type (i.e. a 50m buffer either side of the interface of ‘rainforest’ and ‘native grassland’ would be categorised as ‘rainforest edge’). See Table C.2 for the full list of habitat categories and types.
Slope	Continuous - ranging 0–90 degrees.	6	Calculated from Tasmania 25 metre Digital Elevation Model (DPIPWE, 2010) using ArcGIS “Slope: 3D analyst” tool.

Table C.2. List of TasVeg 3.0 habitat categories used in the analysis and how these habitats were grouped for the calculation of forest edge.

Habitat category	Grouped habitat type
Cleared land	Open Habitat
Highland and treeless vegetation	Open Habitat
Moorland, sedgeland, rushland and peatland	Open Habitat
Native grassland	Open Habitat
Non-native vegetation	Open Habitat
Other natural environments	Open Habitat
Saltmarsh and wetland	Open Habitat
Scrub, heathland and coastal complexes	Open Habitat
Plantation	Forest Habitat
Rainforest	Forest Habitat
Wet eucalypt forest and woodland	Forest Habitat
Dry eucalypt forest and woodland	Forest Habitat
Non eucalypt forest and woodland	Forest Habitat
Shelter belt	Forest Edge Habitat
Plantation edge	Forest Edge Habitat
Rainforest edge	Forest Edge Habitat
Wet eucalypt forest and woodland edge	Forest Edge Habitat
Dry eucalypt forest and woodland edge	Forest Edge Habitat

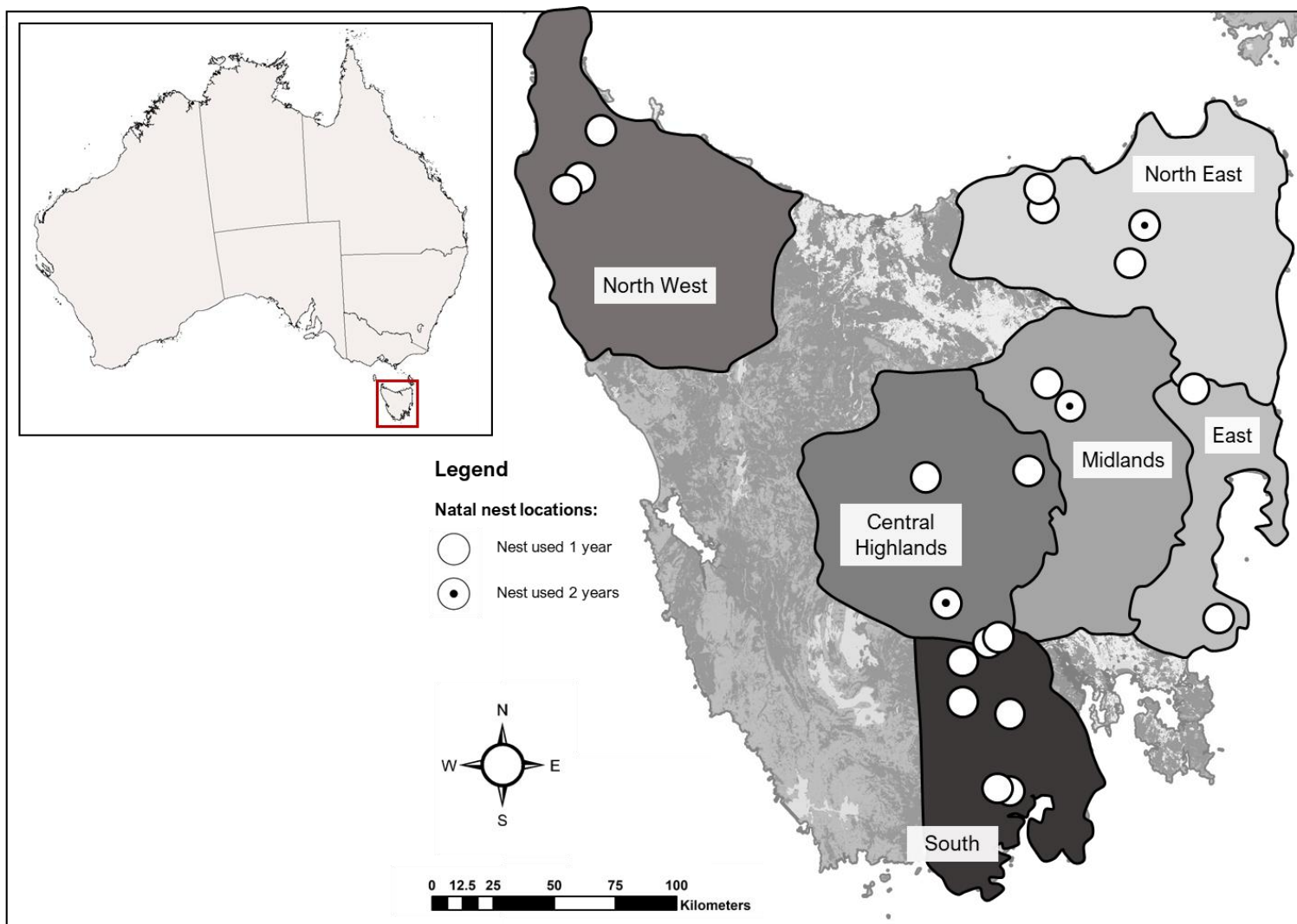


Figure C.1. Study location in Tasmania (Australia) with the spatial distribution of study nest sites. The region classification used to compare model performance in different areas of Tasmania are shown.

Table C.3. Tasmanian wedge-tailed eagle nestlings tracked during their post-fledging dependence period (PFDP) in this study with the estimated date of fledging, the total number of GPS fixes, the length of the PFDP (\* indicate juvenile eagles that were still in the PFDP at the time of analysis), the area of the MCP used for estimation of available habitat and the median and maximum distances between each 15-min GPS fix.

ID	Fledging date	GPS fixes	PFDP duration (days)	Available habitat area (km <sup>2</sup> )	Step distance (m)	
					Median	Max
Edward	30/01/2018	17000	408*	30.3	69.4	5465.1
Eggbert	15/02/2018	5299	193	26.7	28.6	3683.2
Eli	23/01/2018	5480	219	31.7	95.8	4964.6
Ellen	12/01/2018	8214	255	45.9	42.4	7078.2
Emilio	16/01/2018	6871	223	26.7	43.9	5695.0
Emma	22/01/2018	7481	254	33.6	35.0	5491.3
Engelbert	01/02/2018	17834	441*	94.5	62.7	5007.3
Enid	09/02/2018	13789	347*	10.4	32.4	3154.5
Erika	13/01/2018	7432	241	41.1	27.7	4763.7
Erin	24/01/2018	8428	261	76.8	66.8	3162.2
Ernie	02/02/2018	18191	440*	14.5	83.0	3862.2
Ethan	03/01/2018	8007	244	44.0	59.8	4677.0
Ethel	07/02/2018	7556	235	25.0	61.6	4616.2
Eva	24/01/2018	16093	449*	42.2	55.2	5044.5
Ezio	23/02/2018	5413	209	15.3	37.2	2456.0
Walden	01/02/2017	5560	147	18.5	11.5	3527.9
Wanda	25/01/2017	6699	197	13.0	37.2	4161.0
Willow	14/03/2017	22373	573	67.8	54.1	5427.9
Winifred	14/02/2017	7798	222	21.0	57.3	5138.5
Woldja	23/01/2017	7064	216	35.2	72.8	5324.6
Wollowra	06/03/2017	20531	575	23.0	30.1	3979.1
Wyatt	04/02/2017	8365	233	20.4	74.9	5061.9
Total mean		10521.7	299.2	34.4	51.8	4624.6

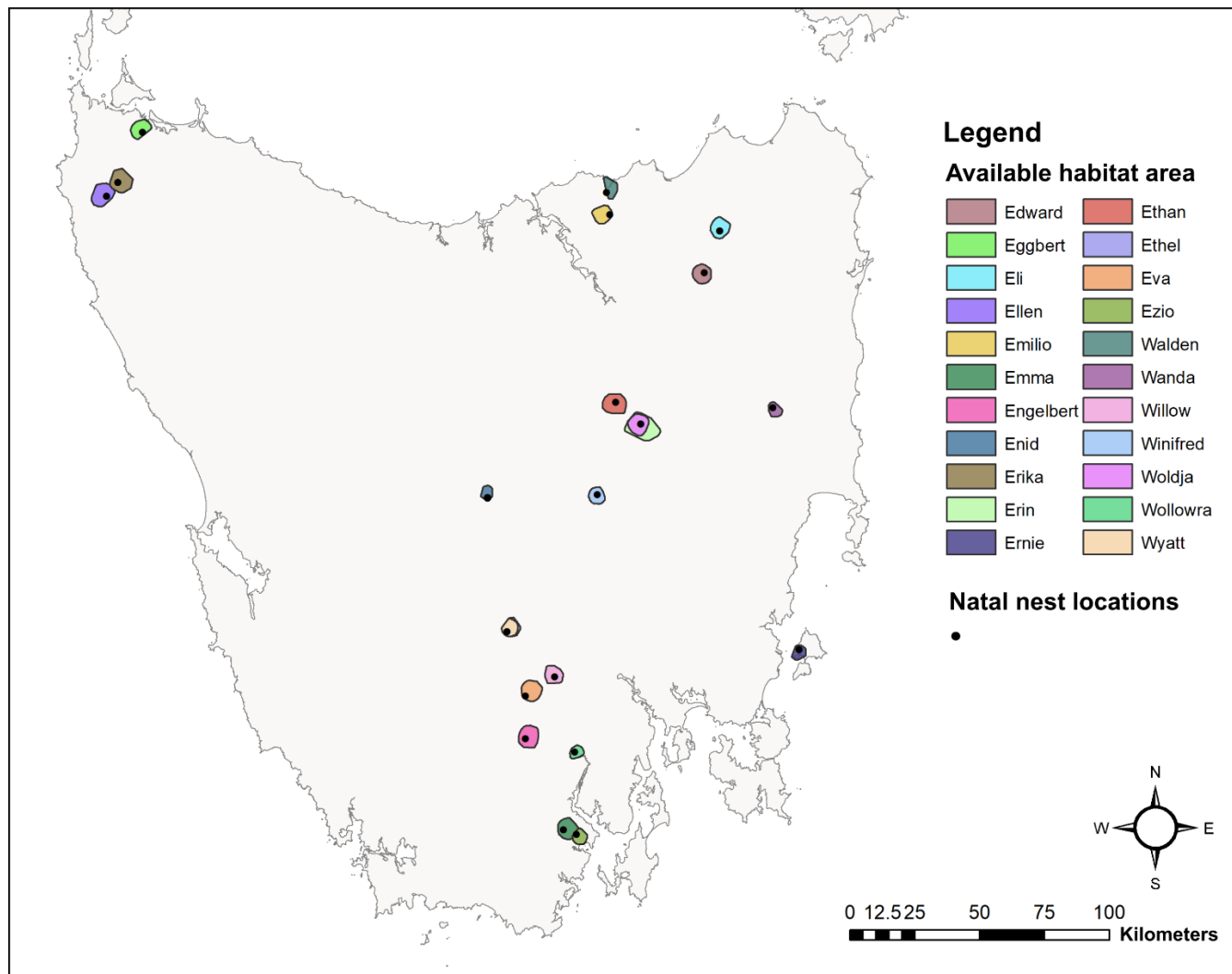


Figure C.2. Map of Tasmania with the available habitat area (buffered 95% MCP) for each eagle shown. The location of each respective natal nest is also indicated.

Table C.4. Details of each behavioural state as estimated by the HMM. The step length parameters of each behaviour, the proportion of time spent performing each behaviour and the mean altitude of each behaviour (calculated following guidelines in *Poessel et al., 2018*) are detailed.

HMM state	Behaviour	Step length parameter (mean $\pm$ SD)	% time in state (mean $\pm$ SD)	Altitude (mean $\pm$ SD)
1	Perching	10( $\pm$ 8)	41.8( $\pm$ 6.9)	20.6( $\pm$ 5.3)
2	Short flights	166( $\pm$ 96)	39.3( $\pm$ 6.8)	22.8( $\pm$ 5.9)
3	Long flights	795( $\pm$ 414)	18.9( $\pm$ 8.7)	63.5( $\pm$ 23.6)

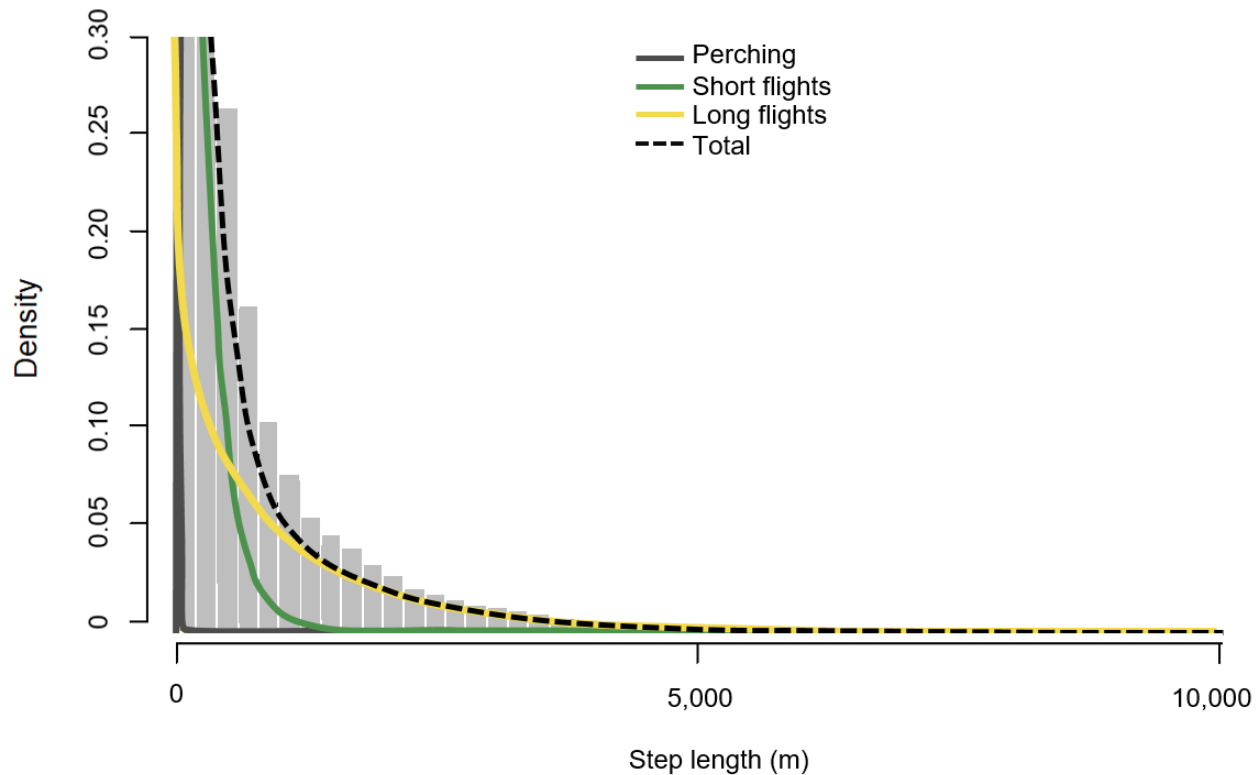


Figure C.3. Estimated state-dependent distributions of step length (km) in each of the behavioural states. All step lengths in the perching behaviour (dark grey) are very short. Steps  $>1$  km were categorised within 'long flights'. Note that there are some short steps included in both 'short flights' and 'long flights', which is a result of the probability of changing behavioural state (i.e. if a bird is performing a 'long flight' it is most likely to stay in this behavioural state). Therefore, if there are a limited number of short steps within a period of 'long flights', these short steps will be categorised as 'long flights'.

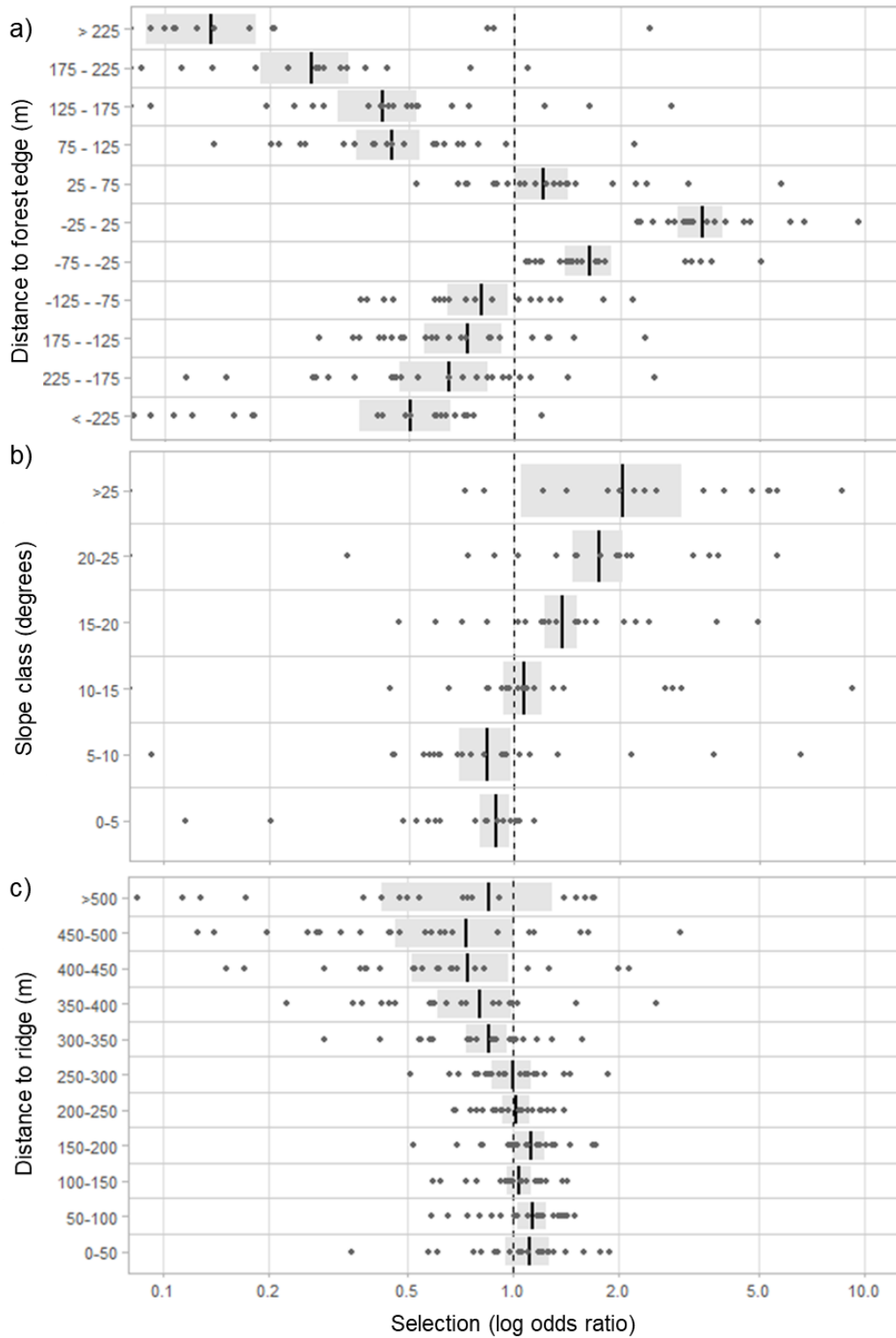


Figure C.4. Plots showing the habitat selection ratios for different ordinal categories of distance to forest edge (a), slope (b) and distance to ridgeline (c), when GPS data was not separated by behaviour. The selection ratio ( $w_i$ ) is indicated with 95% CI shown by shaded bars.  $w_i$  values above one (dashed line) indicate proportionally more time was spent in this habitat relative to its availability.  $w_i$  values below one indicate less time was spent performing the behaviour relative to the habitat availability.  $w_i$  values for each individual eagle are plotted as points.

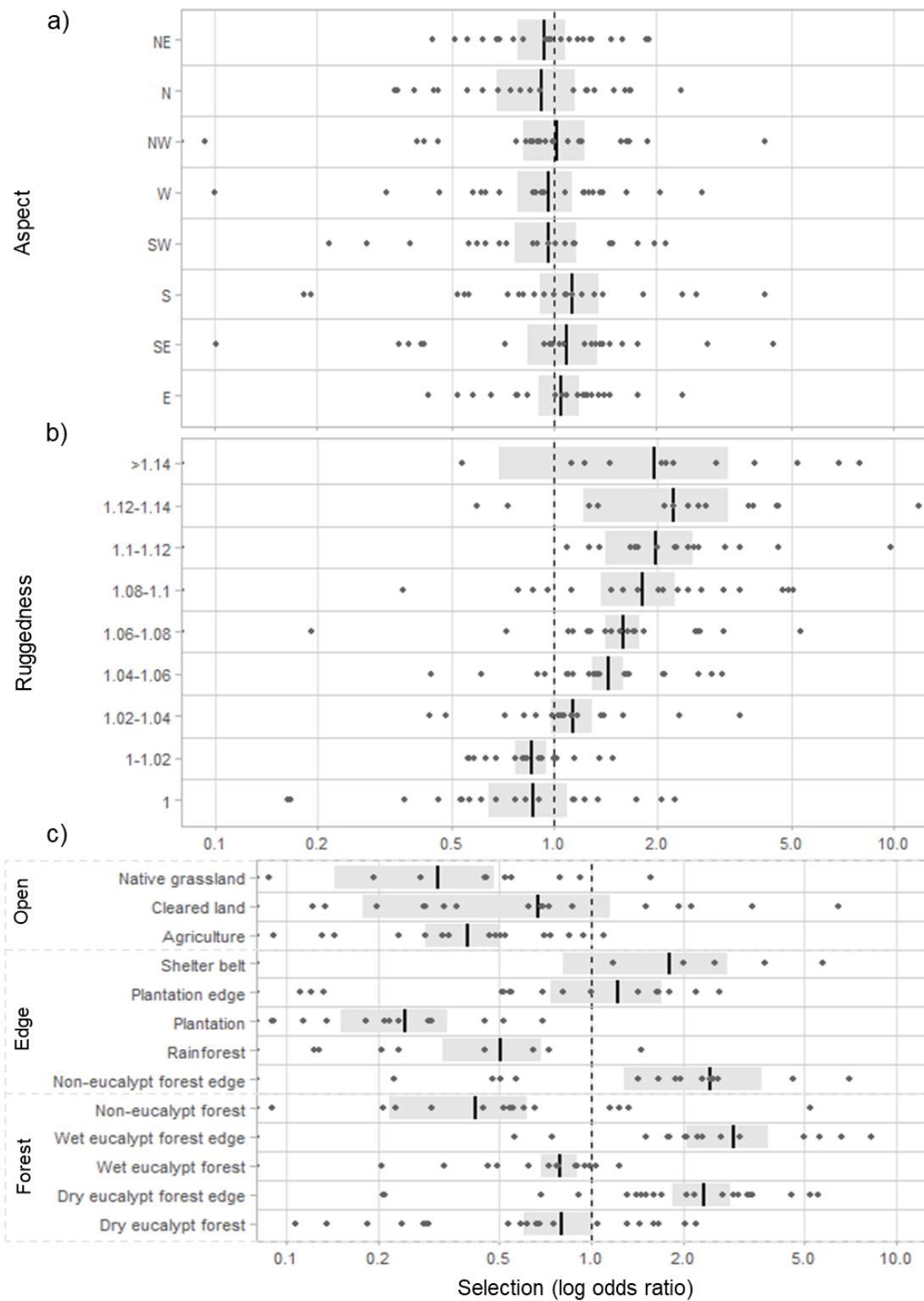


Figure C.5. Plots showing the habitat selection ratios for different categories of aspect (a), ruggedness (b) and land cover type (c), when GPS data was not separated by behaviour. The selection ratio ( $w_i$ ) is indicated with 95% CI shown by shaded bars.  $w_i$  values above one (dashed line) indicate proportionally more time was spent in this habitat relative to its availability.  $w_i$  values below one indicate less time was spent performing the behaviour relative to the habitat availability.  $w_i$  values for each individual eagle are plotted as points.

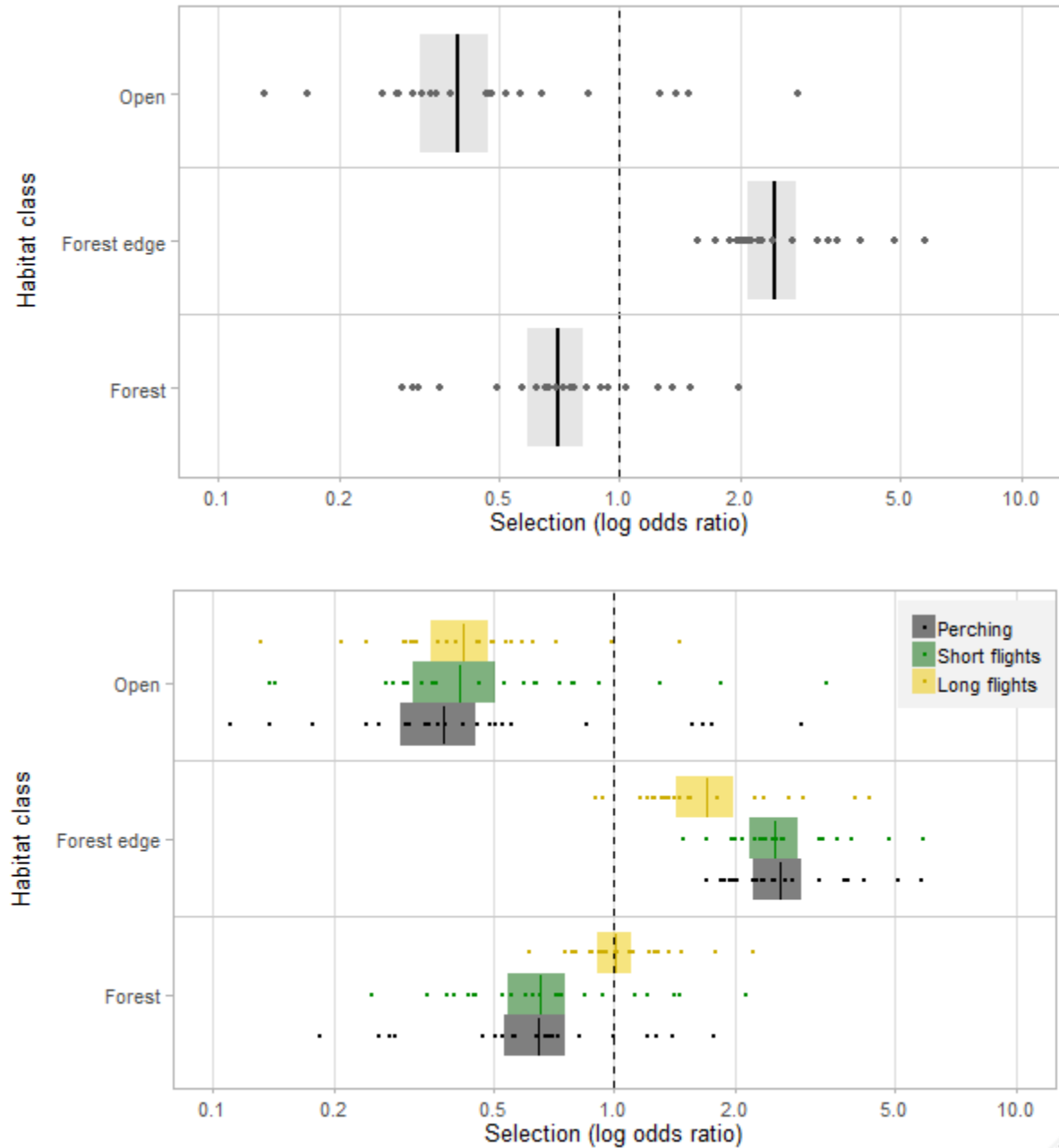


Figure C.6. Plots showing the habitat selection ratios for all GPS data (top) and separated for behavioural state (bottom) for land cover types grouped into three categories (open, forest edge and forest; see Table C.2). The selection ratio value ( $w_i$ ) is indicated with 95% CI shown by shaded bars.  $w_i$  values above one (dashed line) indicate proportionally more time was spent in this habitat relative to its availability.  $w_i$  values below one indicate less time was spent performing the behaviour relative to the habitat availability.  $w_i$  values for each individual eagle are plotted as points.

Table C.5. Perching model performance for each of the LOOCV eagles. None of the expected vs. observed regression intercepts ( $b_0$ ) were significantly different from 0. Five regression slopes were not significantly different from 0 (<sup>a</sup>) and one slope was significantly different from 1 (<sup>b</sup>).  $\chi^2$  tests could not be computed for three eagles due to SV bin 6 involving no available habitat areas in their natal area (\*).

Eagle	Expected vs. observed regression			$\chi^2$	
	$b_0$	$b_1$	$R^2$	$\chi^2$	$p$
All animals	-0.011	1.067	0.903	11.086	0.050
Edward	-0.004	1.025	0.782	16.755	0.005
Eggbert	0.032	0.810 <sup>a</sup>	0.373	*	*
Eli	0.016	0.906	0.759	5.082	0.406
Ellen	-0.018	1.108	0.866	11.266	0.046
Emilio	0.054	0.678	0.663	18.141	0.003
Emma	-0.057	1.345	0.786	24.292	>0.001
Engelbert	0.030	0.820	0.847	15.948	0.007
Enid	0.013	0.924	0.865	19.312	0.002
Erika	0.061	0.632 <sup>a</sup>	0.558	43.491	>0.001
Erin	0.073	0.561 <sup>a</sup>	0.395	39.411	>0.001
Ernie	0.039	0.766 <sup>a</sup>	0.559	17.985	0.003
Ethan	0.027	0.840	0.854	*	*
Ethel	-0.043	1.258	0.903	9.865	0.079
Eva	0.003	0.981 <sup>a</sup>	0.544	7.560	0.182
Ezio	0.025	0.850	0.858	3.308	0.653
Walden	-0.012	1.070	0.623	49.850	0.000
Wanda	-0.013	1.076	0.885	11.499	0.042
Willow	-0.075	1.452 <sup>b</sup>	0.726	13.719	0.017
Winifred	0.007	0.956	0.961	6.033	0.303
Woldja	0.012	0.931	0.763	13.163	0.022
Wollowra	-0.010	1.058	0.729	*	*
Wyatt	-0.041	1.245	0.836	11.019	0.051

Table C.6. Short flight model performance for each of the LOOCV eagles. None of the expected vs. observed regression intercepts ( $b_0$ ) were significantly different from 0. Six regression slopes were not significantly different from 0 (<sup>a</sup>) and one slope was significantly different from 1 (<sup>b</sup>).  $\chi^2$  tests could not be computed for two eagles due to SV bin 6 involving no available habitat areas in their natal area (\*).

Eagle	Expected vs. observed regression			$\chi^2$	
	$b_0$	$b_1$	$R^2$	$\chi^2$	$p$
All animals	-0.009	1.053	0.985	4.405	0.493
Edward	0.012	0.930	0.970	10.253	0.636
Eggbert	0.029	0.826 <sup>a</sup>	0.510	113.782	>0.001
Eli	0.016	0.904	0.945	5.696	0.863
Ellen	0.003	0.982	0.836	35.518	0.037
Emilio	0.000	1.000	0.978	5.136	0.887
Emma	-0.061	1.164	0.948	26.064	0.122
Engelbert	0.013	0.922	0.903	23.785	0.160
Enid	0.007	0.957	0.973	28.742	0.088
Erika	0.077	0.537 <sup>a</sup>	0.164	232.419	>0.001
Erin	0.058	0.651 <sup>a</sup>	0.250	234.292	>0.001
Ernie	0.010	0.943	0.920	12.792	0.512
Ethan	0.007	0.957	0.691	*	*
Ethel	-0.019	1.113	0.962	8.296	0.736
Eva	0.011	0.932 <sup>a</sup>	0.479	24.728	0.143
Ezio	0.049	0.705 <sup>a</sup>	0.477	20.388	0.236
Walden	-0.014	1.083	0.806	37.886	0.027
Wanda	-0.015	1.088	0.881	34.212	0.044
Willow	-0.004	1.022 <sup>a</sup>	0.503	55.913	0.002
Winifred	-0.002	1.014	0.980	6.961	0.803
Woldja	0.005	0.968	0.747	55.175	0.002
Wollowra	-0.006	1.036	0.773	*	*
Wyatt	-0.009	1.052	0.933	7.838	0.759

Table C.7. Long flight model performance for each of the LOOCV eagles. None of the expected vs. observed regression intercepts ( $b_0$ ) were significantly different from 0. Two regression slopes were significantly different from 1 (<sup>b</sup>).  $\chi^2$  tests could not be computed for two eagles due to SV bin 6 involving no available habitat areas in their natal area (\*).

Eagle	Expected vs. observed regression			$\chi^2$	
	$b_0$	$b_1$	$R^2$	$\chi^2$	$p$
All animals	0.007	0.956	0.944	6.338	0.275
Edward	0.008	0.954	0.996	1.656	0.894
Eggbert	0.002	0.990	0.913	*	*
Eli	0.023	0.862	0.929	11.762	0.038
Ellen	0.015	0.908	0.898	32.945	0.000
Emilio	0.027	0.836 <sup>b</sup>	0.988	8.556	0.128
Emma	0.003	0.981	0.973	2.841	0.724
Engelbert	0.028	0.829	0.941	9.994	0.075
Enid	0.029	0.828	0.969	9.441	0.093
Erika	0.034	0.799	0.757	21.956	0.001
Erin	0.010	0.938	0.813	10.159	0.071
Ernie	0.021	0.872	0.971	21.479	0.001
Ethan	0.002	0.988	0.988	*	*
Ethel	0.012	0.927	0.978	2.632	0.757
Eva	0.030	0.820	0.910	8.094	0.151
Ezio	0.027	0.837	0.695	6.804	0.236
Walden	-0.020	1.121 <sup>b</sup>	0.994	0.644	0.986
Wanda	0.006	0.966	0.934	10.788	0.056
Willow	0.000	1.001	0.955	6.667	0.247
Winifred	0.013	0.921	0.971	19.209	0.002
Woldja	-0.007	1.045	0.989	2.270	0.811
Wollowra	0.035	0.793	0.821	12.107	0.033
Wyatt	0.030	0.819	0.920	11.843	0.037

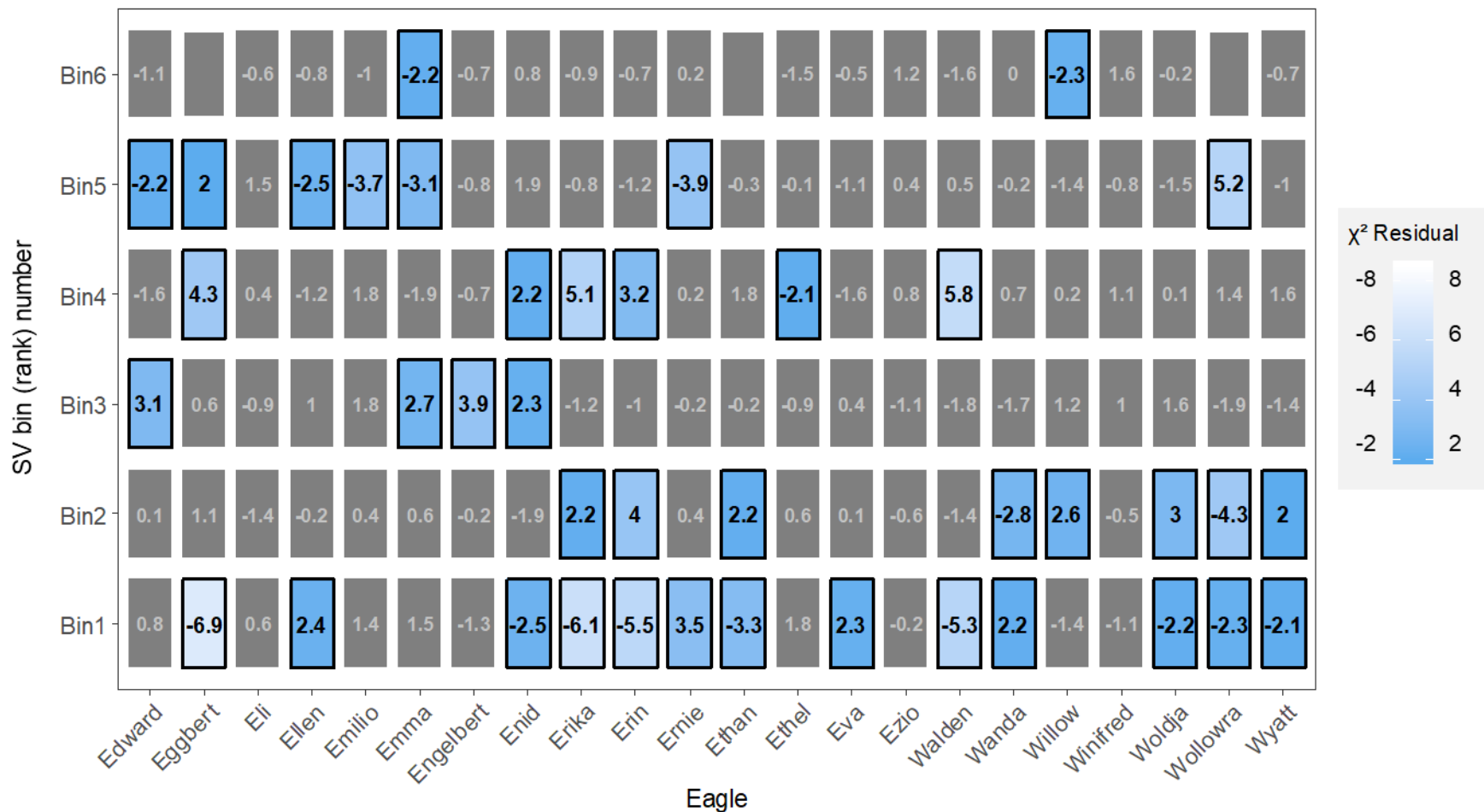


Figure C.7. Perching selection value (SV) bin performance for each individual using residual values from  $\chi^2$  tests. Residual values between -2 and 2 are shaded in grey and indicate the corresponding bird used habitats classified within the SV bin as expected based on the modelled selection values. Grid cells bordered in black indicate those SV bins where use different from the expected modelled proportion. Negative values indicate where an SV bin was used less than predicted and positive where the SV bin was used more than predicted.

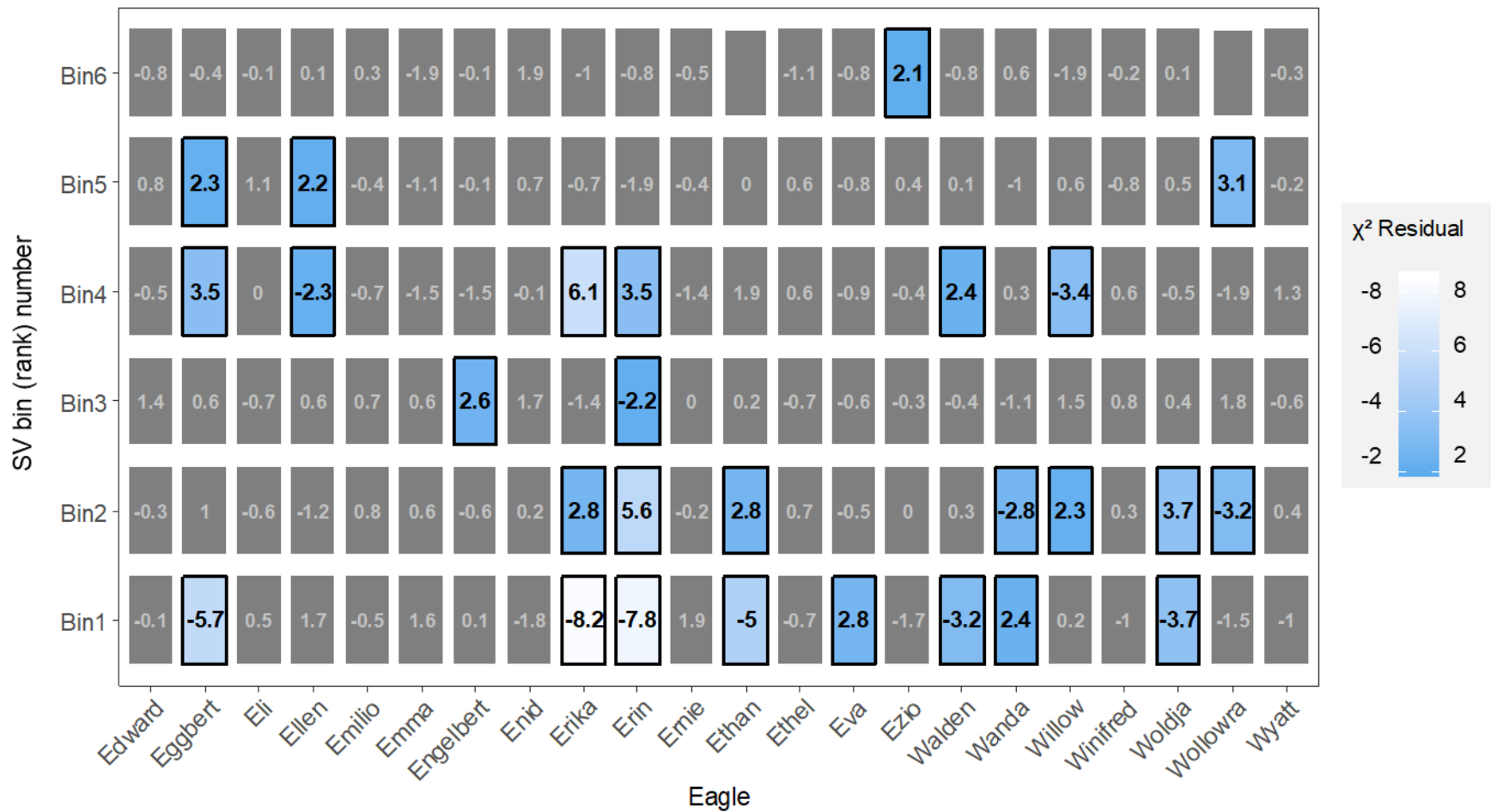


Figure C.8. Short flight selection value (SV) bin performance for each individual using residual values from  $\chi^2$  tests. Residual values between -2 and 2 are shaded in grey and indicate the corresponding bird used habitats classified within the SV bin as expected based on the modelled selection values. Grid cells bordered in black indicate those SV bins where use different from the expected modelled proportion. Negative values indicate where an SV bin was used less than predicted and positive where the SV bin was used more than predicted.

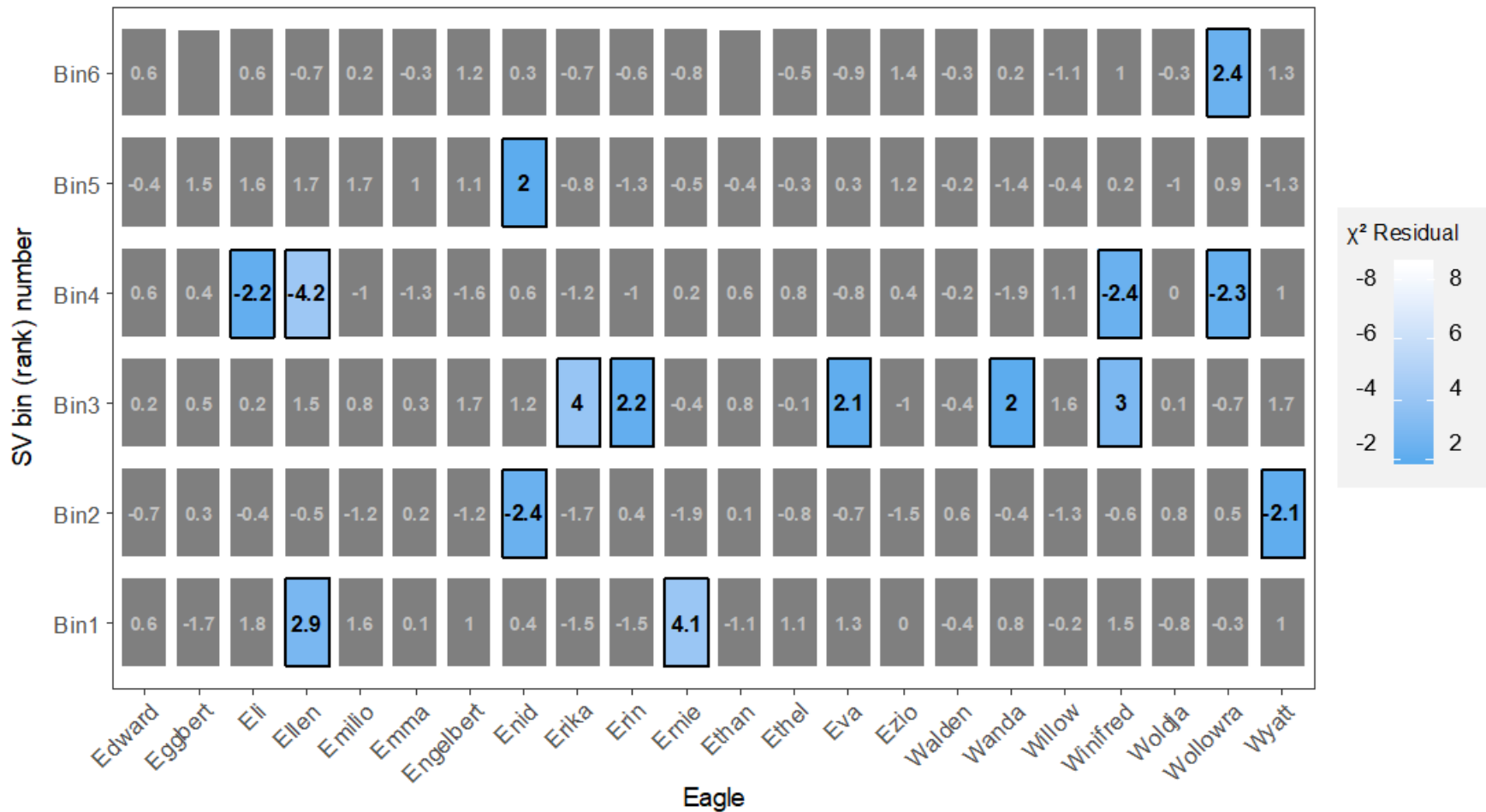


Figure C.9. Long flight selection value (SV) bin performance for each individual using residual values from  $\chi^2$  tests. Residual values between -2 and 2 are shaded in grey and indicate the corresponding bird used habitats classified within the SV bin as expected based on the modelled selection values. Grid cells bordered in black indicate those SV bins where use different from the expected modelled proportion. Negative values indicate where an SV bin was used less than predicted and positive where the SV bin was used more than predicted.

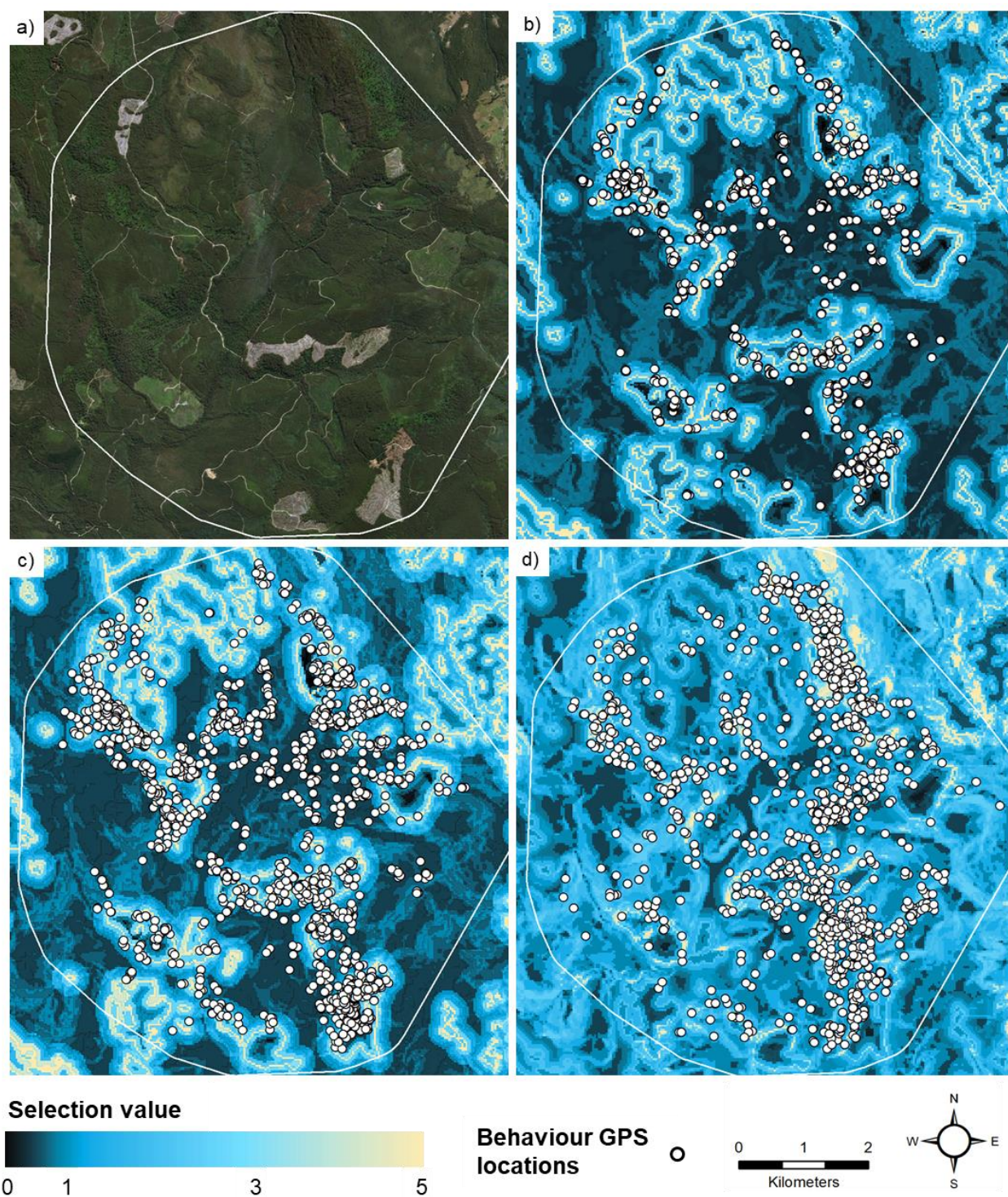


Figure C.10. Example mapped predictions of juvenile Tasmanian wedge-tailed eagle space use for an eagle whose data was not included in the model prediction (Emma). a) Satellite image of the available habitat area (buffered 95% MCP shown by white border). b) Modelled perching habitat use with all perching GPS locations shown as white dots. c) Modelled short flight habitat use with all short flight GPS locations shown as white dots. d) Modelled long flight habitat use with all long flights GPS locations shown as white dots.



Figure C.11. Example mapped predictions of juvenile Tasmanian wedge-tailed eagle space use for an eagle whose data was not included in the model prediction (Ethan). a) Satellite image of the available habitat area (buffered 95% MCP shown by white border). b) Modelled perching habitat use with all perching GPS locations shown as white dots. c) Modelled short flight habitat use with all short flight GPS locations shown as white dots. b) Modelled long flight habitat use with all long flights GPS locations shown as white dots.

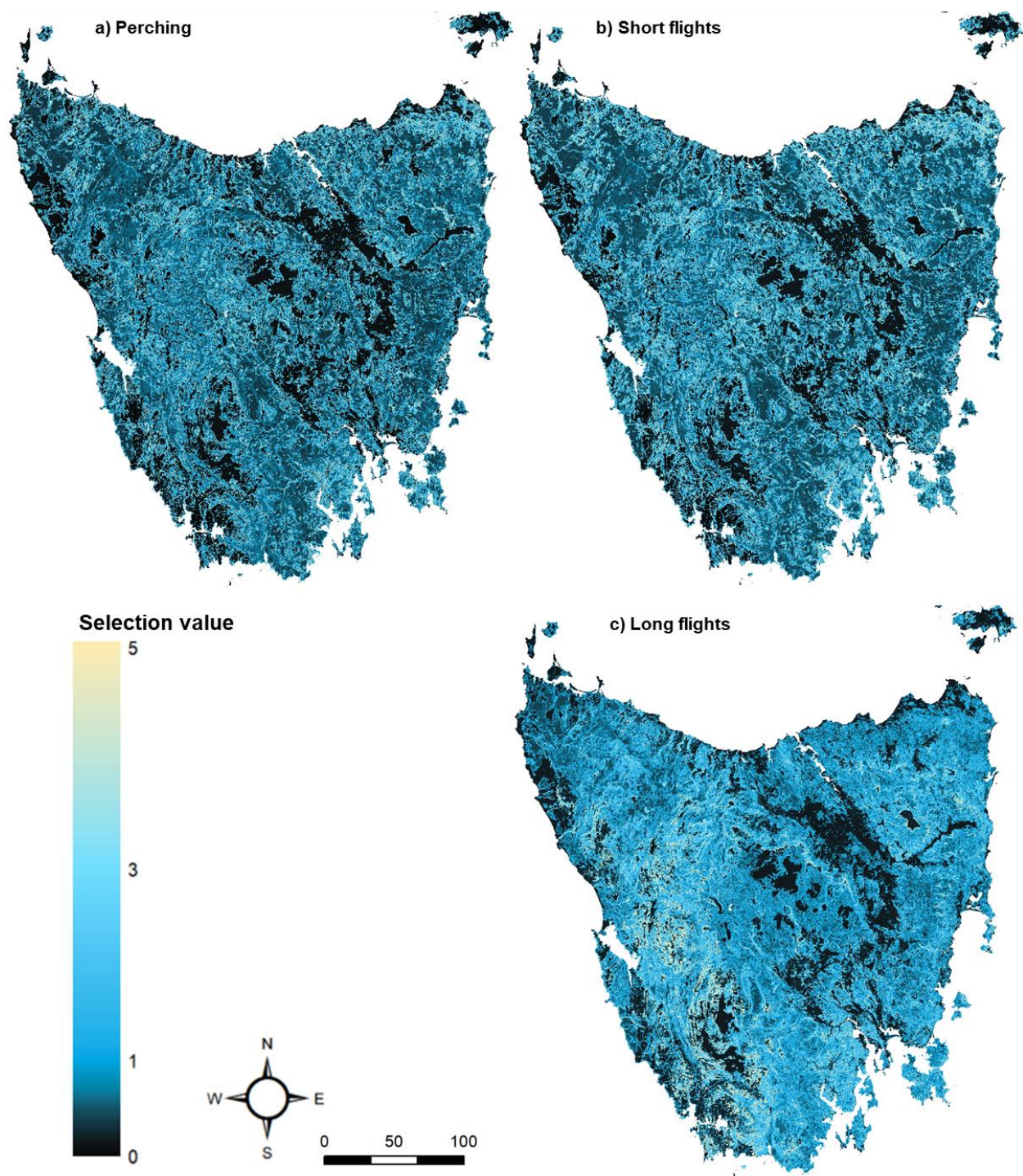


Figure C.12. Modelled predictions of juvenile Tasmanian wedge-tailed eagle space use for perching (a), short flights (b) and long flights (c) based on the bivariate habitat selection values (SVs).

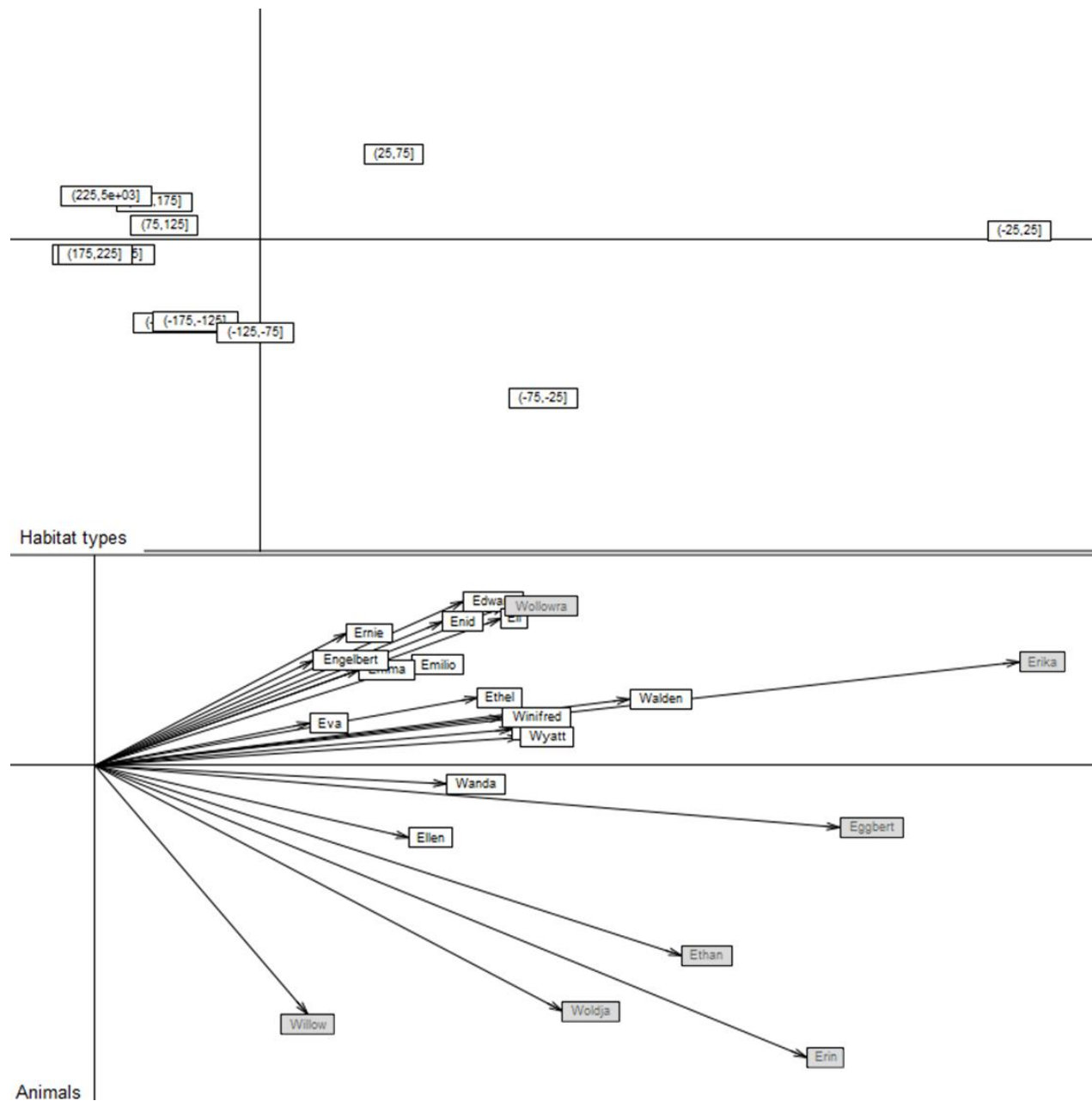


Figure C.13. Eigen plot indicating the relative strength of selection by each individual when performing short flights for different categories of distance to forest edge. Birds shaded in grey are those that the short flight model did not accurately predict where short flights were performed. The shaded birds were mostly located in very flat landscapes (except ‘Willow’ and ‘Wallowra’) and exhibited a stronger selection for areas closer to the forest edge.

## Appendix D

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### Supplementary materials to Chapter 5



Image C. Young

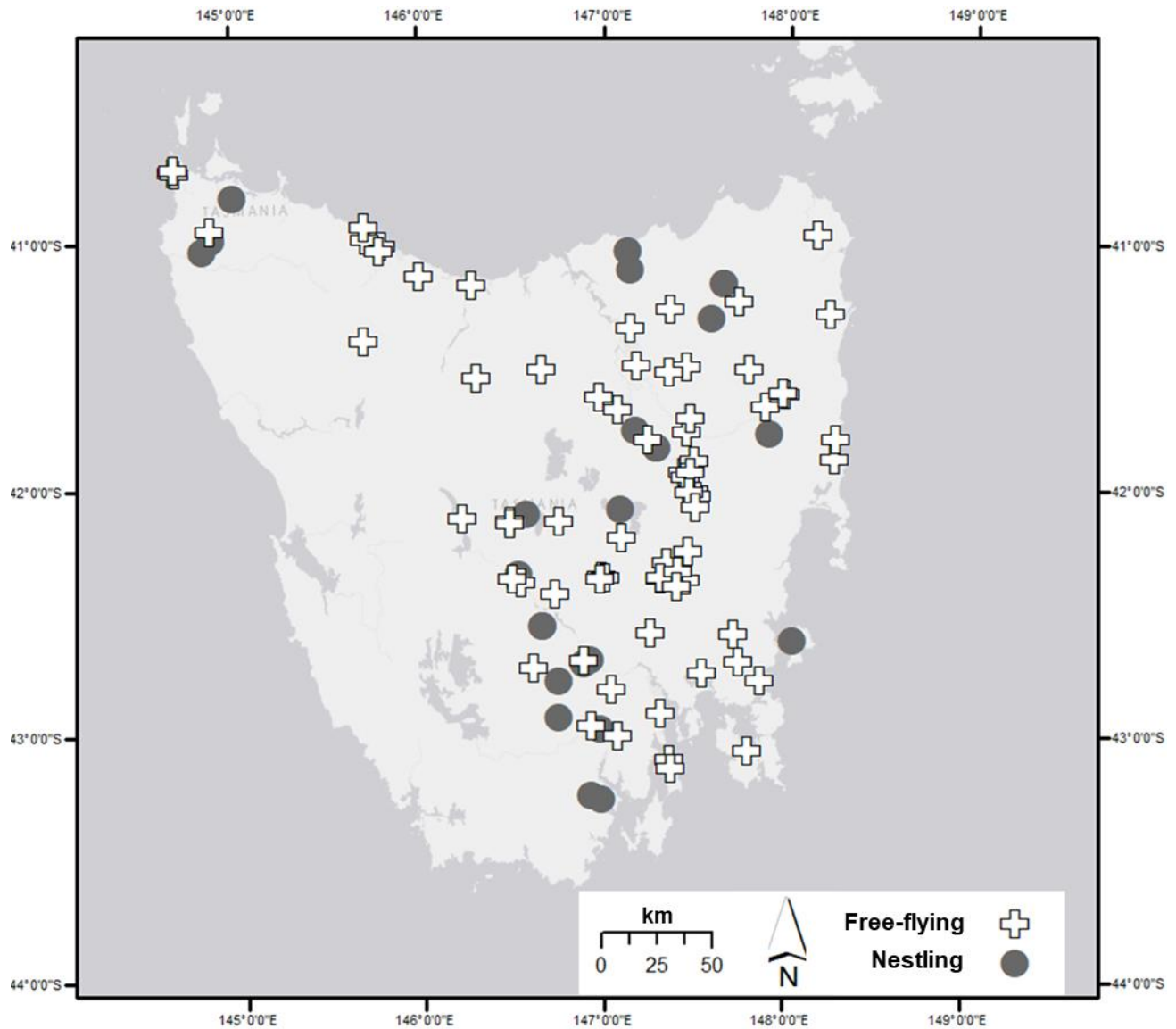
*D.1. Sample locations*

Figure D.1.1. Location of eagles sampled in the study. Nestling ( $n = 25$ ) locations were the corresponding natal nest (some nests were used more than once in successive breeding seasons). Approximate locations were recorded for 75 free-flying birds found dead or injured.

## D.2. Morphometric measurements

Table D.2.1. Morphometric measurement specifics with the associated measurement tool and the sample of birds where the measurement was taken (i.e. nestling and free-flying). Each measurement was based on established techniques described in Harmata *et al.*, (2013) and Wink (2007).

Morphometric	Measurement details	Tool	Sample
Bill depth	Depth of bill at the distal end of the cere.	Calliper	Free-flying
Bill length	Dorsal measurement from tip of the bill to the proximal end of the cere.	Calliper	Free-flying + Nestling
Bill width	Lateral width of bill at the distal end of the cere.	Calliper	Free-flying
Exposed culmen	Dorsal measurement from tip of the bill to the distal end of the cere.	Calliper	Free-flying + Nestling
Forearm length	Ventral length of the right ulna.	Rule	Free-flying
Hallux breadth	Anterior-posterior width of the right hallux claw taken at the junction with the skin.	Calliper	Free-flying + Nestling
Hallux length	Dorsal surface of the right hallux claw from the junction with the skin to the tip.	Calliper	Free-flying + Nestling
Hallux width	Lateral width of the right hallux claw taken at the junction with the skin.	Calliper	Free-flying + Nestling
Head length	Dorsal measurement from the medial back of the skull to the distal edge of the upper mandible.	Calliper	Free-flying + Nestling
Head width	Lateral width at the widest points of the skull.	Calliper	Free-flying
Tail length	Base of the central rectrices to their tips.	Rule	Free-flying
Tarsus breadth	Anterior-posterior measurement of the right tarsometatarsus at the narrowest point	Calliper	Free-flying + Nestling
Tarsus length	Length of right tarsometatarsus	Calliper	Free-flying + Nestling
Tarsus width	Lateral width of the right tarsometatarsus at the narrowest point	Calliper	Free-flying + Nestling
Wing chord	Folded wrist to the tip of the longest primary on the right wing.	Rule	Free-flying

### D.3. Molecular sexing

The sex of wedge-tailed eagles in this study was determined by a PCR assay based on the methods of Wadley (2009) outlined below. DNA was extracted from liver and/or thigh muscle tissue (free-flying birds) or a blood sample (nestlings) using a Qiagen DNEasy Blood and Tissue Kit (Qiagen Inc.), following the provided protocol. We then used primers developed by Wadley (2009) to co-amplify the sex-linked CHD-W and CHD-Z genes in wedge-tailed eagles (Table D.3.1).

Table D.3.1. Primers for PCR-based sex assay in wedge-tailed eagles

Primer name	Sequence	Source
CHD internal F1	TATCGTCAGTTTCCCTTCA	(Wadley, 2009)
CHD internal R1	TCGGAACAACCTTGAATTCTCA	(Wadley, 2009)

Samples were amplified using MangoTaq and PCR reagents from Bioline Aust. Pty. Ltd. Each reaction for PCR contained 2  $\mu$ L template DNA, 0.5  $\mu$ L of each primer (10 $\mu$ M), 0.5  $\mu$ L dNTP mix (2mM each), 0.3  $\mu$ L MgCl<sub>2</sub> (50 mM), 2  $\mu$ L 5x reaction buffer and 0.08  $\mu$ L MangoTaq polymerase, made up to 10  $\mu$ L total with MilliQ water. The following thermocycler protocol was used: initial activation for 2 minutes at 94°C; then 40 repeats of denaturation for 20 seconds at 94°C, followed by annealing for 10 seconds, followed by extension for 60 seconds at 65°C; then a final extension step for 10 minutes at 65°C. The annealing temperature for the first cycle was 58°C, decreasing in 0.2°C increments every cycle to 50°C. PCR products were visualised by electrophoresis on a 1% agarose gel alongside a 100bp Hyperladder (Bioline Inc.). As the CHD-W and CHD-Z genes vary in size (Griffiths *et al.*, 1996), two distinct bands were present in the

heterogametic female birds (a strong band at  $\approx 500$  bp, and a weaker band at  $\approx 250$  bp, which was not always visible on the gel), and a single strong band in the male birds at  $\approx 250$  bp (Figure D.3.1). Unclear results were repeated until sex could be accurately identified. If a clear result could not be achieved the bird was not included in the study.

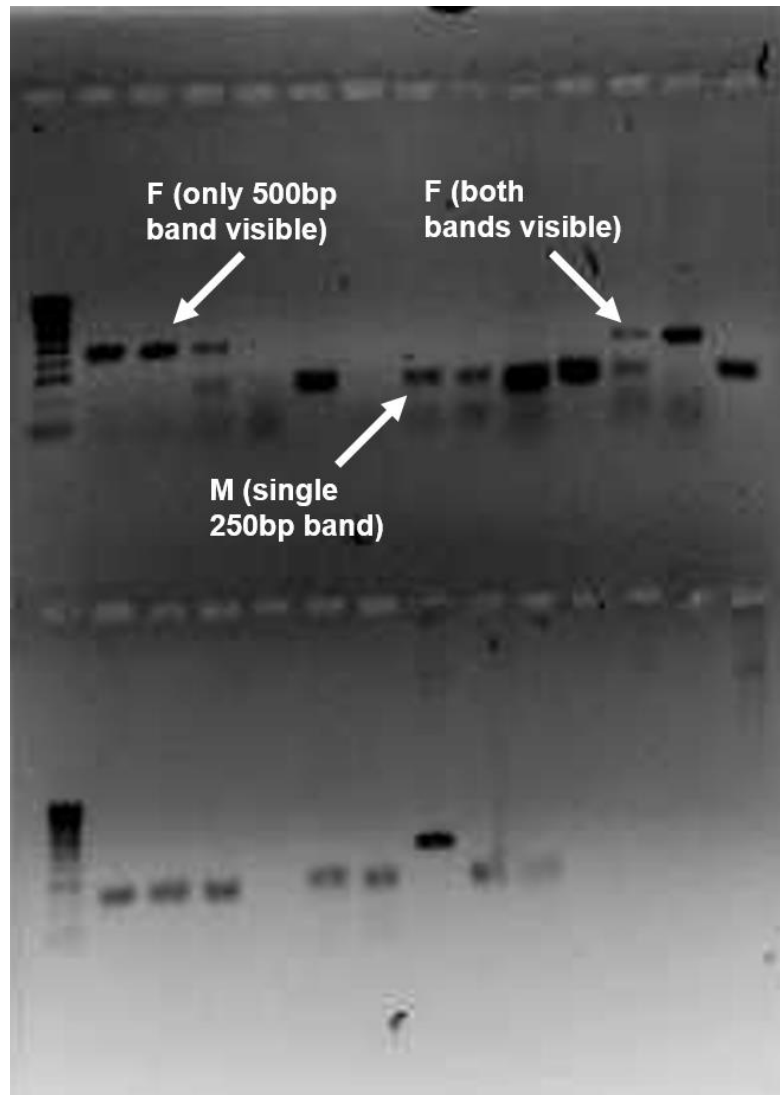


Figure D.3.1. Annotated PCR gel image indicating the bands used to identify sex. Females are clearly indicated by the presence of a strong band at  $\approx 500$  bp, as well as a weaker band at  $\approx 250$  bp, which is not always visible. Males are indicated by a single strong band at  $\approx 250$  bp.

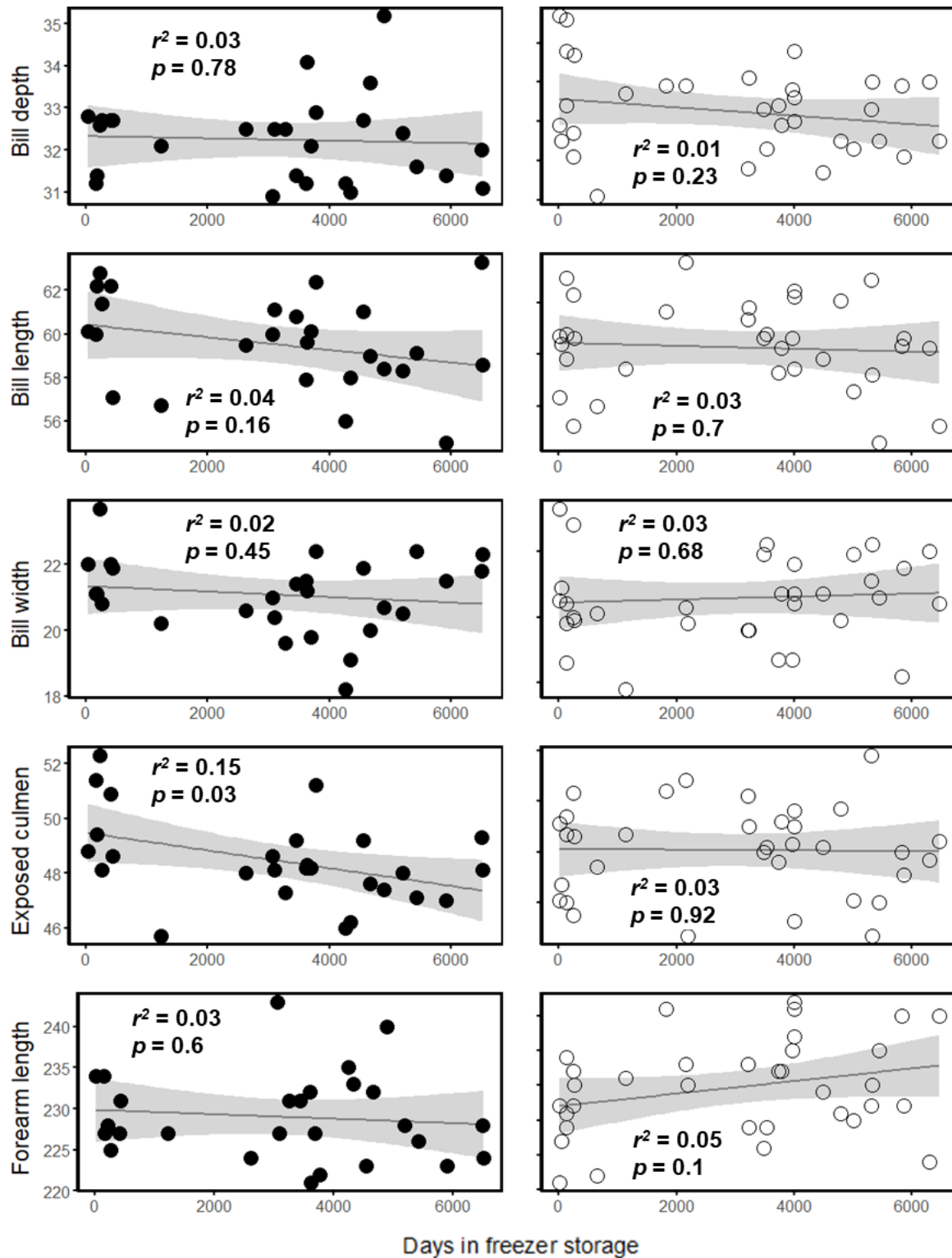
*D.4. Time in freezer storage*

Figure D.4.1. Variation in each morphometric measurement (mm) for all free-flying birds in relation to the number of days spent in freezer storage (range 0–6518 days). Left plots show data from males and right plots show data from females. Shaded area represents the 95% CI.

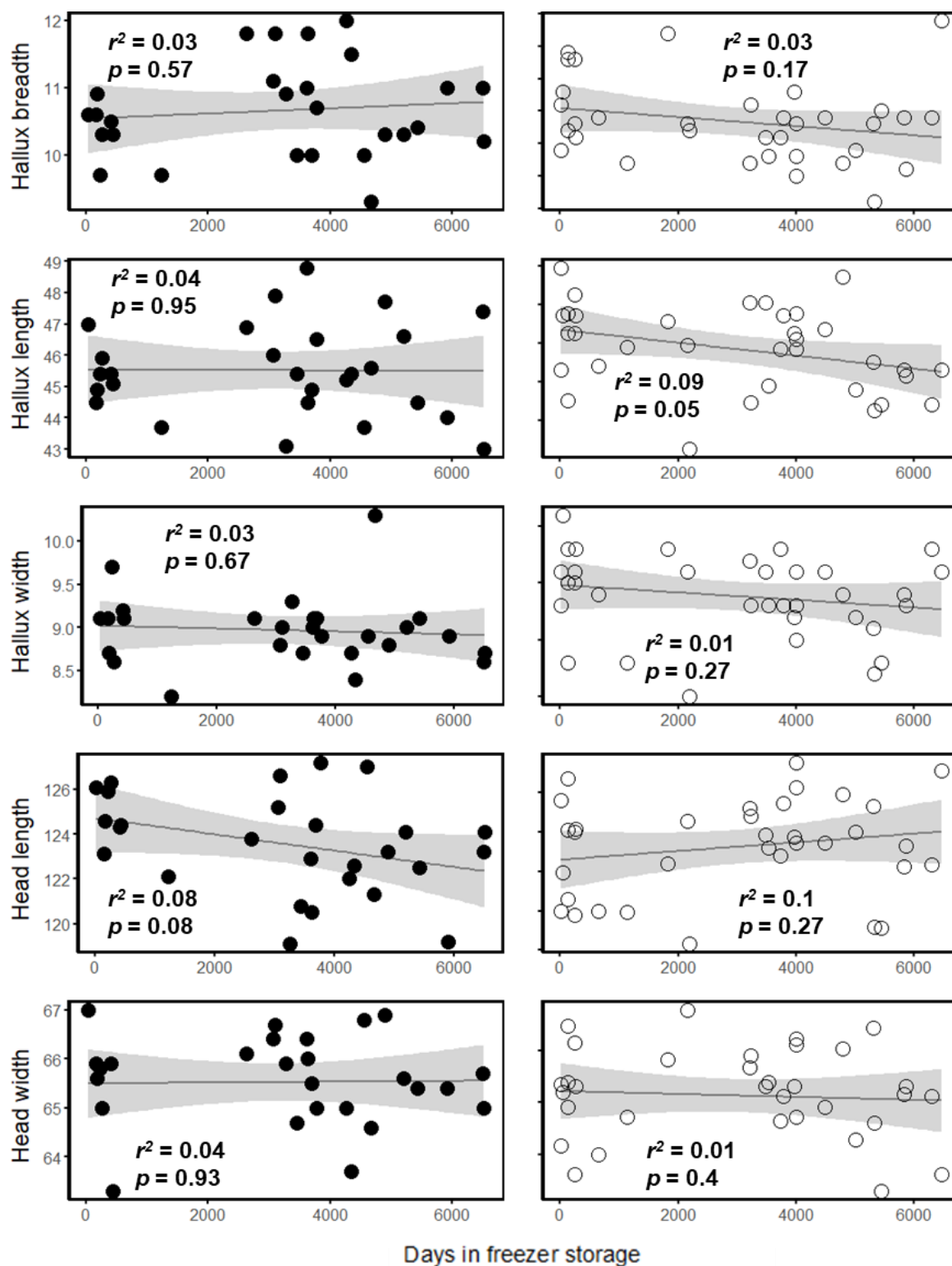


Figure D.4.2. Variation in each morphometric measurement (mm) for all free-flying birds in relation to the number of days spent in freezer storage (range 0–6518 days). Left plots show data from males and right plots show data from females. Shaded area represents the 95% CI.

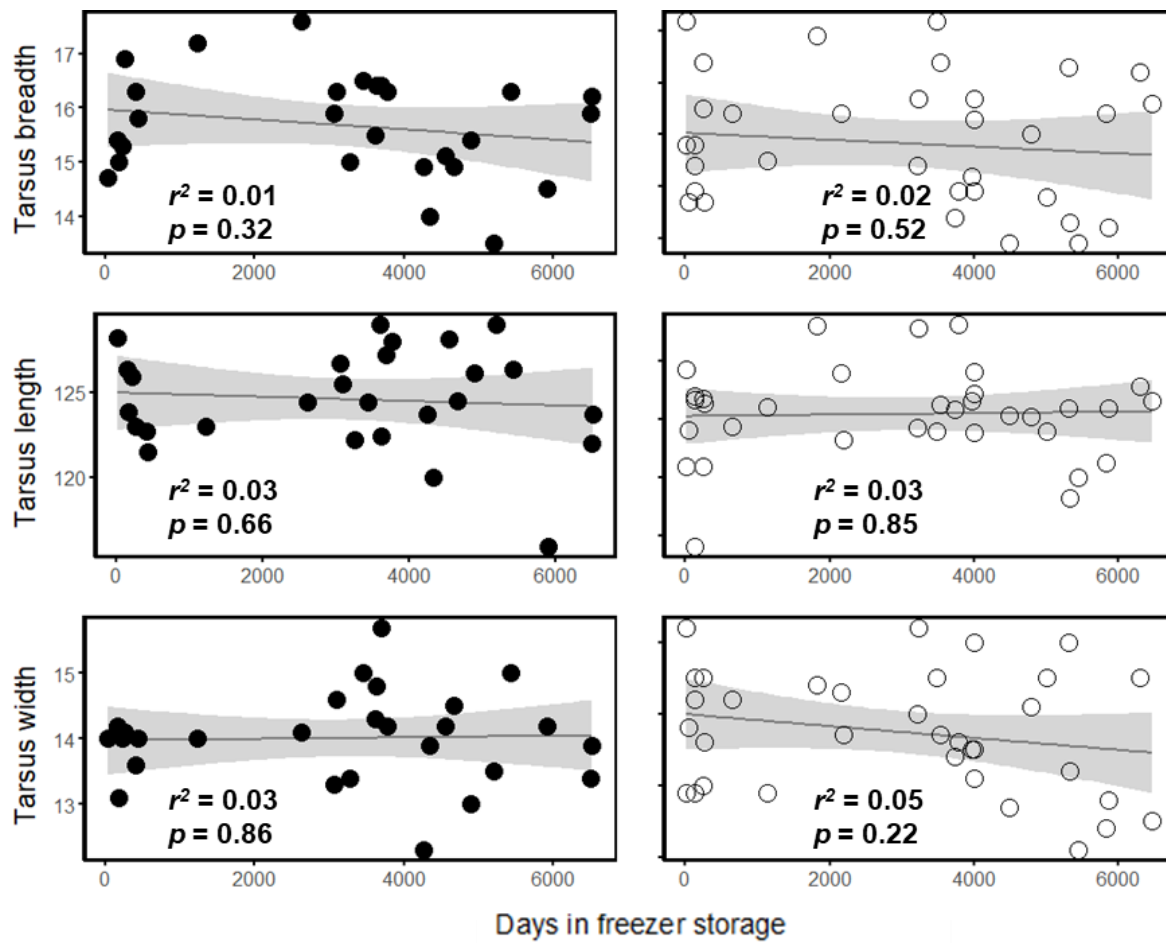


Figure D.4.3. Variation in each morphometric measurement (mm) for all free-flying birds in relation to the number of days spent in freezer storage (range 0–6518 days). Left plots show data from males and right plots show data from females. Shaded area represents the 95% CI.

## D.5. DFA diagnostics

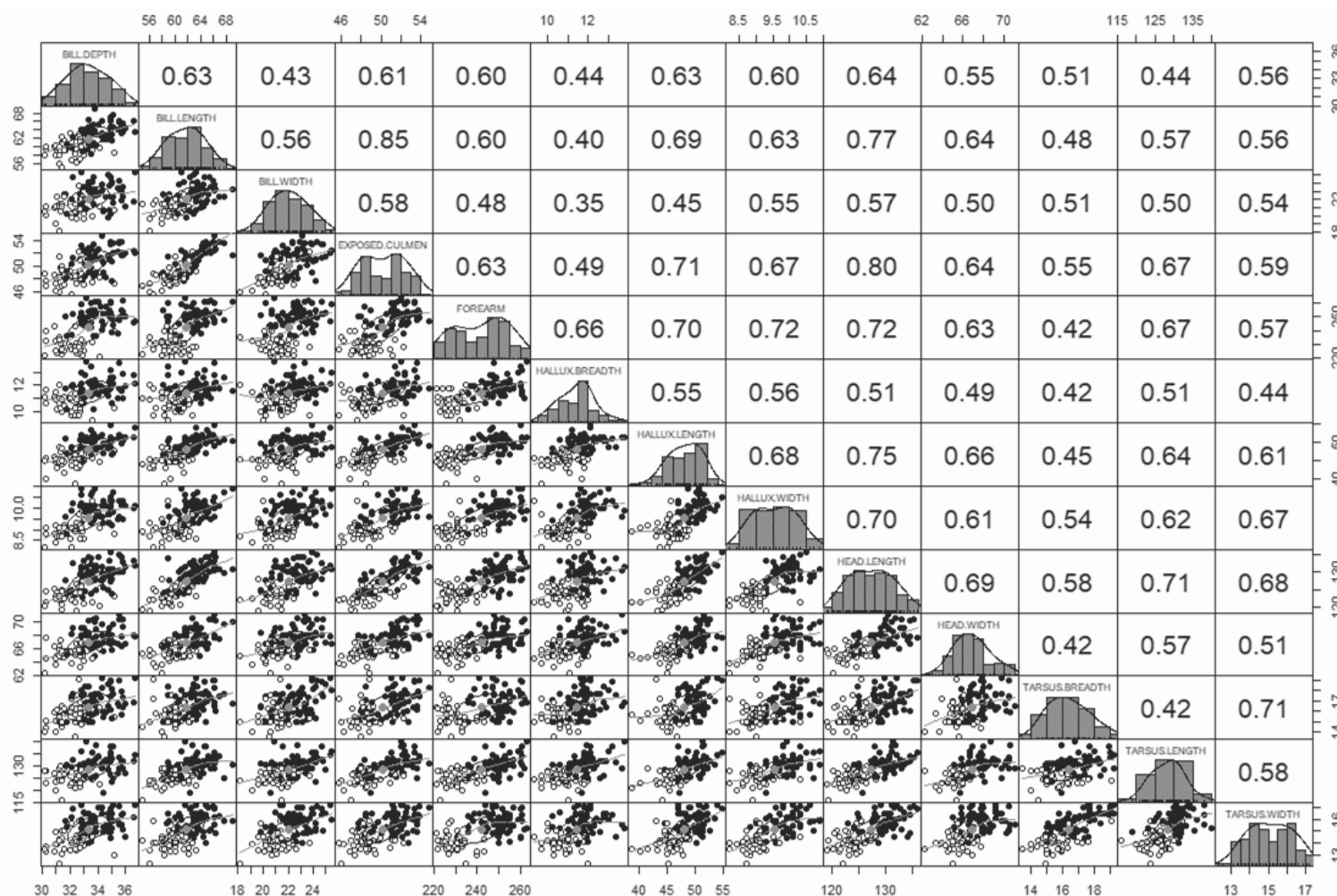


Figure D.5.1. Free-flying bird data diagnostics for all morphometric measurements considered for inclusion in the DFA. A correlation matrix is shown from top-right (Pearson's). The central band shows distribution histograms. From bottom-left are pairwise plots of each morphometric with confirmed genetic sex shown by colour (male = black, female = white).

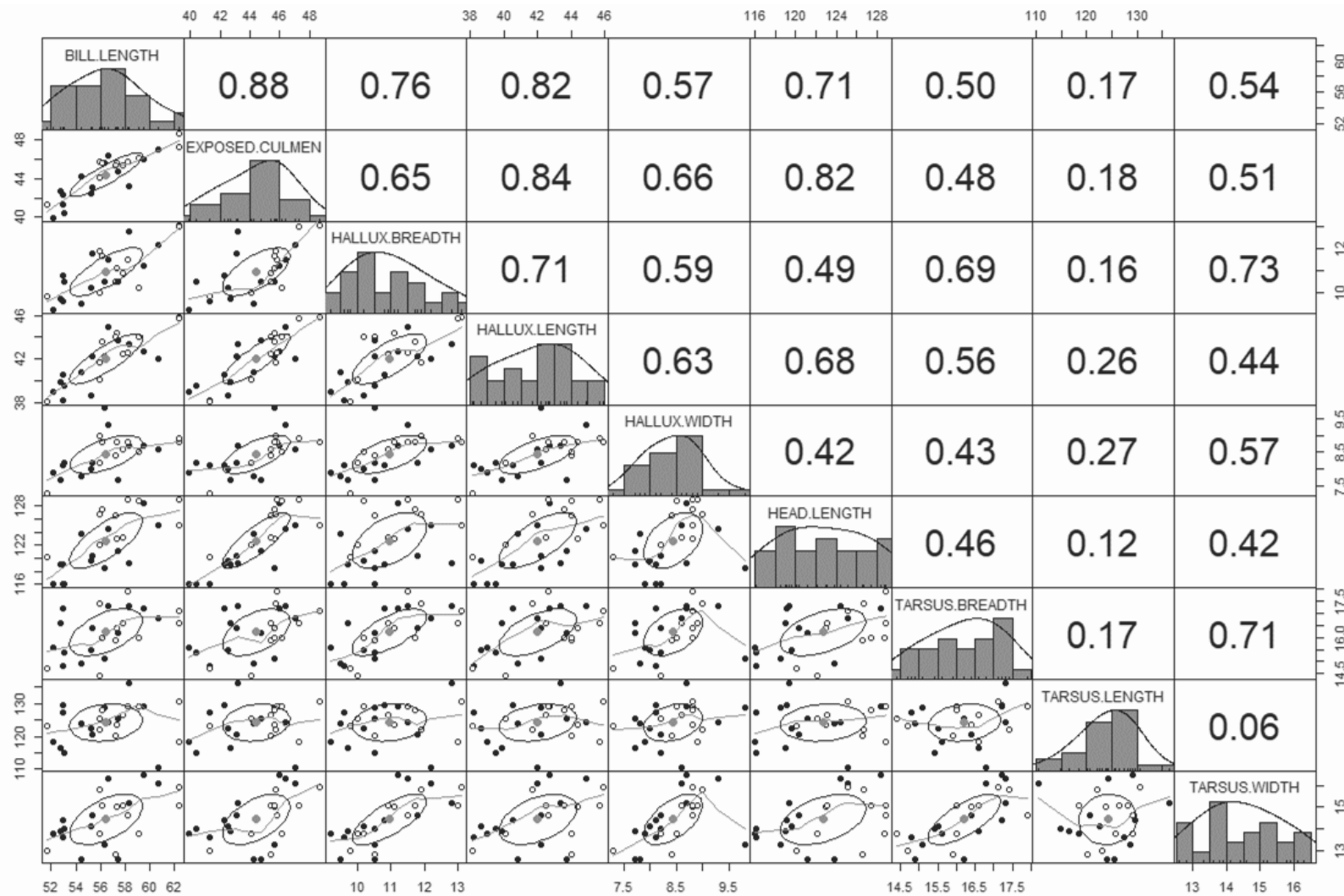


Figure D.5.2. Nestling data diagnostics for all morphometric measurements considered for inclusion in the DFA. A correlation matrix is shown from top-right (Pearson's). The central band shows distribution histograms. From bottom-left are pairwise plots of each morphometric with confirmed genetic sex shown by colour (male = black, female = white).

## Appendix E

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Image C. Young

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