New insights into wild deer population genetics, ecology and impacts: implications for management in south eastern Australia

Christopher Davies
BSc (Hons)

Submitted in total fulfilment of the requirements for the degree
of
Doctor of Philosophy

School of Health and Life Sciences

Federation University Australia

PO Box 3191
Northways Rd, Churchill
Victoria 3842
Australia

December 2019

Statement of authorship and originality

Except where explicit reference is made in the text of the thesis, this thesis contains no material published elsewhere or extracted in whole or in part from a thesis by which I have qualified for or been awarded another degree or diploma. No other person's work has been relied upon or used without due acknowledgement in the main text and the list of references of the thesis. No editorial assistance has been received in the production of the thesis without due acknowledgement. Except where duly referred to, the thesis does not include material with copyright provisions or requiring copyright approvals.

Signature:

Print name: Christopher Davies

Date: 20/12/19

Preface

This thesis is a compilation of my own work, driven by my interest into the ecology and impacts of introduced deer in Victoria. My interest in deer initially lead me to undertake an honours project, focussed on deer as a vector for parasites that can affect domestic livestock. During my honours year it became apparent that little was known about the ecology of wild deer throughout south east Australia. My PhD study was therefore developed to fill knowledge gaps of deer ecology, with a focus on developing and optimising ecological tools to generate data to improve deer management strategies. There are many methods available which can be used to collect ecological data on invasive species, such as deer. For my study, I chose methods from four quite discrete fields; global positioning systems (GPS) tracking, population genetics, camera trapping and spatial modelling. These methods were chosen as they are commonly used in ecological studies of invasive species.

During my candidature significant attempts were made to deploy GPS collars onto sambar deer to investigate their movement patterns. Movement pattern data is extremely useful and can provide insights into habitat preferences, dispersal ability and other information useful for management. Significant time (around 12 months) was spent applying for ethics approval, gaining relevant approvals, permits and licenses to perform this work as well as performing collaring attempts. Unfortunately all attempts were unsuccessful and the investigation of sambar deer movement patterns had to be abandoned. This highlights the difficulties of working with cryptic deer species inhabiting difficult terrain.

The other fields of research pursued (population genetics, camera trapping and spatial modelling) were more successful, the results of which are presented and discussed in this thesis. As the three methods employed in this study are taken from very different fields, a number of experts were enlisted to guide the respective data chapters. The population genetic studies (Chapters two and three) were guided by my primary supervisor Dr Fiona Hogan. I conducted all scat collections from across Victoria, including French Island and Mount Cole and performed all DNA isolations (over 300 in total). Population structure analysis for chapter three was undertaken with the assistance of Dr Faye Wedrowicz and Dr Carlo Pacioni. The camera trapping study (Chapter four) involved deploying camera traps in Baw Baw National Park, which I conducted myself. Occupancy and detectability data analysis for chapter four was performed with the assistance of Dr Hugh Davies. Spatial modelling (Chapter five) which focussed on modelling deer-vehicle collision risk across Victoria was directed by Dr Casey Visintin.

Chapter’s two to five are written as independent scientific publications, therefore there is some unavoidable repetition within the thesis as a whole. Minor changes have been made to the formatting of the published papers to keep style consistent within the thesis.

Acknowledgements

Without the support of particular people this thesis would not have been possible. Thank you to my supervisors Dr Fiona Hogan, Associate Professor Wendy Wright and Dr Faye Wedrowicz. I really appreciate the time you spent helping me over the life of this PhD.

Thanks to Elaine Thomas and Dan Brown from Parks Victoria who helped arrange the delivery of sambar deer tissue samples. Thankyou Fiona Tegart, Ben Webb, Rachel Rachelie and other staff from the resource centre at Federation University for helping me with all things laboratory related. Special thanks to Dr Jordan Hampton, despite not being able to capture any deer, I enjoyed working with you and look forward to working with you in the future. If we had our time again, I'm sure we would have managed to catch one.

Some individuals went above and beyond for me during this project, particularly my brother Dr Hugh Davies who provided support, guidance and feedback on camera trapping data. Also, Dr Faye Wedrowicz provided considerable time helping me conduct genetic analyses, manipulate GIS data and produce figures and tables. Faye, I really could not have done this without you, I really admire your intelligence, modesty and work ethic, thanks so much!

Dr Casey Visintin, thanks for all the time you committed to helping me out. I learnt a lot from the time we spent performing the collision modelling, you are a legend. Looking forward to a catch up and pint with you.

Thank you to my friends and family, your support was extremely important to me. Mum, Dad, Michael and Hugh thanks for helping me through the frustrations and stress of a challenging journey. Last but not least, Victoria, thanks for putting up with me over the life of this PhD, you provided support when I needed it most and I'm looking forward to our lives together post-PhD.

Christopher Davies was supported by an Australian Government Research Training Program (RTP) Stipend and RTP Fee-Offset Scholarship through Federation University Australia. This study was funded by the Holsworth Wildlife Research Endowment – Equity Trustees Charitable Foundation and Federation University Australia's School of Health and Life Sciences. Sampling was performed under the provisions of the Wildlife Act 1975 and National Parks Acts 1975 from the Department of Land Water and Planning (Permit no. 1000 7699).

Table of contents

Publications during enrolment

First authored publications:

1. **Christopher Davies**, Wendy Wright, Faye Wedrowicz and Fiona E. Hogan (2019) A DNA toolbox for non-invasive genetic studies of sambar deer (*Rusa unicolor)*. *Australian Mammalogy*, doi: https://doi.org/10.1071/AM18032

2. **Christopher Davies**, Wendy Wright, Faye Wedrowicz, Carlo Pacioni and Fiona E. Hogan (under review) Delineating genetic management units of sambar deer (*Rusa unicolor*) using opportunistic tissue sampling and targeted scat collection. *Wildlife Research*.

3. **Christopher Davies**, Wendy Wright, Fiona E. Hogan and Hugh Davies (2020) Detectability and activity patterns of sambar deer (*Rusa unicolor*) in Baw Baw National Park, Victoria. *Australian Mammalogy*, https://doi.org/10.1071/AM19029

4. **Christopher Davies**, Wendy Wright, Fiona E. Hogan and Casey Visintin (2019) Predicting deer-vehicle collision risk across Victoria, Australia. *Australian Mammalogy*, doi: https://doi.org/10.1071/AM19042

Other publications during candidature:

Jordan O. Hampton, Neal A. Finch, Kurt Watter, Matthew Amos, Tony Pople, Andrew Moriarty, Andrew Jacotine, Daryl Panther, Clark McGhie, **Chris Davies**, Jim Mitchell and David M. Forsyth (2018). A review of the methods to capture and restrain introduced wild deer in Australia. *Australian Mammalogy* **41**, 1-11, doi: https://doi.org/10.1071/AM17047

Contribution to published works

Thesis Chapter	Publication Title	Status	Nature and % of student contribution	Co-author name(s) Nature and % of co-authors contribution
2	A DNA toolbox for non-invasive genetic studies of sambar deer (*Rusa unicolor*	Published	83%. Concept, laboratory work, collecting data, data analysis, writing of manuscript, review of manuscript in response to editors and reviewers comments, response to editors and reviewers comments, review of final proofs	Dr Fiona Hogan, concept, reviewing and commenting on manuscript, 10%, Assoc Prof Wendy Wright, reviewing manuscript, 2% Dr Faye Wedrowicz, reviewing manuscript, 5%
3	Delineating genetic management units of sambar deer (*Rusa unicolor*) using opportunistic tissue sampling and targeted scat collection	Submitted, under first review	83%. Concept, sample collection, laboratory work, data analysis, writing of manuscript	Dr Fiona Hogan, concept, reviewing and commenting on manuscript, 5% Assoc Prof Wendy Wright, reviewing and commenting on manuscript, 2% Dr Faye Wedrowicz, concept, data analysis, reviewing and commenting on manuscript, 5% Dr Carlo Pacioni, data analysis, reviewing and commenting on manuscript, 5%
4	Detectability and activity patterns of sambar deer (*Rusa unicolor*) in Baw Baw National Park, Victoria	Published	80%. Concept, field work, data analysis, writing of manuscript, review of manuscript in response to editors and reviewers comments, response to editors and reviewers comments	Assoc Prof Wendy Wright, reviewing and commenting on manuscript, 5% Dr Fiona Hogan, reviewing and commenting on manuscript, 5% Dr Hugh Davies, concept, data analysis, reviewing and commenting on manuscript, 10%
5	Predicting deer-vehicle collision risk across Victoria, Australia	Published	80%. Concept, data analysis, writing of manuscript, review of manuscript in response to editors and reviewers comments, response to editors and reviewers comments, review of final proofs	Assoc Prof Wendy Wright, input into manuscript 5% Dr Fiona Hogan, input into manuscript, 5% Dr Casey Visintin, concept, data analysis, input into manuscript, 10%

List of figures

List of tables

Abstract

In south east Australia, populations of wild deer have emerged as problematic invasive species due to their increasing impact on social, economic and ecological values. However, compared to other invasive species, deer are poorly researched throughout their introduced range which is directly constraining their management.

The aim of this thesis is to provide new tools and information to improve the management of wild deer in south east Australia, with a particular focus on sambar deer. A multi-disciplined approach was used to provide new insights into deer population genetics, ecology and vehicle collision risk. A genetic approach was used to collect DNA from deer scats and investigate sambar deer population structure. Camera traps were used to investigate sambar deer ecology and a spatial modelling framework used to investigate deer-vehicle collisions (DVC).

This thesis develops a genetic toolbox that has wide applications for future studies of deer abundance and dispersal, information that is critical to improve management efforts. Sambar deer in the State of Victoria were found to exhibit pronounced population structure and three distinct management units were identified. Camera traps were applied to provide new insights into sambar deer ecology and activity patterns, providing information to guide the application of control efforts. The modelling framework represents the first examination of DVC risk and identified three areas of increased DVC risk across the Victorian road network.

This study provides tools to collect empirical ecological data on contemporary deer populations. New information on deer population connectivity, activity patterns and collision risk is also presented. Future work, applying the genetic, camera trapping and spatial modelling methodologies described here has much scope for further improving our understanding of wild deer ecology and impacts. Similar approaches could be used to inform the management of other terrestrial invasive species in south east Australia.

Chapter 1 – Introduction

Mature sambar stag, Baw Baw National Park,
October 2016

Photo credit: Christopher Davies

Chapter 1 – Introduction

1.1 Global extinction crisis

Many biologists suggest life on earth is currently experiencing an extinction event, where more than three quarters of species are forecast to be lost over a geologically short time period (Barnosky *et al.* 2011). The current accelerated rate of species extinctions is thought to be driven directly by anthropogenic activities, including climate change, overexploitation of resources and the introduction and spread of pathogens and invasive species (Davies *et al.* 2006).

Australia has the highest rate of recent terrestrial mammal extinctions of all continents on Earth, with 29 species lost since European settlement in 1788, representing 35% of global mammal extinctions recorded since 1500 (Woinarski *et al.* 2015). In addition, 56 Australian endemic terrestrial mammal species meet the International Union for the Conservation of Nature (IUCN) Red List criteria for listing as threatened (IUCN 2013). Invasive species have contributed directly to these declines and extinctions.

1.2 Impact of invasive species

A species is defined as invasive when it is introduced to, and threatens environmental, agricultural and/or social values in an area outside its natural range (Invasive Plants and Animals Committee, 2016). Alongside other threatening processes, including habitat loss and landscape fragmentation, invasive species are recognised as key causes of biodiversity loss. Invasive species jeopardise the functionality of ecosystems by threatening the survival, abundance and evolutionary development of native species and communities (Allendorf and Lundquist 2003; Mooney and Cleland 2001).

Invasive predators such as the red fox (*Vulpes vulpes*) and feral cat (*Felis catus*) have been implicated in many extinctions recorded in Australia, and have decimated populations of mammals, birds and reptiles (Abbott 2011; Doherty *et al.* 2017). Other, non–predatory, invasive mammals have also caused significant environmental damage. For example, the European rabbit (*Oryctolagus cuniculus*), introduced to Australia during 1859 has been listed as a key threatening process due its impact on native vegetation and its role in land degradation (Commonwealth of Australia, 2016). Larger animals that have been introduced to Australia, including water buffalo (*Bubalus bubalis*), feral pigs (*Sus scrofa*), goats (*Capra hircus*), camels

(*Camelus dromedarius*) and deer degrade ecosystems and damage vegetation through trampling, browsing and wallowing (Petty *et al.* 2007; Spencer *et al.* 2006).

1.3 Deer

Deer are mammalian herbivores belonging to the Cervidae family, within the order artiodactyla (Hernández Fernández and Vrba 2005). Other families within the order include: Suidae (pigs), Camelidae (camels) and Bovidae (buffalo, antelopes, wildebeest, gazelles, sheep, goats, cattle and others). Although there are similarities in appearance between the cervids and the bovids, the antlers of deer are a distinctive feature. Unlike the horns of bovids, deer antlers are temporary and regularly regrown (Hernández Fernández and Vrba 2005).

Deer are widely distributed and are indigenous to all continents except Australia and Antarctica (Hernández Fernández and Vrba 2005). Deer are ecologically, physiologically and behaviourally diverse and inhabit a range of different habitats from tropical forests to subalpine meadows (Weber and Gonzalez 2003; Leslie 2011). The diversity and richness of deer species makes them of great interest to biologists and the relationship between different deer species is useful for understanding the process of evolution (Hernández Fernández and Vrba 2005).

Deer can cause substantial deleterious ecological impacts. In the United States of America (USA), browsing by native white tailed deer (*Odocoileus virginianus*) has been shown to alter ecosystems (Rooney 2001; Horsley *et al.* 2003). When overabundant, this species has caused considerable negative economic and ecological impacts (Côté *et al.* 2004; Waller 1997). The example of white tailed deer in the USA illustrates how deer can cause significant issues, even within ecosystems that have evolved with the presence of deer. The expansion of native roe deer (*Capreolus capreolus*) populations in the United Kingdom (UK) has also been linked with deleterious impacts to native ecosystems (Ward 2005; Putman *et al*. 2011). In areas where deer have been introduced, their ecological impacts are often more pronounced. Several deer species have been widely translocated outside of their natural home ranges, including to Australia (Moriarty 2004).

Deer were introduced to Australia by the Acclimatisation Society during the 1860's to provide hunting opportunities (Bentley 1957). Six wild deer species have since established populations in Australia; fallow deer (*Dama dama*), red deer (*Cervus elaphus*), rusa deer (*Rusa timorensis*), hog deer (*Axis porcinus*), chital deer (*Axis axis*) and sambar deer (*Rusa unicolor*) (Bentley 1957; Moriarty 2004). Wild deer are present in all of Australia's States and Territories (Fig. 1.1) and occupy a range of different habitat types (Moriarty 2004). Western Australia (WA) and the Northern Territory (NT) have lower numbers of wild deer when compared to other areas of Australia. However, sambar deer are present on the Cobourg peninsula in the NT and small self-sustaining populations of fallow and red deer exist in WA. Of the six established species of deer, four (sambar deer, fallow deer, red deer and hog deer) are present in the south-eastern State of Victoria (Davis *et al.* 2016; Forsyth *et al.* 2009).

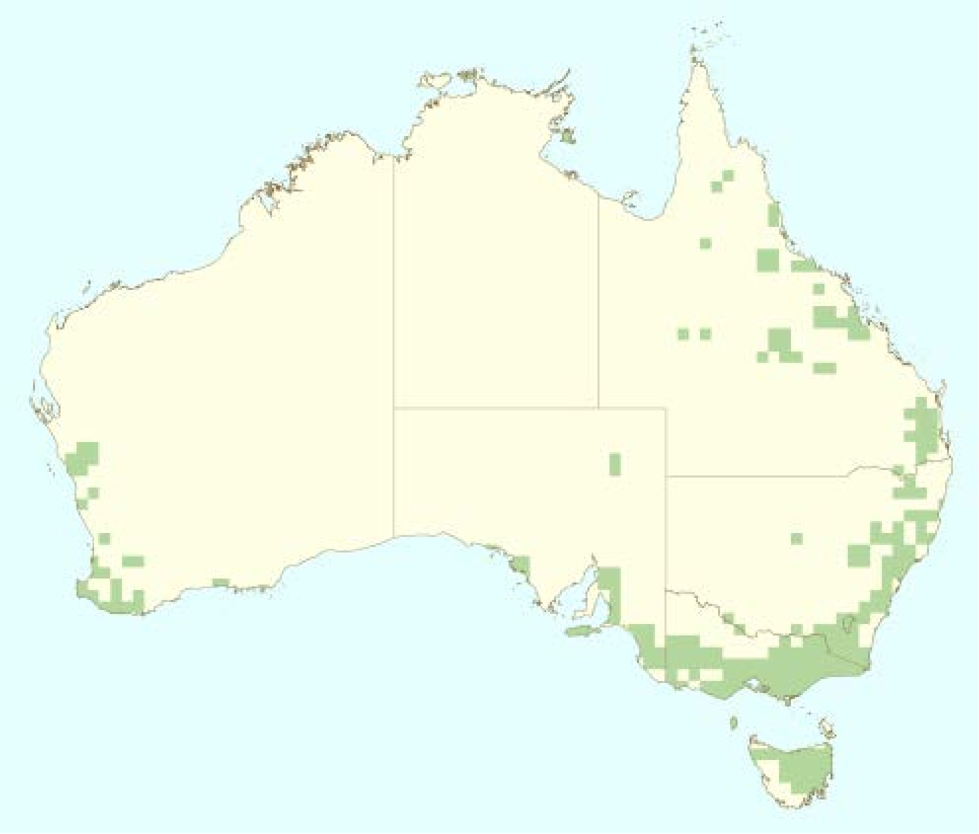

Fig. 1.1: Distribution of introduced deer (all species) in Australia. Green shading denotes deer occurrence. Source: Commonwealth of Australia (2011).

Sambar deer are Asia's most widespread deer species with a native range including forested areas of India, Sri Lanka, Southern Nepal, Burma, China, Myanmar, Thailand, Malaysia, Indonesia and Taiwan (Leslie 2011). Despite their widespread distribution, sambar deer are considered vulnerable in their native range and populations are reported to be decreasing (Timmins *et al.* 2015). Threats include deforestation associated with development and illegal hunting (Timmins *et al.* 2015). Sambar are large species of deer, with males reaching up to 320 kilograms and females reaching around 225 kilograms (Leslie 2011). Only male sambar deer grow antlers which are typically three-tined, rough and corrugated (Fig. 1.2) (Bentley 1957).

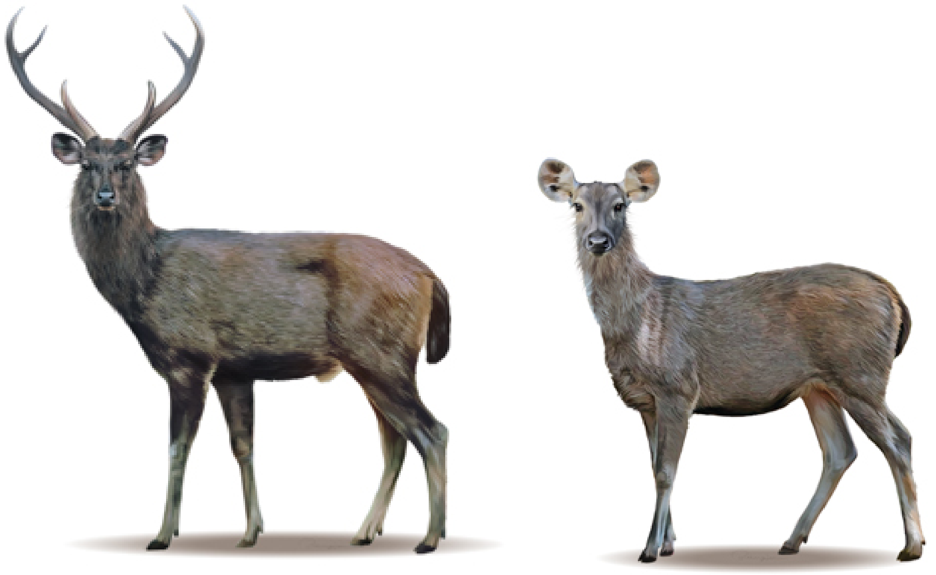

Fig. 1.2: a) Male sambar deer (stag) b) Female sambar deer (hind). (Game Management Authority 2019).

Several recent publications have documented the widespread distribution of sambar deer in Victoria, Australia (Forsyth *et al.* 2015; Gormley *et al.* 2011). Forsyth *et al.* (2015) estimated the distribution of sambar deer, rusa deer and sika deer in Victoria using historical information recorded in published journal articles, books and the Victorian Biodiversity Atlas. The report concluded that sambar deer populations are well established in Victoria and are likely to consist of four reproductively isolated populations: Eastern Victoria, Mt Cole, Timboon and French Island. Occupancy modelling conducted by Gormley *et al.* (2011) also demonstrated a wide distribution of sambar deer in Victoria, and identified several areas of suitable habitat not thought to be inhabited by sambar deer at the time of the study, including the Grampians and Great Otway National Parks.

Across their Victorian range sambar deer have a preference for densely forested habitats (Forsyth *et al.* 2015), reflecting the habitat preferences observed in their native range (Yen *et al.* 2019). Sambar deer are Victoria's most abundant introduced deer species and there is much concern surrounding their negative social, economic and ecological impacts (Davis *et al*. 2016; DEDJTR 2018; Parliament of Victoria 2017).

1.4 Deer impacts

1.4.1 Browse impacts

Due to their varied diet and ability to consume large amounts of forage, wild deer have caused significant changes to vegetation composition and structure in ecosystems around the world. In the USA, browsing by white tailed deer has been shown to reduce plant diversity in bog and fen ecosystems (Pellerin *et al.* 2006). Due to selective browsing, deer can also impact the structure and composition of forest understoreys (Habeck and Schultz 2015; Martin *et al.* 2010). For example, Long *et al.* (2007) reported a decrease in the abundance of palatable species and concurrent increase in unpalatable plant species due to browsing by white tailed deer in the USA.

Deer browsing can also alter recruitment in forest ecosystems. Bradshaw and Waller (2016) demonstrated a sharp decline in the number of maple (*Acer*) and aspen (*Populus*) saplings with increasing densities of white tailed deer in the USA. Browsing of saplings by white tailed deer has resulted in the recruitment failure of long lived conifer species including eastern hemlock (*Tsuga canadensis*) and northern white cedar (*Thuja occidentalis*) (Rooney 2001). As these cedar species support distinct bird and floral communities, recruitment failure is a substantial conservation concern (Rooney 2001). White tailed deer browsing has also been found to reduce the growth and abundance of understorey species in northern hardwood forests of the USA, including lily-of-the-valley (*Maianthemum canadense*) and white flowered trillium (*Trillium grandi-florum*) (Rooney 2001).

The impact of deer browsing has also been documented outside of the US. Studies investigating the ecological impacts of deer in the United Kingdom (Stewart 2001), Italy (Motta 1996), Japan (Akashi *et al.* 2015) and New Zealand (Husheer *et al.* 2003) demonstrate the ability of deer to change forest structure and composition through selective browsing. Furthermore, deer browsing has caused reductions in small mammal densities (Flowerdew and Ellwood 2001) and songbird abundance (Newson *et al.* 2012) in forested woodlands in the United Kingdom. The browse impacts of deer can be particularly damaging in ecosystems that have evolved without the presence of large ungulate herbivores, such as Australian ecosystems.

1.4.2 Browse impacts in Australia

To date, research focussed on the browse impacts of deer in Australian ecosystems has been limited. This is due, in part to the difficulties in differentiating browsing by deer from browsing by native herbivores (Bennett and Coulson 2008). Bennett (2008) identified that sambar deer consumed more forage than native herbivores in a protected water catchment located within the Yarra Ranges National Park in Victoria. Bennett (2008) also showed that, in areas where sambar deer occur in high densities, they significantly reduced plant biomass in the forest understorey.

Deer browsing is damaging a range of ecosystems in Australia, including some that are classified as threatened. In the East Gippsland region of Victoria, deer are adversely affecting several Ecological Vegetation Classes (EVC)[1] including, littoral rainforest, salt marsh and warm temperate rainforest (Peel *et al*. 2005). Sambar deer browsing of rainforest seedlings is also considered to be a significant threat to the functionality of rainforest areas in East Gippsland (EGRCMN 2016). Another study conducted by Keith and Pellow (2005) in the Royal National Park, New South Wales (NSW) showed that 88% of the plant species displayed evidence of damage caused by deer, and concluded that deer herbivory is a significant threat to the growth and survival of established native plant species.

In addition to their ecological impacts, deer browsing also damages agricultural and silvicultural systems (Moore *et al.* 1999). Deer browsing impacts to silvicultural systems have been widely documented in Europe and the USA (Miller *et al.* 2009; Murray *et al.* 2016) but few studies have quantified browse impacts to Australian plantations (Davis *et al.* 2016). Likewise, few studies have examined and identified the specific agricultural impacts of wild deer in Australia. However, agricultural impacts of deer including wallowing in pasture, damage to fencing and browsing impacts have been identified in both Victoria and NSW (Claridge 2016; Lindeman and Forsyth 2008).

1.4.3 Antler rubbing

Male deer use their antlers to display dominance and mark their home range by rubbing and thrashing vegetation (Bentley 1957). Each year, male deer remove a layer of velvet from their newly grown antlers by rubbing on trees and shrubs. Both native plant communities and commercial plantations are at risk of damage due to antler rubbing by deer. Bilney (2013)

---

[1] An EVC is the standard term for classifying vegetation types in Victoria.

identified antler rubbing by sambar deer as a cause of mortality to stands of endangered yellow wood (*Acronychia oblongifolia*) in East Gippsland, showing that sambar deer are directly threatening plant communities that are already at risk due to land clearing and climate change. Antler rubbing by sambar deer has also caused damage to stands of threatened shiny nematolepis (*Nematolepis wilsonii*) in the Upper Yarra water catchment (Bennett 2008). While both these studies demonstrate the direct impact of antler rubbing on specific plant species, they did not investigate the overall impact of antler rubbing on vegetation structure and composition in each relevant EVC.

Antler rubbing by deer can also cause direct economic impacts to producers by damaging plant stock. Lindeman and Forsyth (2008) identified that sambar deer antler rubbing and thrashing was responsible for damage to a Christmas tree farm (*Pinus radiata*) and a fruit orchard in East Gippsland. Most of the work investigating the impacts of antler rubbing has focussed on sambar deer in Victoria and NSW, with little information available regarding other deer species, or in different locations in Australia. There is also little information available regarding which trees and shrubs are favoured for rubbing by each deer species, nor the overall impact of antler rubbing on vegetation recruitment, composition and structure.

Claridge (2016) identified a comprehensive list of native plant species from NSW and the Australian Captial Territy (ACT) impacted by antler rubbing. Plant species severely rubbed by deer included alpine plum pine (*Podocarpus lawrencii*) and hazel pomaderris (*Pomaderris aspera*). Worryingly, alpine plum pine is an important food source of the critically endangered mountain pygmy possum (*Burramys parvus*). Deer also impact their environment due to other aspects of their behaviour and ecology including the formation of game trails, wallows and pugging (damage to soil structure associated with deer hooves).

1.4.4 Game trail formation/wallows/pugging

Sambar deer create wallows in wet areas and regularly coat themselves in mud (Leslie 2011). Wallowing behaviour has been observed in both male and female sambar deer in their native and introduced ranges (Leslie 2011; Peel *et al.* 2005). Recent surveys in Victoria’s largest national park, the Alpine National Park, revealed that sambar deer are destroying endangered alpine bogs through wallowing and trampling (Phillipson *et al.* 2015). Alpine sphagnum bogs and associated fens are endangered ecological communities under the Australian Government’s Environmental Protection and Biodiversity Conservation (EPBC) Act 1999. Sphagnum bogs provide refuge for a number of endemic flora and fauna species, including the critically endangered corroboree frog (*Pseudophryne corroboree*) (Wahren *et al.* 2001). Peel *et al.* (2005) also raised concerns about the formation of game trails by sambar deer in the forested landscapes of East Gippsland, suggesting these trails destroy refuges for small mammals and allow access for introduced predators.

1.4.5 Deer as a vector for disease and parasites

There is concern from biosecurity agencies that introduced deer populations in Australia may act as vectors for diseases and parasites that are harmful to native wildlife, domestic livestock and humans (Cripps *et al.* 2018; Daszak *et al.* 2000). As deer are biologically similar to domestic livestock, particularly ruminant animals such as cattle, sheep and goats, they can be affected by the same diseases (Böhm *et al.* 2007). In the United Kingdom, wild deer have been shown to spread damaging pathogens, including *Mycobacterium avium subsp. paratuberculosis*, the causative agent of Johne's disease (Böhm *et al.* 2007). Foot and mouth disease is a key concern and may be spread by wild deer if it were to establish in Australia (Murray and Snowdon 1976). An outbreak of foot and mouth diseases in Australia would decimate the country's livestock industry and could result in billions of dollars in direct costs associated with production loss and containment (Doran *et al*. 2005).

Cripps *et al*. (2018) established five notifiable diseases (bovine tuberculosis, foot and mouth disease, malignant catarrhal fever, surra and screw worm fly infestation) with a high risk of transmission between wild deer and domestic livestock in Australia. Of these diseases, only malignant catarrhal fever is currently present in Australian wild deer (Tomkins *et al.* 1997). Cripps *et al.* (2018) highlighted that disease data was lacking for sambar deer, and called for future work investigating diseases that are currently present within this population.

Slee and Presidente (1981) investigated parasites of Victorian sambar deer and identified liver fluke (*Fasciola hepatica*) and a number of gastrointestinal nematodes. In Europe, wild deer have been identified as vectors for anthelmintic resistant nematodes (Chintoan-Uta *et al.* 2014). A recent study identified that the pathogenic microsporidian *Enterocytozoon bieneusi* is present within a small proportion of sambar deer residing in Melbourne's water catchments (Zhang *et al.* 2018). *Cryptosporidium* and *giardia* species have also been documented within deer populations in Australia (Ryan and Power 2012).

1.4.6 Vehicle collision risk

Globally, wildlife-vehicle collisions (WVCs) are responsible for the death of billions of animals each year, along with substantial personal and economic costs associated with human injuries and death (Forman and Alexander 1998; Huijser *et al.* 2007). The involvement of cervid species in WVCs is a significant issue in the USA, with annual costs associated with vehicle repair and human medical costs estimated at over eight billion dollars (Huijser *et al.* 2007; Snow *et al.* 2018). Therefore, vehicle collisions represent the most costly of all deer-related damage caused in the USA (Huijser *et al.* 2007).

Several mitigation strategies have been employed in order to reduce the risk of deer vehicle collisions. Fencing has proven effective at reducing the rate of deer vehicle collisions in the USA (Bissonette and Rosa 2012). Other mitigation strategies include speed limit reductions during high risk times of the day (Meisingset *et al.* 2014; Visintin *et al.* 2018), audio and visual deterrents (Huijser *et al.* 2007) and reducing deer populations in high risk areas (Hothorn *et al.* 2012).

In Australia, the risk of deer vehicle collisions is increasing due to the rapid growth of deer populations, increasing human population and development near forested areas (DEDJTR 2018). Wild deer are known to have caused vehicle collisions resulting in human fatalities in Australia, with documented cases including a collision between a car and a rusa deer near the Royal National Park in NSW, and another near Wollongong, NSW in 2012 (Cox 2018). More research is required to identify deer collision hot spots, so that mitigation strategies such as speed limit reduction and roadside fencing can be applied to help reduce deer collision risk.

1.5 Deer management

Deer management is a broad term that refers to actions undertaken to alter deer population dynamics and deer impacts to meet specific objectives. Deer management objectives may include eradication, containment, impact reduction or sustaining a healthy population for recreational hunting (DEDJTR 2018). Some of the more common deer management approaches are described below.

1.5.1 Ground and aerial shooting

Where deer are considered problematic, such as within sensitive ecosystems, or overabundant, land managers attempt to regulate populations through population control (Vercauteren *et al.* 2006). Population control usually involves the shooting of deer either from the ground or from a helicopter. In New Zealand, culling from helicopters effectively reduced the population and impacts of red deer in some areas of the South Island (Tanentzap *et al.* 2009).

Despite being extremely effective in open areas, culling from helicopters is not feasible in areas with dense tree cover. Therefore it may not be practicable in densely forested areas of Australia which are habitats favoured by sambar, hog and red deer. Culling from helicopters may however be feasible in areas with little tree cover such as Australian alpine and subalpine environments. Helicopter culling has recently been utilised in attempts to control wild deer populations in both Queensland (Pople *et al.* 2017) and Victoria (Parks Victoria 2019) but the effectiveness of the method to reduce deer populations has not been empirically tested in Australia.

1.5.2 Exclusion fencing

Fencing, which physically prevents deer from entering specific areas, has been successfully utilised to exclude deer from sensitive areas in the USA, Europe and Australia (Bennett and Coulson 2008; Vercauteren *et al.* 2006). While fencing has been demonstrated to be effective at small scales it is unrealistic and expensive for large areas; and transport of fencing materials to remote areas is difficult and costly (Vercauteren *et al.* 2006). Exclusion fencing of study plots has been used to investigate the effect of deer browsing on vegetation and to assess vegetation damage caused by deer in Victoria (Bennett and Coulson 2008). Exclusion fencing of important areas could be applied alongside control mechanisms such as shooting to improve the ecological outcomes of deer management programs and directly protect endangered plant communities and sensitive alpine bogs. Fencing could also be applied along roadsides to reduce the risks of deer vehicle collisions, a mitigation strategy that has proven highly effective at reducing collisions involving mule deer (*Odocoileus hemionus*) in the USA (Bissonette and Rosa 2012).

1.5.3 Fertility control

Fertility control has been used in some areas of the USA to stabilise rapidly increasing populations of white tailed deer (Raiho *et al.* 2015). This method involves either remotely darting wild deer with a contraceptive agent or capturing deer and administering a contraceptive injection or implant (Garside *et al.* 2014). Fertility control does not reduce deer numbers but reduces population growth rate so is best used in combination with traditional control methods such as culling (Raiho *et al.* 2015). Most studies that have investigated the efficiency of fertility control have been focussed on white tailed deer in the USA (Miller *et al.* 2000; Raiho *et al.* 2015; Turner Jr *et al.* 1996), as a result, little is known about the effectiveness of fertility control for other deer species. Fertility control may play a part in managing deer populations in Australia but further research is required to assess cost effectiveness.

1.5.4 Complexities of deer management in Australia

There are two dominant and conflicting perceptions regarding the presence of deer in Australian ecosystems. Some people consider deer as invasive pests that are associated with damage to ecological and agricultural systems (Lindeman and Forsyth 2008), whilst others consider introduced deer as iconic game species; invaluable additions to the Australian environment (Bentley 1957). Deer management is therefore a complex issue and a highly polarised debate exists between hunting advocates and invasive species managers (Davis *et al.* 2016).

Although associated with many negative impacts, introduced deer populations in Australia also have some positive social and economic impacts. Deer hunting is a popular recreational activity in south east Australia and is valued by many people as a pastime as well as a source of wild meat (Finch *et al.* 2014). Over the last decade the number of licensed recreational hunters in Victoria has grown, with many people taking up deer hunting (Moloney and Turnbull 2017). Deer hunting has a positive economic impact, particularly in small regional areas due to increased trading opportunities for retail and hospitality sectors (Finch *et al.* 2014). In Victoria each hunter is required to hold a game license to hunt deer, for which a fee is paid (approximately $57.80 per year). This fee generates revenue for the State Government. Game hunting and its associated flow on effects was estimated to have raised $295 million dollars in Victoria during 2013 (Henderson *et al.* 2014), a figure that is likely to have increased over the last six years.

1.6 Knowledge gaps currently impeding deer management in south east Australia

Victoria’s draft deer management strategy listed a number of key knowledge gaps that are currently impeding our ability to effectively manage deer. The strategy grouped the many identified knowledge gaps into four broad categories: 1) impacts 2) management systems and tools 3) distribution and abundance 4) community engagement and awareness (DEDJTR 2018).

Within the impacts category, the strategy outlined that more information is required on specific deer impacts to priority assets, the relationship between deer density and the damage they cause, the disease risk that deer pose to livestock, the relationship between deer carcasses and wild dogs, and the rate of vehicle collisions involving deer (DEDJTR 2018). In regard to management systems and tools, the strategy listed a number of knowledge gaps, including the efficacy of alternative deer control options, such as guardian dogs, exclusion fencing, poisons, repellents and fertility control. Additionally, the strategy highlighted that little is known about what are the best practice methods for both monitoring and controlling deer impacts (DEDJTR 2018). The strategy also highlighted a limited understanding of deer abundance and distribution and suggested that further investigation of the rate and location of deer spread is required.

Currently, invasive species managers use a number of different tools to collect information on the species of interest. The presence of invasive species is commonly determined by conducting surveys looking for evidence of the target species including footprints and scats (Forsyth *et al.* 2009). Camera traps have also been used effectively to detect invasive species (Gormley *et al.* 2011; Rendall *et al.* 2014). These tools have been applied to monitor introduced deer species in Australia. However, there is much scope to develop and improve these tools.

1.7 Tools for deer management

A number of different tools and techniques are used to collect ecological information from populations of wild deer. Current methodologies for the estimation of deer abundance (pre and post control) include direct counts, where deer are observed along transects using spotlights (Garel *et al.* 2010), thermal imagers (Focardi *et al.* 2001) or from aircraft (Storm *et al.* 2011). However, direct counts are not without bias and are difficult to conduct in densely forested environments (Brinkman *et al.* 2013). Accordingly, indirect methods such as faecal pellet counts are commonly used to provide estimates of abundance, especially in monitoring programs designed to determine whether control programs have been effective (Acevedo *et al.* 2010; Forsyth *et al.* 2007).

1.7.1 Scat surveys

One of the most common methods used to estimate the abundance and density of wild deer is the faecal pellet count (or scat survey), where the total number of deer scat pellets, and/or total number of pellet groups are counted along transects. The work of Forsyth *et al.* (2007) demonstrated that pellet counts could be useful for estimating deer abundance in challenging environments, showing that three parameters (total number of pellets, total number of pellet groups and pellet frequency) were related to deer density and could therefore be used as indices of deer abundance. In contrast, the accuracy and reliability of faecal pellet indices to estimate deer abundance and density has been questioned, especially when decay rates of faecal pellets, which may be habitat and season specific, are not accounted for (Hemami and Dolman 2005).

Another limitation of scat surveys is the misidentification of the target species (Adams *et al.* 2011; Heise-Pavlov and Meade 2012). This is a significant issue, particularly if scats from the target species appear similar to other species that occupy the same environment. The issue of scat misidentification was demonstrated by Spitzer *et al.* (2019) who showed 41% of roe deer (*Capreolus capreolus*) scats were misidentified during their study in Europe. In Australia, many areas are occupied by multiple deer species resulting in potential scat misidentification during scat surveys. Incorporating a genetic approach into scat surveys can be used to accurately assign the species of origin to scats and identify individuals of the same species, preventing scat misidentification and improving monitoring strategies that incorporate scat surveys.

1.7.2 Camera trapping

Motion sensor camera traps are used extensively for surveillance and wildlife research (Meek *et al.* 2014). Once they are deployed in the field, cameras take photographs when they are triggered by heat or motion. Camera traps can gather large amounts of important ecological information on a range of species, over large study areas for long periods of time and are ideal for detecting rare and cryptic species (Meek *et al.* 2014). They have been used for both species conservation and pest management purposes (Bischof *et al.* 2014; Gormley *et al.* 2011). At the simplest level camera traps can be used to detect species presence in a specific location. This is important for invasive species monitoring programs and for assessing the effectiveness of control operations. Camera traps can also provide insights into species behaviour, activity patterns and reproductive status (Meek *et al.* 2015). In Victoria, camera traps have been used to collect data to inform models of current and potential future distributions of sambar deer (Gormley *et al.* 2011) and to determine their presence in sensitive areas including alpine peat bogs (Phillipson *et al.* 2015) and littoral rainforest (EGRCMN 2016).

1.8 Priority knowledge required to guide deer management strategies

1.8.1 Population structure and connectivity

In recent years, population genetics has been applied to help understand invasive species and inform their management (Rollins *et al.* 2006). Population genetics can be applied to define the size, geographical extent and connectivity of populations of pest species (Abdelkrim *et al.* 2005; Fraser *et al.* 2013). By exploring allele frequencies in different populations, the genetic relatedness of individuals within and between populations can demonstrate connectivity between different populations of pest species (Veale *et al.* 2014). This information can be used to determine whether complete eradication of a pest population is feasible, or an ongoing effort to reduce numbers is a better control strategy (Fraser *et al.* 2013; Rollins *et al.* 2006). Eradication may be possible for small, isolated populations but is considered unfeasible in large, widespread populations, especially if the species is cryptic (Myers *et al.* 2000). Studying the population genetics of invasive species can also provide information on invasion history (Rollins *et al.* 2011), characterise demographics and outline dispersal patterns in different landscapes (Estoup and Guillemaud 2010).

1.8.2 Abundance

Abundance data is critical for the effective management of invasive species and is required to determine how many animals must be removed to stop population growth (Hone *et al*. 2010). Furthermore, abundance estimates are essential to determine the threshold of which deer populations cause negative impacts. In Victoria, current abundance estimates are calculated from hunter surveys for each individual deer species. However, these estimates are likely to be biased due to both under-reporting and over-reporting during the survey process (Moloney and Turnbull 2017). Scat surveys can provide estimates of deer abundance but can be constrained by scat misidentification. For this reason, land managers require more robust methods to estimate the abundance of wild deer pre and post control, to assess which control methods are the most effective.

1.8.3 Vehicle collision risk

Spatial modelling incorporates the use of mathematical models to help understand complex ecological processes and make predictions on how systems may change over time (Elith and Leathwick 2009). Species Distribution Models (SDMs) are one of the most commonly used spatial modelling methods and have been used to investigate the distribution of native and introduced species (Gormley *et al*. 2011). SDMs estimate the relationship between species records at specific sites and environmental covariates to make predictions about the distribution of a species (Elith and Leathwick 2009; Franklin and Miller 2010). SDMs are commonly used to guide decisions involving the management of invasive species (Mainali *et al.* 2015). SDMs can be used to determine where to carry out eradication and control efforts and prevent future invasions. SDMs are also an essential tool to predict areas of potential occupancy to target surveillance and management of invasive species. Globally, SDMs have been used to estimate the distribution of deer for conservation purposes (Quevedo *et al.* 2017). In Victoria, species distribution modelling was used to estimate the current and future distributions of sambar deer to help focus control efforts and improve monitoring (Gormley *et al.* 2011).

SDM's can also be incorporated into other modelling approaches and used to predict where wildlife vehicle collisions are likely to occur (Visintin *et al.* 2016). This approach has proven successful at predicting the collision risk of native species across the Victorian road network allowing mitigation efforts (such as fencing, speed limit reduction) to be applied in high risk areas. Currently, no published studies have used a modelling approach to estimate the collision risk of wild deer across the Victorian road network, despite increasing records of deer vehicle collisions.

1.9 Problem statement

To date, research has not been sufficient to underpin the effective management of wild deer in south east Australia. Land managers require both robust tools to monitor deer populations as well an increased understanding of deer ecology and impacts in order to generate and implement effective management strategies. Establishing new tools and improving the methods currently used to monitor wild deer populations will greatly improve the capacity to effectively manage wild deer in the future. Likewise, a better understanding of deer ecology and impacts will help develop and apply mitigation strategies in ecosystems put at risk by the presence of deer.

1.10 Thesis aims

The overarching aim of this thesis is to provide tools and information to guide the management of wild deer in south east Australia, with a particular focus on sambar deer (Victoria's most abundant and damaging introduced deer species). The outcomes of this study will provide, 1) genetic, camera trap and modelling tools to enhance deer monitoring, and 2) new information about Victorian deer populations, including sambar deer population structure, activity patterns and insights into the vehicle collision risk posed by deer in general.

1.11 Thesis structure

The thesis is structured as follows. The thesis consists of four self-contained data chapters, all of which have either been published or are currently under review in peer-reviewed journals.

In chapter one, the introduction, I provide the context for the issues of deer management and identify key research priorities to improve deer management in south east Australia. Chapter one also establishes the aims of this thesis.

Genetic data can be used to address complex ecological questions and has been applied to improve the management of invasive species. In chapter two I develop a ‘genetic toolbox’ which optimises the isolation of DNA from wild deer scats. Additionally, chapter two presents a suite of molecular markers which can genetically distinguish scats of sambar deer from other sympatric deer species and provide a unique DNA profile for individual sambar deer.

The genetic toolbox developed in chapter two was then applied to explore contemporary population structure of sambar deer in Victoria. Specifically, in chapter three microsatellite genotyping (using DNA sourced from deer scats and tissue) is applied to determine whether the Victorian sambar population is a single homogenous unit or divided into discrete management units. This information has wide applications to inform the implementation of deer control operations.

Deer management interventions such as culls undertaken in protected areas require information to determine their timing and scale to ensure resources are spent effectively. In chapter four I investigate the predictors of sambar deer detectability and detail sambar deer activity patterns in Baw Baw National Park (BBNP). In chapter four I also explore the effectiveness of the camera trap survey method, and assess how reliably a single camera can detect deer when present.

Globally, vehicle collisions represent one of most serious impacts of wild deer. Expanding deer populations in Victoria are posing a risk to motorists and deer vehicle collisions are likely to cause significant impacts in coming years. In chapter five I utilise a spatial modelling approach to predict where vehicle collisions involving wild deer are most likely to occur in Victoria. The clear identification of high risk areas will allow mitigation strategies to be implemented and reduce the risk of deer vehicle collisions in the future.

To conclude, in chapter six, I highlight the advances made by my research. I integrate my findings from chapters two, three, four and five and discuss their implications for the management of wild deer in Australia, with a particular focus on sambar deer in Victoria. I then identify the important remaining knowledge gaps and provide suggestions for future research.

1.12 References

Abbott, I. (2011). The importation, release, establishment, spread, and early impact on prey animals of the red fox *Vulpes vulpes* in Victoria and adjoining parts of south-eastern Australia. *Australian Zoologist* **35**, 463-531.

Abdelkrim, J., Pascal, M., Calmet, C., and Samadi, S. (2005). Importance of assessing population genetic structure before eradication of invasive species: Examples from insular Norway rat populations. *Conservation Biology* **19**, 1509-1518.

Acevedo, P., Ferreres, J., Jaroso, R., Durn, M., Escudero, M. A., Marco, J., and Gortzar, C. (2010). Estimating roe deer abundance from pellet group counts in Spain: An assessment of methods suitable for Mediterranean woodlands. *Ecological indicators* **10**, 1226-1230.

Adams, J. R., Goldberg, C. S., Bosworth, W. R., Rachlow, J. L., and Waits, L. P. (2011). Rapid species identification of pygmy rabbits (*Brachylagus idahoensis*) from faecal pellet DNA. *Molecular Ecology Resources* **11**, 808-812.

Akashi, N., Unno, A., and Terazawa, K. (2015). Significance of woody browse preferences in evaluating the impact of sika deer browsing on tree seedlings. *Journal of Forest Research* **20**, 396-402.

Allendorf, F. W. and Lundquist, L. L. (2003). Introduction: Population biology, evolution, and control of invasive species. *Conservation Biology* **17**, 24-30.

Barnosky, A., Matzke, N., Tomiya, S., Wogan, G. O. U., Swartz, B., Quental, T., Marshall, C., McGuire, J., Lindsey, E., Maguire, K., Mersey, B., and Ferrer, E. A. (2011). Has the Earth's sixth mass extinction already arrived? *Nature* **471**, 51-57.

Bennett, A. (2008) The impacts of sambar (*Cervus unicolor*) in the Yarra Ranges National Park. Ph.D. Thesis, The University of Melbourne, Melbourne.

Bennett, A. and Coulson, G. (2008). Evaluation of an exclusion plot design for determining the impacts of native and exotic herbivores on forest understoreys. *Australian Mammalogy* **30**, 83-87.

Bentley, A. (1957). A brief account of the deer in Australia. *The Journal of Wildlife Management* **21**, 221-225.

Bilney, R. J. (2013). Antler rubbing of yellow-wood by sambar in East Gippsland, Victoria. *Victorian Naturalist, The* **130**, 68-74.

Bischof, R., Hameed, S., Ali, H., Kabir, M., Younas, M., Shah, K. A., Din, J. U., and Nawaz, M. A. (2014). Using time-to-event analysis to complement hierarchical methods when assessing determinants of photographic detectability during camera trapping. *Methods in Ecology and Evolution* **5**, 44-53.

Bissonette, J. A. and Rosa, S. (2012). An evaluation of a mitigation strategy for deer-vehicle collisions. *Wildlife Biology* **18**, 414-423, 410.

Böhm, M., White, P. C., Chambers, J., Smith, L., and Hutchings, M. (2007). Wild deer as a source of infection for livestock and humans in the UK. *The Veterinary Journal* **174**, 260-276.

Bradshaw, L. and Waller, D. M. (2016). Impacts of white-tailed deer on regional patterns of forest tree recruitment. *Forest Ecology and Management* **375**, 1-11.

Brinkman, T. J., Person, D. K., Smith, W., Chapin, F. S., McCoy, K., Leonawicz, M., and Hundertmark, K. J. (2013). Using DNA to test the utility of pellet-group counts as an index of deer counts. *Wildlife Society Bulletin* **37**, 444-450.

Chintoan-Uta, C., Morgan, E. R., Skuce, P. J., and Coles, G. C. (2014). Wild deer as potential vectors of anthelmintic-resistant abomasal nematodes between cattle and sheep farms. *Proceedings of the Royal Society: Biological Sciences* **281**.

Claridge, A.W. (2016). Ecological and agricultural impacts of introduced deer in the Australian Alps. A final report to the Australian Alps Liason Committee. New South Wales Parks and Wildlife Service, Queanbeyan, NSW 2620, Australia.

Commonwealth of Australia (2011). Feral Deer. Australian Government, Canberra.

Commonwealth of Australia (2016). Threat abatement plan for competition and land degradation by rabbits. Australian Government, Canberra.

Côté, S. D., Rooney, T. P., Tremblay, J.-P., Dussault, C., and Waller, D. M. (2004). Ecological impacts of deer overabundance. *Annual Review of Ecology, Evolution, and Systematics* **35**, 113-147.

Cox, A. (2018). Feral deer putting NSW drivers at risk. Available at: https://invasives.org.au/blog/feral-deer-putting-nsw-drivers-at-risk/ [accessed 16 July 2018].

Cripps, J. K., Pacioni, C., Scroggie, M. P., Woolnough, A. P., and Ramsey, D. S. L. (2018). Introduced deer and their potential role in disease transmission to livestock in Australia. *Mammal Review*.

Daszak, P., Cunningham, A. A., and Hyatt, A. D. (2000). Emerging infectious diseases of wildlife - threats to biodiversity and human health. *Science* **287**, 443-449.

Davies, R., Orme, C. D. L., Olson, V., Thomas, G., Ross, S., Ding, T.-S., Rasmussen, P., Stattersfield, A., Bennett, P., Blackburn, T., Owens, I. P. F., and Gaston, K. J. (2006). Human impacts and the global distribution of extinction risk. *Proceedings - Royal Society. Biological Sciences* **273**, 2127-2133.

Davis, N. E., Bennett, A., Forsyth, D. M., Bowman, D. M. J. S., Lefroy, E. C., Wood, S. W., Woolnough, A. P., West, P., Hampton, J. O., and Johnson, C. N. (2016). A systematic review of the impacts and management of introduced deer (family Cervidae) in Australia. *Wildlife Research* **43**, 515-532.

Department of Economic Development Jobs Trasnsport and Resources (DEDJTR) (2018). Draft deer management strategy. Melbourne, Victoria.

Doherty, T., Dickman, C., Johnson, C., Legge, S., Ritchie, E., and Woinarski, J. C. Z. (2017). Impacts and management of feral cats (*Felis catus*) in Australia. *Mammal Review* **47**, 83-97.

Doran, R. J. and Laffan, S. W. (2005). Simulating the spatial dynamics of foot and mouth disease outbreaks in feral pigs and livestock in Queensland, Australia, using a susceptible-infected-recovered cellular automata model. *Preventive Veterinary Medicine* **70**, 133-152.

East Gippsland Rainforest Conservation Management Network (EGRCMN) (2016). Submisison to the inquiry into the control of invasive animals on crown land. East Gippsland, Victoria.

Elith, J. and Leathwick, J. (2009). Species Distribution Models: Ecological Explanation and Prediction Across Space and Time. *Annual Review of Ecology, Evolution and Systematics* **40**, 677-697.

Estoup, A. and Guillemaud, T. (2010). Reconstructing routes of invasion using genetic data: why, how and so what? *Molecular Ecology* **19**, 4113-4130.

Finch, N., Murray, P., Hoy, J., and Baxter, G. (2014). Expenditure and motivation of Australian recreational hunters. *Wildlife Research* **41**, 76-83.

Flowerdew, J. R. and Ellwood, S. A. (2001). Impacts of woodland deer on small mammal ecology. *Forestry* **74**, 277-287.

Focardi, S., De Marinis, A. M., Rizzotto, M., and Pucci, A. (2001). Comparative evaluation of thermal infrared imaging and spotlighting to survey wildlife. *Wildlife Society Bulletin* **29**, 133-139.

Forman, R. and Alexander, L. E. (1998). Roads and their major ecological effects. *Annual Review of Ecology and Systematics* **29**, 207-231.

Forsyth, D. M., Barker, R. J., Morriss, G., and Scroggie, M. P. (2007). Modeling the relationship between fecal pellet indices and deer density. *Journal of Wildlife Management* **71**, 964-970.

Forsyth, D. M., McLeod, S. R., Scroggie, M. P., and White, M. D. (2009). Modelling the abundance of wildlife using field surveys and GIS: non-native sambar deer (*Cervus unicolor*) in the Yarra Ranges, south-eastern Australia. *Wildlife Research* **36**, 231-241.

Forsyth, D. M., Stamation, K., and Woodford, L. (2015). Distributions of sambar deer, rusa deer and sika deer in Victoria. Arthur Rylah Institute. Melbourne, Victoria.

Franklin, J. and Miller, J. A. (2010) 'Mapping species distributions: Spatial inference and prediction.' (Cambridge University Press: New York, United States of America.)

Fraser, E. J., Macdonald, D. W., Oliver, M. K., Piertney, S., and Lambin, X. (2013). Using population genetic structure of an invasive mammal to target control efforts – An example of the American mink in Scotland. *Biological Conservation* **167**, 35-42.

Game Management Authority (2019) Game deer species: sambar deer. Available at: https://www.gma.vic.gov.au/hunting/deer/game-deer-species [Accessed 30 June 2019].

Garel, M., Bonenfant, C., Hamann, J., Klein, F., and Gaillard, J. (2010). Are abundance indices derived from spotlight counts reliable to monitor red deer *Cervus elaphus* populations? *Wildlife Biology* **16**, 77-84.

Garside, D., Holt, W. V., and Brown, J. L. (2014) 'Fertility control in wildlife: Review of current status, including novel and future technologies.' (Springer.)

Gormley, A. M., Forsyth, D. M., Griffioen, P., Lindeman, M., Ramsey, D. S. L., Scroggie, M. P., and Woodford, L. (2011). Using presence-only and presence–absence data to estimate the current and potential distributions of established invasive species. *Journal of Applied Ecology* **48**, 25-34.

Habeck, C. W. and Schultz, A. K. (2015). Community-level impacts of white-tailed deer on understorey plants in North American forests: a meta-analysis. *AoB PLANTS* **7**, plv119.

Heise-Pavlov, S. R. and Meade, R. D. (2012). Improving reliability of scat counts for abundance and distribution estimations of Lumholtz's Tree-kangaroo (*Dendrolagus lumholtzi*) in its rainforest habitats. *Pacific Conservation Biology* **18**, 153-163.

Hemami, M. R. and Dolman, P. M. (2005). The disappearance of muntjac (*Muntiacus reevesi*) and roe deer (*Capreolus capreolus*) pellet groups in a pine forest of lowland England. *European Journal of Wildlife Research* **51**, 19-24.

Henderson, W., Morison, J., Bailey, H., and Schirmer, J. (2014). Estimating the economic impact of hunting in Victoria in 2013. Melbourne, Victoria.

Hernández Fernández, M. and Vrba, E. S. (2005). A complete estimate of the phylogenetic relationships in Ruminantia: A dated species-level supertree of the extant ruminants. *Biological Reviews of the Cambridge Philosophical Society* **80**, 269-302.

Hone, J., Duncan, R. P., and Forsyth, D. M. (2010). Estimates of maximum annual population growth rates (rm) of mammals and their application in wildlife management. *Journal of Applied Ecology* **47**, 507-514.

Horsley, S., Stout, S., and deCalesta, D. S. (2003). White-tailed deer impact on the vegetation dynamics of a northern hardwood forest. *Ecological Applications* **13**, 98-118.

Hothorn, T., Brandl, R., and Müller, J. (2012). Large-scale model-based assessment of deer-vehicle collision risk. *PloS one* **7**, e29510.

Huijser, M. P., McGowen, P. T., Fuller, J., Hardy, A., and Kociolek, A. (2007). Wildlife-vehicle collision reduction study: report to Congress. Western Transportation Institute. Bozeman, Montana, USA.

Husheer, S. W., Coomes, D. A., and Robertson, A. W. (2003). Long-term influences of introduced deer on the composition and structure of New Zealand Nothofagus forests. *Forest Ecology and Management* **181**, 99-117.

Invasive Plants and Animals Committee (2016). Australian pest animal strategy 2017-2027. Australian Government, Canberra, ACT.

IUCN (2013). Guidelines for using the IUCN categories and criteria. International Union for the Conservation of Nature. Gland, Switzerland.

Keith, D. and Pellow, B. (2005). Effects of Javan rusa deer (*Cervus timorensis*) on native plant species in the Jibbon-Bundeena area, Royal National Park, New South Wales. *Proceedings of the Linnean Society of New South Wales* **126**, 99-110.

Leslie, D. M. (2011). *Rusa unicolor* (Artiodactyla: Cervidae). *Mammalian Species,* 1-30.

Lindeman, M. J. and Forsyth, D. M. (2008) Agricultural impacts of wild deer in Victoria. Arthur Rylah Institute for Environmental Research. Melbourne, Victoria.

Long, Z., Pendergast, T., and Carson, W. (2007). The impact of deer on relationships between tree growth and mortality in an old-growth beech-maple forest. *Forest Ecology and Management* **252**, 230-238.

Mainali, K., Warren, D., Dhileepan, K., McConnachie, A., Strathie, L., Hassan, G., Karki, D., Shrestha, B., and Parmesan, C. (2015). Projecting future expansion of invasive species: comparing and improving methodologies for species distribution modeling. *Global Change Biology* **21**, 4464-4480.

Martin, J.-L., Stockton, S. A., Allombert, S., and Gaston, A. J. (2010). Top-down and bottom-up consequences of unchecked ungulate browsing on plant and animal diversity in temperate forests: lessons from a deer introduction. *Biological Invasions* **12**, 353-371.

Meek, P. D., Ballard, G. A., and Fleming, P. J. S. (2015). The pitfalls of wildlife camera trapping as a survey tool in Australia. *Australian Mammalogy* **37**, 13-22.

Meek, P. D., Ballard, G., Claridge, A., Kays, R., Moseby, K., O'Brien, T., O'Connell, A., Sanderson, J., Swann, D. E., Tobler, M., and Townsend, S. (2014). Recommended guiding principles for reporting on camera trapping research. *Biodiversity and Conservation* **23**, 2321-2343.

Meisingset, E., Loe, L., Brekkum, Ø., and Mysterud, A. (2014). Targeting mitigation efforts: The role of speed limit and road edge clearance for deer-vehicle collisions. *Journal of Wildlife Management* **78**, 679-688.

Miller, B. F., Campbell, T. A., Laseter, B. R., Ford, W. M., and Miller, K. V. (2009). White-tailed deer herbivory and timber harvesting rates: Implications for regeneration success. *Forest Ecology and Management* **258**, 1067-1072.

Miller, L., Johns, B., and Killian, G. (2000). Immunocontraception of white-tailed deer with GnRH vaccine. *American Journal of Reproductive Immunology and Microbiology* **44**, 266-274.

Moloney, P. D. and Turnbull, J. D. (2017). Estimates of harvest for deer in Victoria: results from surveys of Victorian Game licence holders in 2016. Unpublished client report for the Game Management Authority, Arthur Rylah Institute for Environmental Research, Department of Environment, Land, Water and Planning, Heidelberg, Victoria.

Mooney, H. A. and Cleland, E. E. (2001). The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences* **98**, 5446-5451.

Moore, N. P., Hart, J., and Langton, S. D. (1999) Factors influencing browsing by fallow deer *Dama dama* in young broad-leaved plantations. *Biological Conservation* **87**, 255-260.

Moriarty, A. (2004). The liberation, distribution, abundance and management of wild deer in Australia. *Wildlife Research* **31**, 291-299.

Motta, R. (1996). Impact of wild ungulates on forest regeneration and tree composition of mountain forests in the Western Italian Alps. *Forest Ecology and Management* **88**, 93-98.

Murray, B., Webster, C., Jenkins, M., Saunders, M., and Haulton, G. S. (2016). Ungulate impacts on herbaceous-layer plant communities in even-aged and uneven-aged managed forests. *Ecosphere* **7**, e01378.

Murray, M. D. and Snowdon, W. A. (1976). The role of wild animals in the spread of exotic diseases in Australia. *Australian Veterinary Journal* **52**, 547-554.

Myers, J. H., Simberloff, D., Kuris, A. M., and Carey, J. R. (2000). Eradication revisited: dealing with exotic species. *Trends in Ecology & Evolution* **15**, 316-320.

Newson, S. E., Johnston, A., Renwick, A. R., Baillie, S. R., and Fuller, R. J. (2012). Modelling large-scale relationships between changes in woodland deer and bird populations. *Journal of Applied Ecology* **49**, 278-286.

Parks Victoria (2019). ‘Deer control program - Parks Victoria trialling aerial deer shooting program.’ (Parks Victoria, Melbourne, Victoria.) Available at: https://parkweb.vic.gov.au/explore/parks/mount-buffalo-national-park/plans-and-projects/deer-control-program [Accessed 30 July 2019].

Parliament of Victoria (2017). Inquiry into the control of invasive animals on Crown land. Victorian Government, Melbourne.

Peel, B., Bilney, R. J., and Bilney, R. J. (2005). Observations of the ecological impacts of sambar (*Cervus unicolor*) in East Gippsland, Victoria, with reference to destruction of rainforest communities. *The Victorian Naturalist* **122**, 189-200.

Pellerin, S., Huot, J., and Ct, S. (2006). Long-term effects of deer browsing and trampling on the vegetation of peatlands. *Biological Conservation* **128**, 316-326.

Petty, A. M., Werner, P. A., Lehmann, C. E. R., Riley, J. E., Banfai, D. S., and Elliott, L. P. (2007). Savanna responses to feral buffalo in Kakadu National Park, Australia. *Ecological Monographs* **77**, 441-463.

Phillipson, S., Spencer, K., and Hill, A. (2015). Monitoring changes in the levels of deer abundance and impacts in the Alpine National Park. Bairnsdale, Victoria.

Pople, T., Brennan, M., Amos, M., Kearns, B., McBride, K., and Blokland, A. (2017) Management of an expanding chital deer population in North Queensland. In ‘Proceedings of the 17th Australasian Vertebrate Pest Conference.’ (Ed. Buckmaster, T.) pp. 88-89. (Invasive Species Cooperative Research Centre: Canberra.)

Putman, R., Langbein, J., Green, P., and Watson, P. (2011). Identifying threshold densities for wild deer in the UK above which negative impacts may occur. *Mammal Review* **41**, 175-196.

Quevedo, P., von Hardenberg, A., Pastore, H., Álvarez, J., and Corti, P. (2017). Predicting the potential distribution of the endangered huemul deer *Hippocamelus bisulcus* in North Patagonia. *Oryx* **51**, 315-323.

Raiho, A., Hooten, M., Bates, S., Hobbs, N. T., and Umapathy, G. (2015). Forecasting the effects of fertility control on overabundant ungulates: White-tailed deer in the National capital region. *PloS one* **10**, e0143122.

Rendall, A., Sutherland, D., Cooke, R., White, J., and Boldgiv, B. (2014). Camera trapping: A contemporary approach to monitoring invasive rodents in high conservation priority ecosystems. *PloS one* **9**, e86592.

Rollins, L. A., Woolnough, A. P., and Sherwin, W. B. (2006). Population genetic tools for pest management: a review. *Wildlife Research* **33**, 251-261.

Rollins, L. A., Woolnough, A. P., Sinclair, R., Mooney, N. J., and Sherwin, W. B. (2011). Mitochondrial DNA offers unique insights into invasion history of the common starling. *Molecular Ecology* **20**, 2307-2317.

Rooney, T. P. (2001). Deer impacts on forest ecosystems: a North American perspective. *Forestry* **74**, 201-208.

Ryan, U. and Power, M. (2012). *Cryptosporidium* species in Australian wildlife and domestic animals. *Parasitology* **139**, 1673-1688.

Slee, K. J. and Presidente, P. J. (1981). Biological and pathological features of sambar in Victoria. Part II Parasitological and Pathological findings. *Australian Deer* **6**.

Snow, N. P., Zhang, Z., Finley, A. O., Rudolph, B. A., Porter, W. F., Williams, D. M., and Winterstein, S. R. (2018). Regional-based mitigation to reduce wildlife–vehicle collisions. *The Journal of Wildlife Management* **82**, 756-765.

Spencer, P. B. S., Hampton, J., Lapidge, S. J., Mitchell, J., Lee, J., and Pluske, J. R. (2006). An assessment of the genetic diversity and structure within and among populations of wild pigs (*Sus scrofa*) from Australia and Papua New Guinea. *Journal of Genetics* **85**, 63-66.

Spitzer, R., Churski, M., Felton, A., Heurich, M., Kuijper, D. P. J., Landman, M., Rodriguez, E., Singh, N. J., Taberlet, P., van Beeck Calkoen, S. T. S., Widemo, F., and Cromsigt, J. P. G. M. (2019). Doubting dung: eDNA reveals high rates of misidentification in diverse European ungulate communities. *European Journal of Wildlife Research* **65**, 28.

Stewart, A. J. A. (2001). The impact of deer on lowland woodland invertebrates: a review of the evidence and priorities for future research. *Forestry* **74**, 259-270.

Storm, D., Samuel, M., Van Deelen, T., Malcolm, K., Rolley, R., Frost, N., Bates, D., and Richards, B. (2011). Comparison of visual-based helicopter and fixed-wing forward-looking infrared surveys for counting white-tailed deer *Odocoileus virginianus*. *Wildlife Biology* **17**, 431-440.

Tanentzap, A. J., Burrows, L. E., Lee, W. G., Nugent, G., Maxwell, J. M., and Coomes, D. A. (2009). Landscape-level vegetation recovery from herbivory: Progress after four decades of invasive red deer control. *Journal of Applied Ecology* **46**, 1064-1072.

Timmins, R., Kawanishi, K., Giman, B., Lynam, A., Chan, B., Steinmetz, R., Sagar Baral, H., and Samba Kumar, N. (2015). *Rusa unicolor* (errata version published in 2015). The IUCN Red List of Threatened Species 2015.)

Tomkins, N. W., Jonsson, N. N., Young, M. P., Gordon, A. N., and McColl, K. A. (1997). An outbreak of malignant catarrhal fever in young rusa deer (*Cervus timorensis*). *Australian Veterinary Journal* **75**, 722-723.

Turner Jr, J. W., Turner, J., Kirkpatrick, J., and Liu, I. K. M. (1996). Effectiveness, reversibility, and serum antibody titers associated with immunocontraception in captive white-tailed deer. *Journal of Wildlife Management* **60**, 45-51.

Veale, A. J., Gleeson, D. M., and Clout, M. N. (2014). Measuring connectivity of invasive stoat populations to inform conservation management. *Wildlife Research* **41**, 395-406.

Vercauteren, K., Lavelle, M., and Hygnstrom, S. (2006). Fences and deer-damage management: A review of designs and efficacy. *Wildlife Society Bulletin* **34**, 191-200.

Visintin, C., Golding, N., van der Ree, R., and McCarthy, M. A. (2018). Managing the timing and speed of vehicles reduces wildlife-transport collision risk. *Transportation Research Part D: Transport and Environment* **59**, 86-95.

Visintin, C., van der Ree, R., and McCarthy, M. (2016). A simple framework for a complex problem? Predicting wildlife-vehicle collisions. *Ecology & Evolution* **6**, 6409-6421.

Wahren, C. H. A., Williams, R. J., and Papst, W. A. (2001). Vegetation change and ecological processes in alpine and subalpine sphagnum bogs of the Bogong High Plains, Victoria, Australia. *Arctic, Antarctic, and Alpine Research* **33**, 357-368.

Waller, D. M. (1997). The white-tailed deer: A keystone herbivore. *Wildlife Society Bulletin* **25**, 217-226.

Ward, A. I. (2005). Expanding ranges of wild and feral deer in Great Britain. *Mammal Review* **35**, 165-173.

Weber, M. and Gonzalez, S. (2003). Latin American deer diversity and conservation: A review of status and distribution. *Écoscience* **10**, 443-454.

Woinarski, J. C. Z., Burbidge, A., and Harrison, P. L. (2015). Ongoing unraveling of a continental fauna: Decline and extinction of Australian mammals since European settlement. *Proceedings of the National Academy of Sciences of the United States of America* **112**, 4531-4540.

Yen, S. C., Yen, S.-C., Wang, Y., Yu, P.-H., Kuan, Y.-P., Liao, Y.-C., Chen, K.-H., and Weng, G.-J. (2019). Seasonal space use and habitat selection of sambar in Taiwan. *Journal of Wildlife Management* **83**, 22-31.

Zhang, Y., Koehler, A. V., Wang, T., Haydon, S. R., and Gasser, R. B. (2018). First detection and genetic characterisation of *Enterocytozoon bieneusi* in wild deer in Melbourne's water catchments in Australia. *Parasites & vectors* **11**, 2.

Foreword to chapter 2

Many studies have used genetics to inform the management of invasive species. Genetics can be used estimate abundance, determine population structure, establish if populations are expanding or declining and ascertain the origin of pest species populations. This information is critical to make informed management decisions regarding the spatial and temporal application of control efforts and achieve favourable outcomes in pest species eradication and control.

In order to undertake genetic studies with meaningful results, researchers require genetic tools that are sensitive, accurate and reproducible. This is particularly important for studies that utilise DNA collected non-invasively which can be of low quality and quantity.

The main objective of chapter two was to establish a suite of molecular tools to collect reliable genetic data from the scats of wild deer, with a particular focus on sambar deer. Specifically, this chapter provides detailed guidance about how to locate and extract DNA from deer scats and describes optimised molecular tools which can identify deer species, sex and develop an individual genotype, from degraded scat DNA. In doing so, chapter two establishes a foundation for future genetic studies of sambar deer and other introduced deer.

Termed here ‘the DNA toolbox’, this suite of methods was applied in chapter three of this thesis to collect genetic data from wild sambar deer with the aim of investigating population structure and connectivity. The DNA toolbox has clear potential for further application in future genetic studies of wild deer in south east Australia.

Chapter 2 - A DNA toolbox for non-invasive genetic studies of sambar deer (*Rusa unicolor*)

Fresh sambar deer scat, Hill End, Victoria,
October 2016

Photo credit: Christopher Davies

Davies, C., Wright, W., Wedrowicz, F. and Hogan, F. (2019) A DNA toolbox for non-invasive genetic studies of sambar deer (*Rusa unicolor*). *Australian Mammalogy*, doi: https://doi.org/10.1071/AM18032

Chapter 2 - A DNA toolbox for non-invasive genetic studies of sambar deer (*Rusa unicolor*)

2.1 Abstract

Invasive sambar deer (*Rusa unicolor*) are having significant detrimental impacts on natural environments in south-eastern Australia. Little, however, is known about their ecology, limiting evidence-based management strategies directed at reducing deer impacts. Genetic data, generated from DNA isolated from deer scats, can be used to fill ecological knowledge gaps. This study outlines a non-invasive genetic sampling strategy by which good-quality DNA from a single deer scat can be used to determine (1) species of origin, (2) sex and (3) a unique DNA profile. DNA from deer tissue and sambar deer scat samples were used to develop and optimise molecular methods to collect reliable genetic information. A DNA toolbox is presented that describes how to find, collect and store scat samples, isolate DNA and use molecular markers to generate informative genetic data. Generating genetic data using this approach will support studies aimed at acquiring ecological knowledge about sambar deer. Such knowledge will be critical for developing evidence-based recommendations to improve on-ground management decisions for sambar deer.

2.2 Introduction

Invasive species are a significant threat to biodiversity and a deep understanding of their ecology is required to develop effective management strategies and to reduce associated impacts. The detrimental impact of invasive species has been particularly evident on the Australian continent where they have been implicated in the majority of recent extinctions (Wayne *et al.* 2017). While invasive mammalian predators such as the red fox (*Vulpes vulpes*) and feral cat (*Felis catus*) have played an important and direct role in these extinctions (Doherty *et al.* 2017), habitat degradation caused by invasive herbivores has also resulted in significant biodiversity loss (Legge *et al.* 2011). Invasive herbivores can outcompete native animals for food and water (Davis *et al.* 2008), degrade vegetation (Edwards *et al.* 2010) and cause erosion (Bayne *et al.* 2004). Having established themselves throughout large areas of Australia, invasive deer species are now emerging as a significant threat to native ecosystems (Davis *et al.* 2016).

Deer were first introduced to Australia during the 19th and 20th centuries to provide game for hunting (Bentley 1957; Moriarty 2004). Of the 18 species of deer introduced to Australia, six have successfully established wild populations, including sambar (*Rusa unicolor*), fallow (*Dama dama*), red (*Cervus elaphus*), chital (*Axis axis*), rusa (*Rusa timorensis*) and hog deer (*Axis porcinus*) (Moriarty 2004; Leslie 2011). Wild populations of four deer species (sambar, fallow, red and hog) currently exist in Victoria in south eastern Australia (Davis *et al.* 2016). Increasing numbers of applications to the State’s Department of Environment, Land, Water and Planning (DELWP) to control deer on private land (Lindeman and Forsyth 2008) and rising harvest rates indicate that the distribution and abundance of these species are increasing across Victoria (GMA 2015). There is increasing concern about the potential for wild deer populations in south eastern Australia to cause considerable ecological damage and to act as reservoirs for agriculturally important diseases and parasites (Cripps *et al.* 2018).

Deer have been implicated in a broad range of detrimental impacts on ecosystems around the world (Côté *et al.* 2004). In Australia, deer damage native ecosystems through browsing, thrashing and rubbing of vegetation (Bilney 2013). Wild deer also compete with native fauna for forage (Davis *et al.* 2008), spread weeds (Davis *et al.* 2010) and alter habitats. In addition, deer carrion can provide a source of food for other invasive species, such as red foxes (Forsyth *et al.* 2014). In Victoria, reduction in the biodiversity of native vegetation by sambar deer is listed as a potentially threatening process (DSE 2010). Despite their significant and increasing impact on the environment, resulting from their rapid population growth, sambar remain one of the least studied invasive mammal species in Australia.

There are important knowledge gaps associated with the ecology of all wild deer species in Victoria, including sambar deer (Davis *et al.* 2016). Analysis of harvest and capture rates per unit of effort have been used as indices of abundance for deer in Victoria (Forsyth *et al.* 2018), however, more accurate estimates of abundance using methods such as mark recapture have not been carried out for any of Victoria's wild deer species. Furthermore, there are very few studies describing home range, habitat use and dispersal patterns for invasive wild deer in Victoria (Davis *et al.* 2016). The effectiveness of deer control methods such as culling programs are also poorly understood (State of Victoria 2018). While addressing these knowledge gaps is a vital first step towards the effective management of deer, their cryptic nature makes this a formidable task.

Molecular ecology, where genetic data are used to address ecological questions, is an approach often used to obtain information about invasive species. For example, genetic data have been used widely in New Zealand to inform the management of invasive species including possums (Adams *et al.* 2014), stoats (Veale *et al.* 2014) and rats (Abdelkrim *et al.* 2010). Genetic data can also be used to identify genetic bottlenecks after control efforts, in order to determine whether control programs have effectively reduced population size (Cowled *et al.* 2006). Furthermore, genetic studies that identify the sex of individuals can determine whether males or females display increased dispersal (Hansen *et al.* 2007), allowing control operations to target individuals of the sex with greater dispersal (Rollins *et al.* 2006).

Where invasive collection of DNA (e.g. tissue sampling) from wild animals is challenging, DNA can be sourced non-invasively through the collection of biological material discarded by an animal (e.g. scats). Scats are easily collected from the environment, which enables large areas to be searched and sampled without target animals having to be caught (Waits and Paetkau 2005). Scat collection can therefore, increase sample size, remove sampling bias and reduce sampling costs which are often associated with invasive sampling (Kohn and Wayne 1997). A number of studies have demonstrated how genetic data generated from scat DNA can be used to improve the understanding of deer ecology (Valière *et al.* 2007; Brinkman and Hundertmark 2009).

Despite the many benefits of non-invasive sampling, the use of DNA sourced from discarded biological samples such as scats is associated with some difficulties. These are related to the rapid degradation of DNA following cell death (Alaeddini *et al.* 2010), a process which may be accelerated by environmental factors including rain, or by sample storage conditions (Wedrowicz *et al.* 2013; Agetsuma Yanagihara *et al.* 2017). Studies that utilise non-invasive genetic sampling are, therefore, often constrained by low DNA quantity (DNA amount) and quality (DNA integrity) which can cause errors in the genetic data obtained, leading to incorrect findings and conclusions (Bonin *et al.* 2004). It is therefore necessary to tailor sampling strategies, DNA isolation methods and molecular markers for the target species and the specific environment, to ensure the accuracy of data, knowledge and recommendations (Valière *et al.* 2007).

Here we present a method which can be used to source good quality DNA from a single sambar deer scat collected in a temperate environment. We describe how to find and collect scats suitable for genetic analysis, discuss methods for isolating target DNA from scats and present techniques for assessing DNA quantity and quality following DNA isolation. We evaluate a range of molecular markers for their application and performance in studies of deer, using DNA sourced non-invasively from scats. Finally, we identify a suite of molecular markers which can be used reliably with DNA isolated from scats to 1) confirm that the scat sample originated from sambar deer, 2) determine the sex of the individual and 3) provide a unique DNA profile that can identify individuals. Examples of how genetic data generated using this approach can be used to address ecological questions and inform management strategies of sambar deer in south eastern Australia are also provided.

2.3 Materials and Methods

2.3.1 Sample collection

Tissue samples (ear or liver) were collected from sambar (n=41), red (n=1), fallow (n=6) and hog (n=6) deer shot by park rangers and licenced recreational hunters during control operations in various parks and reserves in Victoria. These samples formed reference samples for our study and were stored in vials containing 20% dimethyl sulfoxide (DMSO) in a saturated salt solution and refrigerated at 4°C.

Deer trails in forested areas of west Gippsland were searched for sambar deer scats that appeared shiny with a visible mucus coating, indicating freshness. When scats were located, a single pellet was picked up with a single use toothpick. A sterile, rayon-tipped swab dipped in Longmire buffer was used to wipe the entire surface of the scat to sample intestinal epithelial cells. The head of the swab was cut off using scissors and stored in a 1.5 mL vial containing

0.5 mL of Longmire buffer. Following swabbing, scats were discarded in the field. All tubes containing swab heads and buffer were stored at 4°C until DNA isolation was undertaken.

2.3.2 DNA isolation

DNA was isolated from tissue samples using the Qiagen® DNeasy Blood and Tissue Kit according to the manufacturer's protocol. DNA was extracted from scat samples using the Qiagen® QIAamp DNA Mini kit following the manufacturer's protocol for DNA Purification from Blood or Body Fluids, with minor changes. Buffer AL (500µL) and Proteinase K (20 µL) were added directly to sample tubes (containing swab heads and Longmire buffer) and DNA was isolated from 400 µL of the resultant solution. DNA was eluted in 100 µL and stored at – 20°C. DNA isolated from both tissues and scats were quantified using the Qubit® fluorometer (Invitrogen) following the manufacturer's protocol. In order to reduce the risk of contamination, all DNA extractions from scats were performed using separate equipment (pipettes and tube racks) in a dedicated facility.

2.3.3 Determining deer species

Scats from different deer species can be difficult to differentiate with a high degree of certainty based on appearance alone (Ramón-Laca *et al.* 2014). Therefore, an important first step when sampling scats, is to confirm the species of origin. Here we amplified a stretch of the mitochondrial control region for 23 known deer tissue samples from Victoria (sambar, n=10; fallow, n=6; red, n=1; and hog, n=6) and 16 scat samples thought to have originated from sambar deer. Polymerase Chain Reaction (PCR) assays consisted of 2 µL DNA template, 10 µL of GoTaq® Green Master Mix (Promega), 0.2 µM of primers; CervtPro and CervCRH (Balakrishnan *et al.* 2003) and 1 µg μL^{-1} of bovine serum albumin (BSA) made up to 20 µL with nuclease free water. DNA amplification was performed in a Veriti® thermal cycler with a 10 minute initial denaturation at 95°C, followed by 35 cycles of: 45 seconds at 95°C, 40 seconds at 54°C and 75 seconds at 72°C followed by a final extension of 10 minutes at 72°C. All PCR amplifications included positive and negative controls.

PCR products were visualised under ultra violet (UV) light on a 2% agarose gel stained with SYBR Safe™ DNA Stain (Invitrogen), and were subsequently purified using the Wizard® SV Gel and PCR Clean-Up System (Promega). Sanger sequencing was performed by the Australian Genome Research Facility (AGRF), Melbourne, Australia. Sequence quality and base calls were assessed manually for all samples. DNA sequences were trimmed in Sequence

Scanner 2.0 (Applied Biosystems) and aligned in MEGA7 (Kumar *et al.* 2016) using MUSCLE (Edgar 2004). Sequences derived from scat DNA were confirmed as sambar deer by comparison with deer sequences generated from reference tissue and BLAST analysis.

2.3.4 Determining sex

The amelogenin locus is found on both the X and Y chromosomes and has been used to genetically assign sex to ungulate DNA using a single primer pair, SE47 and SE48 (Ennis and Gallagher 1994). In North American ungulates, SE47 and SE48 produce X-linked and Y-linked amplicons of approximately 300 and 275 base pairs, respectively (Brinkman and Hundertmark 2009).

Tissue samples from two female and two male sambar deer were used to validate the ability of the primer pair SE47/SE48 to accurately infer sex. Serial dilutions (1:10) of DNA template from two validated females (16.6 ng/µL and 34.2 ng/µL) and two validated males (13.1 ng/µL and 23.0 ng/µL) were used to determine the sensitivity of the assay. The performance of the sex markers to amplify degraded DNA was finally tested by SE47/SE48 PCR amplification of scat DNA isolates (*n*=32) with concentrations ranging between 0.14 to 35.0 ng/µL.

PCR amplifications were performed in 10 µL volumes consisting of 5 µL GoTaq® Green Master Mix, 1 µg of BSA, 0.5 µM each of SE47 and SE48 primers and 1 µL of DNA template, adjusted to 10 µL with water. Thermal cycling began with an initial denaturation step of 15 minutes at 94°C, 35 cycles of 94°C for one minute, 60°C for 30 seconds and 72°C for one minute, followed by a final extension cycle at 72°C for 5 minutes. PCR products were separated on 2% agarose gel stained with SYBR Safe™ DNA Stain and visualised using UV light.

2.3.5 Identifying individual sambar deer

DNA isolated from sambar deer tissue samples were used to test the cross species amplification success of 17 microsatellite markers developed for ungulates: BL42, BM203, BM757, BM848, BMC1009, CSSM43, IDVGA55, INRA121, TGLA126, TGLA53, TGLA57, VH110 (Bonnet *et al.* 2002), Ca18, Ca43 (Gaur *et al.* 2003), CelJP38, OarFCB5 and RT7 (Pérez-Espona *et al.* 2008). All microsatellite loci had been previously used for multi-locus genotyping of cervids including red (Valière *et al.* 2007; Pérez-Espona *et al.* 2008) and chital (Gaur *et al.* 2003) deer. Genotyping was carried out on the Applied Biosystems 3730 DNA analyser and GENEMAPPER 3.7 software (Applied Biosystems) by AGRF, Melbourne, Australia.

Genotypic data were used to estimate the average number of alleles per locus (N_A), observed heterozygosity (H_O), expected heterozygosity (H_E), probability of identity (P_{ID}) and probability of identity among siblings (P_{SIBS}) using GENALEX version 6.503 (Peakall and Smouse 2006). GENEPOP version 4.2 (Rousset 2008) was used to test for linkage disequilibrium (LD) and departures from Hardy-Weinberg equilibrium (HWE) with Bonferroni correction.

2.3.6 Genotyping error rates

Amplification success is often lower and genotyping errors higher when amplifying DNA isolated from samples such as scats. Genotyping errors were estimated by genotyping DNA from a subset of scat samples (*n*=21) with eight microsatellite loci (BL42, BM757, BMC1009, IDVGA55, INRA121, TGLA126, TGLA53 and TGLA57). Error rates were calculated in GIMLET v 1.3.3 (Valière 2002b) using allele frequencies determined in GENALEX (Peakall and Smouse 2006). The threshold rule was applied when assigning consensus genotypes wherein a genotype had to appear at least twice to be accepted. Using the calculated error rates, the PCR repetition batch module of GEMINI v 1.3.0 (Valière 2002a) was used to estimate the minimum number of PCR replicates required to obtain reliable genotypes. Simulations were run (*n*=250) using hypothetical populations of 100 individuals, taking 50 samples on one sampling occasion.

2.4 Results

2.4.1 DNA quantification

The mean DNA concentration for tissue isolates (*n*=54) was 24.6 ng/µL and ranged from 0.90 ng/µL to 75.6 ng/µL, whereas the mean DNA concentration for most scat isolates (*n*=31) ranged from 0.14 ng/µL to 4.96 ng/µL with a mean of 1.68 ng/µL. One additional scat sample (collected immediately after a deer was observed defecating) had a high DNA concentration of 35.0 ng/µL.

2.4.2 Determining deer species

The mitochondrial control region was amplified for reference tissue samples for four deer species. Agarose gel visualisation of the mtDNA control region amplicons (~560 bp) showed a size difference between sambar deer amplicons and the amplicons of red, fallow and hog deer (Fig. 2.1). Sequence data confirmed an insertion of 78 bp within sambar deer DNA sequences compared to other deer species sampled for this study. Aligned and trimmed sequences from reference samples were compared to those published on GenBank revealing six haplotypes for Victorian deer, one haplotype each for sambar, red and hog deer and three haplotypes for fallow deer. Haplotypes have the following GenBank accession numbers: MK473445 (sambar), MK473447 (red), MK473446 (hog), MK473448, MK473449 and MK473450 (fallow).

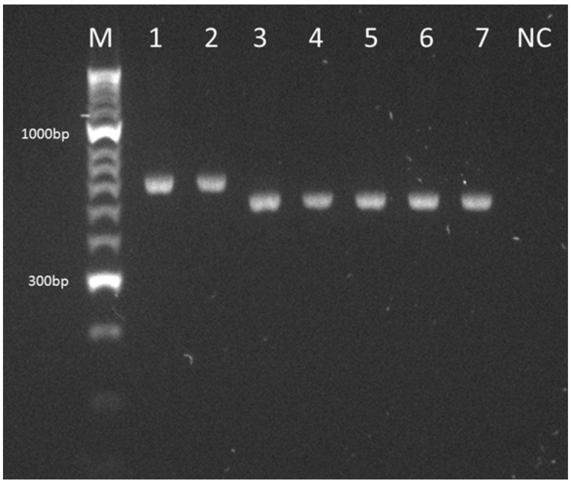

Fig. 2.1: Amplification of the mitochondrial control region using primers CervtPro and CervCRH (Balakrishnan *et al*. 2003) to differentiate sambar deer from other deer species. Lane M, Bioline Hyperladder II, Lanes 1-2: Sambar deer, Lane 3: Red deer, Lanes 4-5: Fallow deer, Lanes 6-7: Hog deer. NC: Negative control.

Comparison between sequences derived from scats (*n*=16) and deer reference sequences, confirmed that all scats originated from sambar deer. BLAST results also showed scat sequences had the highest similarity (based on maximum score and E value) with published sambar deer mitochondrial control region sequences (accession numbers: AF291884.1 and KY946815.1).

2.4.3 Determining deer sex

PCR products produced by the SE47/SE48 primer pair were homozygous for females and heterozygous for males. The observed size difference between the X and Y PCR products was ~50 bp, allowing separation of the two amplicons on a 2% agarose gel. Sex was assigned correctly to samples from individuals of known sex (two females and two males), where samples with a single band at ~290 bp were assigned as female (XX), whilst samples with two bands of ~290 bp and ~240 bp were assigned as male (XY) (Fig. 2.2A). Serial dilutions of sambar deer DNA consistently amplified to a minimum of 0.13 ng/µL (Table 2.1). Clear amplification and separation of X and Y PCR products was achieved for DNA isolated from scats, where 91% (29/32) of samples were successfully assigned sex (four individuals shown, Fig. 2.2B).

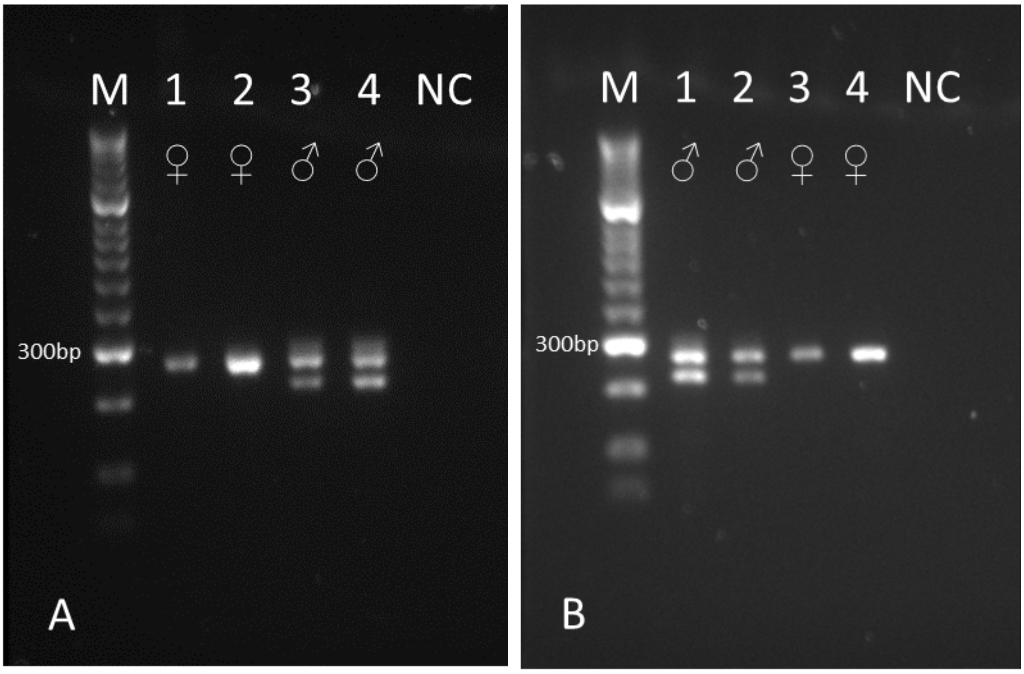

Fig. 2.2: Amplification of the amelogenin locus with SE47 and SE48 primers (Ennis and Gallagher 1994) to determine sambar deer sex. A) Lane M: Bioline Hyperladder II, Lanes 1-2: Female sambar deer DNA, Lanes 3-4: Male sambar deer DNA. B) Lane M: Bioline Hyperladder II, Lanes 1-2: Scat samples identified as male, Lanes 3-4: Sambar scat samples identified as female. NC: Negative control.

Table 2.1: Amplification success of sex markers (SE47 and SE48) for serial dilutions of sambar deer tissue DNA isolates.

+ denotes one band (homozygous) was observed for females and two bands (heterozygous) for males. – denotes that expected amplification was not observed.

Dilution	Sample 1 Female (DNA, ng/µL)	Sample 2 Female (DNA, ng/µL)	Sample 3 Male (DNA, ng/µL)	Sample 4 Male (DNA, ng/µL)
1:1	+ (16.6)	+ (34.2)	+ (13.1)	+ (23.0)
1:10	+ (1.66)	+ (3.42)	+ (1.31)	+ (2.30)
1:100	+ (0.16)	+ (0.34)	+ (0.13)	+ (0.23)
1:1000	- (0.02)	- (0.03)	- (0.01)	- (0.02)

2.4.4 Identifying individual sambar deer

Genotyping of 17 microsatellite markers developed for ungulates demonstrated good cross species amplification success in sambar deer. All markers consistently amplified and were polymorphic except BM203 and CSSM43 (which had poor amplification success) and VH110 and BM848 (which were monomorphic) (Table 2.2). Significant LD was detected between one pair of loci (BMC1009 and OarFCB5).

The final suite of 11 microsatellite loci consistently amplified, showed no evidence of LD and did not deviate from HWE after Bonferroni correction. Allelic diversity among the 11 loci ranged from two to nine alleles per locus, with an average of 4.1 (Table 2.2). The mean observed (H_O) and expected (H_E) heterozygosity were 0.546 and 0.551, respectively (Table 2.2). The power of this marker suite to identify individual sambar deer was high, with a P_{ID} of 2.7×10^{-7} (~1 in 3.6 million) for unrelated individuals and 1.0×10^{-3} (~1 in 959) for full siblings, and are therefore suitable for identifying individuals and conducting population genetic studies of sambar deer in south eastern Australia.

Table 2.2: Characterisation of 17 Cervid microsatellite loci for sambar deer.
n – Number of individuals successfully genotyped, ns – Amplification success, N_A – Number of alleles, H_O – Observed heterozygosity, H_E – Expected heterozygosity. The 11 loci in bold are recommended for multi-locus genotyping.

Locus	*n*	ns (%)	N_A	H_O	H_E
BL42	**32**	**94**	**9**	**0.531**	**0.762**
BM757	**34**	**100**	**4**	**0.647**	**0.587**
Ca18	**33**	**97**	**4**	**0.636**	**0.562**
Ca43	**32**	**94**	**3**	**0.438**	**0.490**
CelJP38	**33**	**97**	**4**	**0.727**	**0.668**
IDVGA55	**34**	**100**	**5**	**0.441**	**0.483**
INRA121	**34**	**100**	**4**	**0.647**	**0.653**
OarFCB5	**34**	**100**	**3**	**0.588**	**0.554**
RT7	**34**	**100**	**4**	**0.735**	**0.652**
TGLA53	**34**	**100**	**3**	**0.471**	**0.515**
TGLA57	**34**	**100**	**2**	**0.147**	**0.136**
TGLA126	40	98	2	0.550	0.425
BMC1009	41	100	2	0.512	0.476
VH110	10	100	1	-	-
BM848	10	90	1	-	-
BM203	10	20	2	-	-
CSSM43	10	70	2	-	-

2.4.5 Assessing genotyping performance for DNA isolated from scats

Genotyping error rates for DNA isolated from deer scats were low in this study (Table 2.3). From 1,344 amplifications the average proportion of positive PCR tests was 95% and varied between 88% and 99% among loci and 66% and 100% among samples. Allelic dropout (ADO) was estimated at 0.9% across loci and 1.6% across samples. No evidence of false alleles was detected. GEMINI simulations indicated that with two PCR replications per locus, the proportion of correct multi locus genotypes would be 93.48%, with three PCR replicates 99.98% and after four replicates 100%.

Table 2.3: Error rates for eight microsatellites based on eight replicates. PCR – Successful amplification rate, ADO – allelic dropout rate, FA – false allele rate.

Locus	PCR (%)	ADO (%)	FA (%)
BL42	88	0.0	0.0
BM757	96	2.5	0.0
BMC1009	92	0.0	0.0
IDVGA55	92	0.0	0.0
INRA121	95	4.8	0.0
TGLA126	97	0.0	0.0
TGLA53	99	0.0	0.0
TGLA57	98	0.0	0.0
Mean	95	1.0	0.0

2.5 Discussion

There is an urgent need to gather information regarding populations of wild deer in south east Australia. Here, we have presented a non-invasive molecular approach that can be used to collect genetic information regarding contemporary sambar deer populations. The 'DNA toolbox', outlined in Fig. 2.3, describes how DNA isolated from a single scat can be reliably used for population genetic studies of sambar deer. Data generated using this approach will fill knowledge gaps regarding sambar deer ecology and provide critical information for the management of this invasive species.

Deer continuously deposit scats in the environment (Košnář and Rajnyšová 2012), therefore, if deer are present in an area their scats should be readily detected. Using a non-invasive sampling approach, genetic material from scats may be collected from protected areas such as national parks where hunting is prohibited. Scats can also be collected by citizen scientists, natural resource managers and other stakeholders, with fewer difficulties (compared with invasive sampling such as shooting or live capture) relating to requirements for the ethical destruction and handling of animals. However, sourcing DNA using a non-invasive approach is not a panacea for genetic studies and a number of significant issues associated with non-invasive sampling must be considered (Taberlet *et al.* 1999). DNA rapidly degrades after cell death, hence the quality and quantity of DNA recovered from a scat is typically lower than that obtained from tissue and blood samples (Taberlet *et al.* 1999; Bonin *et al.* 2004). Decreased DNA quantity and DNA degradation is also exacerbated by weathering, especially rainfall (Agetsuma Yanagihara *et al.* 2017). Consequently, the collection of scats for genetic studies should be conducted in dry conditions and during fine weather. Where possible, scats which appear fresh and undamaged should be sampled over those which appear dry and weathered.

Steps 1 through 3 of the DNA toolbox (Fig. 2.3) provide guidance relating to the collection of scats in order to improve the probability of obtaining good quality DNA from sambar deer scats. Step 1 describes environmental signs (e.g. rubbing of trees, footprints) that indicate recent deer activity, and identify areas in which fresh scats are likely to be found. To sample DNA from sambar deer scats, we recommend a swabbing method which collects epithelial cells shed from the gastrointestinal tract during defecation. These cells coat the outside surface of the scat (Ramón-Laca *et al.* 2015). The swabbing method reduces the amount of non-target DNA collected from other biological organisms that may be present in the scat (e.g. microorganisms, plants, fungi, invertebrates), and minimises the concentration of faecal contaminants (PCR inhibitors) which can reduce PCR success and increase genotyping errors (Waits and Paetkau 2005). The swabbing method yields higher quantities of target DNA (e.g. sambar deer), compared to other methods where slices or slurries of scats are used for DNA extraction (Ramón-Laca *et al.* 2015). Whole sample DNA extractions such as these, however, would enable additional data to be collected regarding an individual's diet and health (e.g. microbiome diversity, pathogen infection).

In step 2 of the DNA Toolkit (Fig. 2.3), we recommend the isolation of DNA using a commercially available DNA extraction kit. Such kits are widely used to isolate DNA from the scats of wild animals due to their simplicity and cost effectiveness (Brinkman *et al.* 2010; Wedrowicz *et al.* 2013; Lounsberry *et al.* 2015). During this study the Qiagen® QIAamp DNA Mini kit was effective at isolating DNA from scat swabs using an adapted blood and body fluid isolation protocol. In this study, the lowest measured DNA concentration was 0.14 ng/µL. It is important to note that, this figure refers to the total DNA present in a sample which may include considerable amounts of foreign DNA and low amounts of target (sambar) DNA. At DNA concentrations ranging from 0.01–0.03 ng/µL, amplification of the amelogenin locus failed, while concentrations above 0.13 ng/µL were successful (Table 2.1). We therefore suggest that scat samples with DNA concentrations less than 0.05 ng/µL are discarded because levels of target DNA are likely to be low. However, in some cases (e.g. when sample size is small) it may be preferable to carry such samples through to step 3 to confirm that sufficient sambar deer DNA has been isolated.

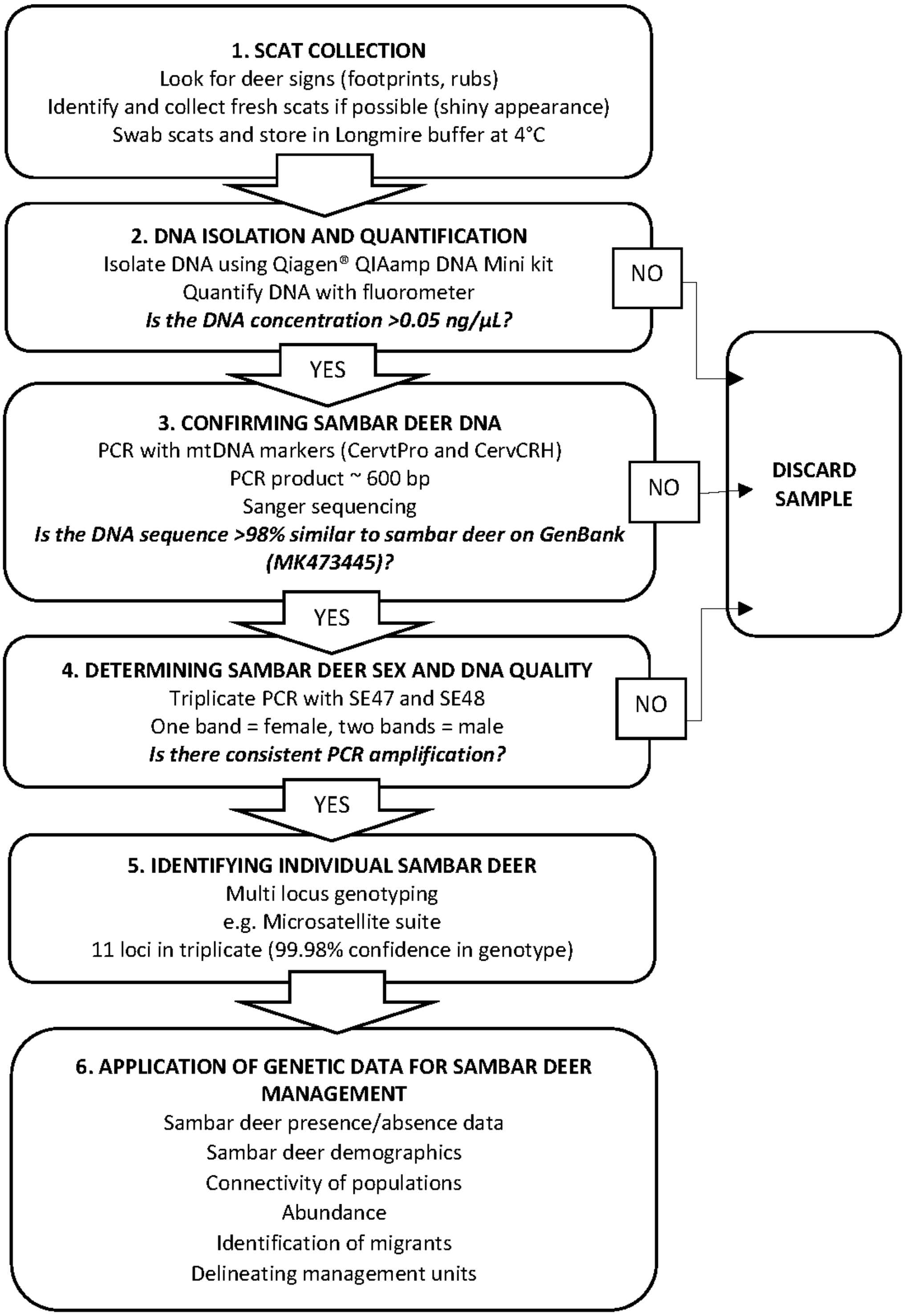

Fig. 2.3: ‘DNA toolbox’ for isolating good quality DNA from a single sambar deer scat to generate genetic data for applied sambar deer management.

Distinguishing deer scats from other Australian mammals through visual cues is relativity easy for the trained eyed. However, assigning deer scats to specific sympatric deer species based on scat morphology alone is quite difficult, mainly due to variation in scat size within different deer age classes (Bowkett *et al.* 2013) and changes in the appearance of scats caused by differences in deer diet (Lunt and Mhlanga 2011). In step 3 of the DNA Toolkit (Fig. 2.3) DNA is amplified using a pair of deer specific molecular markers (CervtPro and CervCRH) which have been reported to specifically amplify the mitochondrial control region for deer and not for other non-deer species (Balakrishnan *et al.* 2003). Positive amplification of this primer pair can therefore be used to confirm that the scat sample is from deer. In addition, due to the presence of a 78 base pair insertion in the mitochondrial control region of sambar deer (Fig. 2.1), it is possible to distinguish sambar deer DNA from the DNA of other deer species (fallow, hog and red) using gel electrophoresis. Complete reliance on this approach to confirm the presence of sambar deer DNA is not ideal, however, as intraspecific variation could exist within the D-loop of the mitochondrial control region, resulting in incorrect species identification (Pun *et al.* 2009). Species identification using scat DNA should therefore be conducted by comparing sequences generated from scats with those from known reference samples (preferably with individuals from a similar region) and/or reference sequences which can be sourced from GenBank. Six haplotype sequences are reported here that can be used to genetically discriminate sambar, fallow, red and hog deer. Mitochondrial sequence data can also be used for phylogenetic studies which can be useful for understanding invasion processes (Rollins *et al.* 2011) and inferring contemporary population structure of invasive species (Mora *et al.* 2018). If preferred, other genetic methods such as the approach taken by Furlan and Gleeson (2017) using quantitative PCR (qPCR), could be employed to identify species, negating the need for DNA sequencing.

Determining the sex of a sampled individual can be useful for understanding ecological and biological processes such as sex ratios, sex-biased dispersal and mating systems (Brinkman and Hundertmark 2009). In step 4 of the DNA Toolkit (Fig. 2.3), we recommend use of the primer pair SE47 and SE48 to assign sex to the sampled individual. When conducting genetic studies with degraded DNA it is important to choose or design molecular markers which amplify short pieces of DNA (generally <300 bp). This is because as DNA degrades, the DNA molecule itself is cleaved into shorter fragments. DNA regions targeted by particular markers may therefore be incomplete. Targeting shorter regions of DNA can thus increase amplification success (Waits and Paetkau 2005). The quality of DNA derived from sambar deer scats may be assessed by amplification with the SE47/SE48 pair in a similar fashion to that conducted by Hogan *et al.* (2008) for owl feathers and Wedrowicz *et al.* (2017) for koala scats.

Multi-locus genotyping with a suite of molecular markers will provide a DNA profile from which an individual can be identified. Here we tested 17 microsatellite markers, developed for Artiodactyls (even toed ungulates, including sheep and cattle), which have been applied in population genetics studies of cervid species including red deer (Valière *et al.* 2007; Pérez-Espona *et al.* 2008) and chital deer (Gaur *et al.* 2003). Our results show that cross species amplification success is good for sambar deer with 15 of the 17 markers tested being polymorphic (Table 2.1). The final suite of 11 microsatellite markers identified had good resolution for the identification of individual sambar deer and it was estimated that the chance of unrelated deer having the same DNA profile was ~1 in 3.6 million. Genotyping error rates were found to be low (Table 2.3), showing that high quality DNA can be collected from scat samples. Our results show that using DNA sourced from scats, these markers have sufficient power for population genetic studies, however, there may be limitations for other applications, including the investigation of evolutionary lineages and/or fine scale dispersion processes.

This study demonstrates that DNA isolated from scats can be used to supplement invasive sampling methods. Such an approach is likely to be useful when collecting DNA from areas where hunting is not permitted and has the additional advantage of allowing large areas to be surveyed with minimal cost. Although scats are much easier to obtain than tissue samples, the methods for sampling, extracting and testing scat DNA are much more complex. Collection of poor samples (incorrect species, highly degraded) will result in increased costs in processing and analysing. It is therefore important to have measures in place to allow samples to be screened, ensuring that 1) DNA from the target is obtained and 2) DNA from the target is of sufficient quality to produce reliable genetic data.

2.5.1 Management implications

There is an urgent need to gather information regarding populations of wild deer in Victoria to inform their management and mitigate their ecological impacts. Genetic data can be used to provide evidence-based recommendations to improve on-ground management. We have demonstrated a toolbox of methods useful to confirm species, determine sex and genetically profile animals using DNA sourced non-invasively from deer scats. Application of the approach described here will facilitate the collection of large amounts of genetic data easily and quickly over large geographical areas. Once genetic data have been collected, contemporary population genetic analysis can detail aspects of deer ecology that have not yet been studied in Victoria, including population structure, the delineation of management units, abundance estimates, migration rates and dispersal characteristics. Furthermore, genetic identification of sambar deer scats will prevent misidentification of deer scats, improving species distribution models and the results of faecal pellet surveys. Conducting future studies on sambar deer using these approaches will allow evidence-based management of wild deer in Victoria and help assess the effectiveness of current control operations.

2.7 References

Abdelkrim, J., Byrom, A.E., and Gemmell, N.J. (2010). Fine-scale genetic structure of mainland invasive *Rattus rattus* populations: Implications for restoration of forested conservation areas in New Zealand. *Conservation Genetics* **11**, 1953-1964.

Adams, A.L., van Heezik, Y., Dickinson, K.J.M., and Robertson, B.C. (2014). Identifying eradication units in an invasive mammalian pest species. *Biological Invasions* **16**, 1481-1496.

Agetsuma Yanagihara, Y., Inoue, E., and Agetsuma, N. (2017). Effects of time and environmental conditions on the quality of DNA extracted from fecal samples for genotyping of wild deer in a warm temperate broad-leaved forest. *Mammal Research* **62**, 201-207.

Alaeddini, R., Walsh, S.J., and Abbas, A. (2010). Forensic implications of genetic analyses from degraded DNA—A review. *Forensic Science International: Genetics* **4**, 148-157.

Balakrishnan, C., Monfort, S., Gaur, A., Singh, L., and Sorenson, M. (2003). Phylogeography and conservation genetics of Eld's deer (*Cervus eldi*). *Molecular Ecology* **12**, 1-10.

Bayne, P., Harden, R., and Davies, I. (2004). Feral goats (*Capra hircus*) in the Macleay River gorge system, north-eastern New South Wales, Australia, impacts on soil erosion. *Wildlife Research* **31**, 519-525.

Bentley, A. (1957). A brief account of the deer in Australia. *The Journal of Wildlife Management* **21**, 221-225.

Bilney, R.J. (2013). Antler rubbing of yellow-wood by sambar in east Gippsland, Victoria. *The Victorian Naturalist* **130**, 68.

Bonin, A., Bellemain, E., Bronken Eidesen, P., Pompanon, F., Brochmann, C., and Taberlet, P. (2004). How to track and assess genotyping errors in population genetics studies. *Molecular Ecology* **13**, 3261-3273.

Bonnet, A., Thévenon, S., Maudet, F., and Maillard, J.C. (2002). Efficiency of semi-automated fluorescent multiplex PCRs with 11 microsatellite markers for genetic studies of deer populations. *Animal Genetics* **33**, 343-350.

Bowkett, A., Jones, T., Laizzer, R., Plowman, A., and Stevens, J. (2013). Can molecular data validate morphometric identification of faecal pellets in Tanzanian forest antelope species? *Conservation Genetics Resources* **5**, 1095-1100.

Brinkman, T.J., and Hundertmark, K.J. (2009). Sex identification of northern ungulates using low quality and quantity DNA. *Conservation Genetics* **10**, 1189-1193.

Brinkman, T.J., Person, D.K., Schwartz, M.K., Pilgrim, K.L., Colson, K.E., and Hundertmark, K.J. (2010). Individual identification of sitka black-tailed deer (*Odocoileus hemionus sitkensis*) using DNA from fecal pellets. *Conservation Genetics Resources* **2**, 115-118.

Côté, S.D., Rooney, T.P., Tremblay, J.-P., Dussault, C., and Waller, D.M. (2004). Ecological impacts of deer overabundance. *Annual Review of Ecology, Evolution and Systematics* **35**, 113-147.

Cowled, B.D., Lapidge, S.J., Hampton, J.O., and Spencer, P.B.S. (2006). Measuring the demographic and genetic effects of pest control in a highly persecuted feral pig population. *The Journal of Wildlife Management* **70**, 1690-1697.

Cripps, J.K., Pacioni, C., Scroggie, M.P., Woolnough, A.P., and Ramsey, D.S.L. (2018). Introduced deer and their potential role in disease transmission to livestock in Australia. *Mammal Review* **49**, 60-77.

Davis, N.E, Forsyth, D.M, and Coulson, G. (2010). Facilitative interactions between an exotic mammal and native and exotic plants: hog deer (*Axis porcinus*) as seed dispersers in south-eastern Australia. *Biological Invasions* **12**, 1079-1092.

Davis, N.E., Bennett, A., Forsyth, D.M., Bowman, D.M.J.S., Lefroy, E.C., Wood, S.W., Woolnough, A.P., West, P., Hampton, J.O., and Johnson, C.N. (2016). A systematic review of the impacts and management of introduced deer (family Cervidae) in Australia. *Wildlife Research* **43**, 515-532.

Davis, N.E., Coulson, G., and Forsyth, D.M. (2008). Diets of native and introduced mammalian herbivores in shrub-encroached grassy woodland, south-eastern Australia. *Wildlife Research* **35**, 684-694.

Doherty, T., Dickman, C., Johnson, C., Legge, S., Ritchie, E., and Woinarski, J.C.Z. (2017). Impacts and management of feral cats (*Felis catus*) in Australia. *Mammal Review* **47**, 83-97.

DSE (2010). Flora and Fauna Guarantee Action Statement: reduction in biodiversity of native vegetation by sambar deer (*Cervus unicolor*). Department of Sustainability and Environment, Melbourne, Australia

Edgar, R.C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**, 1792-1797.

Edwards, G.P., Zeng, B., Saalfeld, W.K., and Vaarzon-Morel, P. (2010). Evaluation of the impacts of feral camels. *Rangeland Journal* **32**, 43-54.

Ennis, S., and Gallagher, T.F. (1994). A PCR-based sex-determination assay in cattle based on the bovine amelogenin locus. *Animal Genetics* **25**, 425-427.

Forsyth, D.M., Caley, P., Davis, N.E., Latham, A.D.M., Woolnough, A.P., Woodford, L.P., Stamation, K.A., Moloney, P.D., and Pascoe, C. (2018). Functional responses of an apex predator and a mesopredator to an invading ungulate: Dingoes, red foxes and sambar deer in south-east Australia. *Austral Ecology* **43**, 375-384.

Forsyth, D.M., Woodford, L., Moloney, P.D., Hampton, J.O., Woolnough, A.P., and Tucker, M. (2014). How does a carnivore guild utilise a substantial but unpredictable anthropogenic food source? Scavenging on hunter-shot ungulate carcasses by wild dogs/dingoes, red foxes and feral cats in south-eastern Australia revealed by camera traps. *PLoS ONE* **9**.

Furlan, E., and Gleeson, D. (2017). Improving reliability in environmental DNA detection surveys through enhanced quality control. *Marine & Freshwater Research* **68**, 388-395.

Gaur, A., Singh, A., Arunabala, V., Umapathy, G., Shailaja, K., and Singh, L. (2003). Development and characterization of 10 novel microsatellite markers from Chital deer (*Cervus axis*) and their cross-amplification in other related species. *Molecular Ecology Notes* **3**, 607-609.

GMA (2015). Estimates of harvest for deer, duck and quail in Victoria: Results from surveys of Victorian game licence holders in 2014, Game Management Authority, Victoria, Australia

Hansen, H., Hess, S., Cole, D., and Banko, P. (2007). Using population genetic tools to develop a control strategy for feral cats (*Felis catus*) in Hawai'i. *Wildlife Research* **34**, 587-596.

Hogan, F., Cooke, R., Burridge, C., and Norman, J. (2008). Optimizing the use of shed feathers for genetic analysis. *Molecular Ecology Resources* **8**, 561-567.

Kohn, M.H., and Wayne, R.K. (1997). Facts from feces revisited. *Trends in Ecology & Evolution* **12**, 223-227.

Košnář, A., and Rajnyšová, R. (2012). Assessment of a daily defecation rate of fallow deer upon a closed population study. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis* **60**, 173-180.

Kumar, S., Stecher, G., and Tamura, K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* **33**, 1870-1874.

Legge, S., Kennedy, M., Lloyd, R.A.Y., Murphy, S., and Fisher, A. (2011). Rapid recovery of mammal fauna in the central Kimberley, northern Australia, following the removal of introduced herbivores. *Austral Ecology* **36**, 791-799.

Leslie, D.M. (2011) *Rusa unicolor* (Artiodactyla: Cervidae). *Mammalian Species* **43**, 1-30.

Lindeman, M.J., and Forsyth, D.M. (2008). Agricultural impacts of wild deer in Victoria. Arthur Rylah Institute for Environmental Research, Department of Sustainability and Environment, Victoria, Australia

Lounsberry, Z.T., Forrester, T.D., Olegario, M.T., Brazeal, J.L., Wittmer, H.U., and Sacks, B.N. (2015). Estimating sex-specific abundance in fawning areas of a high-density Columbian black-tailed deer population using fecal DNA. *The Journal of Wildlife Management* **79**, 39-49.

Lunt, N., and Mhlanga, M.R. (2011). Defecation rate variability in the common duiker: importance of food quality, season, sex and age. *South African Journal of Wildlife Research* **41**, 29-35.

Mora, M., Medina-Vogel, G., Sepúlveda, M.A., Noll, D., Álvarez-Varas, R., and Vianna, J.A. (2018). Genetic structure of introduced American mink (*Neovison vison*) in Patagonia: colonisation insights and implications for control and management strategies. *Wildlife Research* **45**, 344-356.

Moriarty, A. (2004). The liberation, distribution, abundance and management of wild deer in Australia. *Wildlife Research* **31**, 291-299.

Peakall, R., and Smouse, P.E. (2006). GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6**, 288-295.

Pérez-Espona, S., Pérez-Barbería, F.J., McLeod, J.E., Jiggins, C.D., Gordon, I.J., and Pemberton, J.M. (2008). Landscape features affect gene flow of Scottish Highland red deer (*Cervus elaphus*). *Molecular Ecology* **17**, 981-996.

Pun, K.-M., Albrecht, C., Castella, V., and Fumagalli, L. (2009). Species identification in mammals from mixed biological samples based on mitochondrial DNA control region length polymorphism. *Electrophoresis* **30**, 1008-1014.

Ramón-Laca, A., Gleeson, D., Yockney, I., Perry, M., Nugent, G., and Forsyth, D.M. (2014). Reliable discrimination of 10 ungulate species using high resolution melting analysis of faecal DNA. *PLoS ONE* **9**.

Ramón-Laca, A., Soriano, L., Gleeson, D., and Godoy, J.A. (2015). A simple and effective method for obtaining mammal DNA from faeces. *Wildlife Biology* **21**, 195-203.

Rollins, L.A., Woolnough, A.P., and Sherwin, W.B. (2006). Population genetic tools for pest management: a review. *Wildlife Research* **33**, 251-261.

Rollins, L.A., Woolnough, A.P., Sinclair, R., Mooney, N.J., and Sherwin, W.B. (2011). Mitochondrial DNA offers unique insights into invasion history of the common starling. *Molecular Ecology* **20**, 2307-2317.

Rousset, F. (2008). genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources* **8**, 103-106.

State of Victoria (2018). Draft deer management strategy. State of Victoria, Melbourne, Victoria.

Taberlet, P., Waits, L., and Luikart, G. (1999). Non-invasive genetic sampling: look before you leap. *Trends in Ecology & Evolution* **14**, 323-327.

Valière, N. (2002a). Gemini: Software for testing the effects of genotyping errors and multitubes approach for individual identification. *Molecular Ecology Notes* **2**, 83-86.

Valière, N. (2002b). GIMLET: A computer program for analysing genetic individual identification data. *Molecular Ecology Notes* **2**, 377-379.

Valière, N., Bonenfant, C., Toïgo, C., Luikart, G., Gaillard, J.-M., and Klein, F. (2007). Importance of a pilot study for non-invasive genetic sampling: genotyping errors and population size estimation in red deer. *Conservation Genetics* **8**, 69-78.

Veale, A.J., Gleeson, D.M., and Clout, M.N. (2014). Measuring connectivity of invasive stoat populations to inform conservation management. *Wildlife Research* **41**, 395-406.

Waits, L.P., and Paetkau, D. (2005). Non-invasive genetic sampling tools for wildlife biologists: A review of applications and recommendations for accurate data collection. *Journal of Wildlife Management* **69**, 1419-1433.

Wayne, A.F., Wilson, B.A., and Woinarski, J.C.Z. (2017). Falling apart? Insights and lessons from three recent studies documenting rapid and severe decline in terrestrial mammal assemblages of northern, south-eastern and south-western Australia. *Wildlife Research* **44**, 114-126.

Wedrowicz, F., Karsa, M., Mosse, J., and Hogan, F.E. (2013). Reliable genotyping of the koala (*Phascolarctos cinereus*) using DNA isolated from a single faecal pellet. *Molecular Ecology Resources* **13**, 634-641.

Wedrowicz, F., Mosse, J., Wright, W., and Hogan, F. (2017). Validating the use of non-invasively sourced DNA for population genetic studies using pedigree data. *Web Ecology* **17**, 9-18.

Foreword to chapter 3

Understanding the population structure and connectivity of invasive species can inform their management and help assess the likelihood of achieving eradication or control. For example, eradication efforts performed on large populations that are highly connected across a landscape are unlikely to be effective due to rapid reinvasion. Therefore, investigating population structure can help determine the feasibility and scale of eradication efforts and highlight areas where control and asset protection is a more realistic management option.

In Victoria, sambar deer are considered the most problematic introduced deer species and have been associated with range of marked social, economic and ecological impacts. Limited research on all aspects of sambar deer ecology has constrained their management. No previous studies have investigated the population structure of sambar deer in south eastern Australia, or attempted to delineate individual management units.

This chapter applies the ‘DNA toolbox’ described in chapter two to collect genetic data from sambar deer from across Victoria. A population genetics approach is then used to determine the population structure of sambar deer across Victoria and delineate independent management units.

Chapter three has direct implications for the management of sambar deer in Victoria. The three distinct management units will help plan control efforts and contain sambar deer populations to the areas identified. The sampling methodology and genetic approach outlined here could also be replicated to delineate management units for other introduced deer species in Victoria.

Chapter 3 – Delineating genetic management units of sambar deer (*Rusa unicolor*) using opportunistic tissue sampling and targeted scat collection

Male sambar stag wallowing, Hill End, Victoria,
December 2015

Photo credit: Christopher Davies

Davies, C., Wright, W., Wedrowicz, F., Pacioni, C. and Hogan, F. (under review) Delineating genetic management units of sambar deer (*Rusa unicolor*) using opportunistic tissue sampling and targeted scat collection. *Wildlife Research*.

Chapter 3 – Delineating genetic management units of sambar deer (*Rusa unicolor*) using opportunistic tissue sampling and targeted scat collection

3.1 Abstract

Introduced sambar deer (*Rusa unicolor*) are having detrimental impacts on native ecosystems in south eastern Australia. Population genetics can be applied to delineate management units providing information that can help plan and improve control strategies. This study aims to delineate management units of sambar deer in south eastern Australia using population genetic analyses. Sambar deer DNA was sourced opportunistically from tissue samples and targeted scat collection. Samples were collected from three areas in Victoria, south eastern Australia; Mt Cole (MC), French Island (FI) and eastern Victoria (EV). Contemporary population structure was assessed using a suite of 11 polymorphic microsatellite markers; the number of maternal sambar deer lineages in south eastern Australia was investigated through sequencing of the mitochondrial (mtDNA) control region. We identified three distinct genetic clusters. Differentiation between inferred clusters was found to be high with F_{ST} ranging from 0.24 between EV and FI clusters and 0.48 between MC and FI clusters. Two mtDNA haplotypes were identified; R.u1 was found throughout EV and FI, while R.u2 was unique to MC. DNA isolated from scats provided reliable data and proved critical for sampling areas where hunting and culling of deer are not generally undertaken. We establish three genetically distinct sambar deer management units in south eastern Australia, MC, FI and EV. Sambar deer control strategies should be applied to each management unit independently. Management of sambar deer within each independent management unit should be coordinated and planned as a single program/project rather than independent, small scale operations. This may be difficult/infeasible for the EV management unit which is large and geographically complex. Further research may help identify additional fine scale structure in EV allowing smaller, more practicable management units to be identified. Genetic data can be used to identify management units for invasive species which will be critical for the development of future management strategies and improving control operations. The approach outlined here could also be applied to improve the management of other introduced deer species in south eastern Australia.

3.2 Introduction

Similar to habitat loss and fragmentation, invasive species are recognised globally as major drivers of biodiversity loss (Allendorf and Lundquist 2003). Australia has a long history of damaging invasive species introductions (Hoffman and Broadhurst 2016) including accidental arrivals to the continent, as well as those brought intentionally, either as domestic pets and livestock, for recreational hunting opportunities or as biological control agents (Phillips *et al*. 2007). Some of the most damaging examples of invasive species introductions include the feral cat (*Felis catus*), red fox (*Vulpes vulpes*) and cane toad (*Bufo marinus*). These species have been associated with major declines in small mammals across the Australian continent (Woinarski *et al.* 2015). Larger species including feral goats (*Carpa hircus*) and deer have also caused significant damage to Australian ecosystems (Bayne *et al*. 2004; Davis *et al*. 2016), including those already under threat from anthropogenic climate change (Department of Environment 2015). Invasive species can have pronounced economic impacts (Perrings *et al*. 2000; Pimentel *et al*. 2005). For example, in Australia during 2001 the financial costs associated with economic loss and control of invasive species was estimated to be over $9 billion dollars, rising to over $13 billion in 2012 (Hoffman and Broadhurst 2016).

Wild deer, particularly sambar deer (*Rusa unicolor*), are currently causing significant environmental issues across south eastern Australia and pressure exists on land managers to improve deer management practices. Sambar are large tropical deer. The species has a native range in south east Asia including areas of India, Sri Lanka, Thailand, Nepal and China (Leslie 2011), and was first introduced into Australia during the 1800s to provide game for hunting (Bentley 1957). Sambar deer are generalist browsers and have established self-sustaining wild populations in Victoria, New South Wales (NSW), the Australian Capital Territory (ACT) and Northern Territory (NT) (Moriarty 2004). Surveys based on deer harvest by recreational hunters provide evidence of rapidly increasing sambar deer numbers in Victoria over the last decade, with estimates of over 30,000 sambar deer harvested in 2009 rising to almost 90,000 in 2017 (Moloney and Turnbull 2018) and more than 100,000 in 2018 (Moloney and Powell 2019). Increased sambar deer numbers have raised concerns regarding their social, economic and ecological impacts.

The largest sambar deer populations in Australia are thought to occur in Victoria, where the species has been identified as a potentially threatening process (Parliament of Victoria 2017) and have been implicated in damage to native ecosystems (Bilney 2013; Peel *et al.* 2005).

Sambar deer have been shown to browse a wide selection of trees, shrubs, forbs and grasses, including threatened native species in the Yarra Ranges National Park (Forsyth and Davis 2011), and their potential to permanently change vegetation composition and structure has been documented. They also consume a number of weed species, so are likely to disperse environmental weeds across Victorian landscapes (Eyles 2002). Antler rubbing by sambar deer has damaged stands of yellow wood (*Acronychia oblongifolia*) and shiny nematolepsis (*Nematolepis wilsonii*) in the Yarra Ranges and in East Gippsland (Bennett and Coulson 2011; Bilney 2013) linking sambar to the loss of rare and threatened plant species. In the Alpine National Park (ANP) sambar create wallows and game trails and can damage sensitive sub alpine peat communities which are listed as threatened ecosystems (Department of Environment 2015). Another, particularly serious, impact of wild deer is their ability to carry and transmit endemic and exotic diseases to livestock (Cripps *et al.* 2018), wildlife (Ryan and Power 2012) and people (Ng *et al*. 2011).

Based on occurrence records, a recent study investigating sambar deer distribution proposed four reproductively isolated populations in Victoria: eastern Victoria, Mount Cole, Timboon and French Island (Forsyth *et al.* 2015). Of these, the proposed population in eastern Victoria has the largest distribution, estimated to cover over 66,300 km^2. This area encompasses most forested areas east of Melbourne through to the Victoria–NSW border and continues northwards into the ACT (Forsyth *et al.* 2015). The eastern Victorian sambar deer population is thought to have been established by animals released by the Acclimatisation Society and by deer escaping from farms (Moriarty 2004). Known escapes and introductions occurred at Gembrook (undated), Kinglake (1863), Snake Island (1866) and Tooradin (1869-1873) (Bentley 1967). The proposed sambar deer populations at Mount Cole, French Island and Timboon (Fig. 3.1) are smaller than the eastern Victorian population. Releases of sambar deer around Ercildoune in the 1870s and 1880s are thought to have founded the Mount Cole population (Forsyth *et al.* 2015). The origin of the French Island population is uncertain. It may have established from a release during 1859 (Bentley 1967) or from animals originating from the Tooradin release swimming to the island (Forsyth *et al.* 2015). It is likely that the four sambar deer populations proposed by Forsyth *et al*. (2015) are genetically isolated from each other, since barriers to dispersal including large expanses of cleared land, major roads and watercourses exist between them (Forsyth *et al.* 2015). However, no previous studies have investigated the connectivity of sambar deer populations across Victoria.

Managing deer populations is a complex issue in Victoria. Despite clear evidence of the damage they cause to ecological and agricultural systems, they are also recognised as valuable game species (Davis *et al*. 2016). As such, recreational hunters expect healthy populations of deer to hunt. Some land managers have suggested that deer eradication is required where they are impacting sensitive vegetation communities. Current management strategies to reduce deer populations and their associated impacts in Australia include culling (Pople *et al*. 2017; DEDJTR 2018) and the use of fencing to exclude deer from sensitive areas (Bennett and Coulson 2008). A lack of understanding regarding deer distribution, abundance, connectivity and ability to spread to new areas makes the effective management of sambar deer in Victoria difficult. More research is required to investigate these aspects of sambar deer ecology and improve their management.

A molecular approach, where DNA is used to investigate the relatedness between sampled individuals can be used to determine whether the putative populations are indeed genetically isolated and have the potential to be managed independently. Genetic data can be used to delineate population boundaries and assess connectivity between groups (Fraser *et al.* 2013). Mapping genetic structure within populations, to identify individual management units, has previously been used to direct and improve eradication efforts for invasive species including mink (*Neovision vison*), stoats (*Mustela erminea*) and feral pigs (*Sus scrofa*) (Hampton *et al.* 2004b; Mora *et al.* 2018; Veale *et al.* 2014). Small populations, with clear boundaries, and no connectivity represent the best opportunity for successful eradication because reinvasion from other populations is unlikely to occur (Abdelkrim *et al.* 2005). Genetic data can also be used to detect reinvasion pathways that could be subsequently targeted for ongoing control and surveillance (Adams *et al.* 2014). Subsequent to the implication of control actions, genetic data can also be used to determine the success of the program by identifying survivors and re-invaders (Veale *et al.* 2013).

The ecology of sambar deer in Victoria is poorly understood and no previous studies have incorporated a genetic approach for sambar deer research. Here, we use opportunistic sampling of tissue (sourced from hunters) and targeted non-invasive sampling of scats to investigate the contemporary genetic structure of sambar deer in south eastern Australia and attempt to delineate separate management units. This information will determine if sambar deer in Victoria form one large, homogenous population or are divided into distinct populations. Identifying genetically isolated populations will help land managers determine the feasibility of eradication and control efforts by determining the scale of connected sambar deer populations and likelihood of reinvasion after the implementation of management efforts.

3.3 Methods

3.3.1 Sample collection and DNA isolation

Collection of samples for population genetic studies from wild animals can be challenging. Deer, for example, are large, cryptic animals that are inherently difficult and expensive to trap, which limits the ability to collect large numbers of tissue or blood samples for genetic analyses (Hampton *et al.* 2018). DNA from tissue can be provided by recreational hunters, however this requires a good relationship with hunters and restricts sampling to areas where hunting is permitted. Alternatively, deer DNA can be sourced non-invasively through the collection faecal pellets, often referred to as scats (Davies *et al*. 2019). As scats are continuously deposited in the environment, animals do not need to be culled or caught to be sampled. Scat collection therefore allows for targeted, rather than opportunistic DNA collection.

Sambar deer tissue (ear or liver) and scat samples were collected from three of the four Victorian sambar populations proposed by Forsyth *et al.* (2018): eastern Victoria (EV), French Island (FI) and Mount Cole (MC) (Fig. 3.1). Samples were not collected from Timboon due to issues accessing private property. All sambar deer tissue samples from EV (*n*=56) were supplied by licensed recreational hunters and Parks Victoria. Deer scats, presumed to be individual sambar deer, were collected from EV (*n*=33), FI (*n*= 23) and MC (*n*=23). DNA was sampled from scats using a swabbing method described in Davies *et al.* (2019). Attempts were made to prevent swabbing scats from the same individual by employing an exclusion zone of ~100 metres between scat samples, observing the size and shape of scats and discarding subsequent samples that appeared similar in appearance. Geographic locations were recorded for scat samples using a handheld Garmin® GPS device, or georeferenced by recording the nearest road, track or town. DNA was isolated from tissue samples using Qiagen® DNeasy Blood and Tissue Kit and from scat samples using the Qiagen® QIAamp DNA Mini kit following protocols outlined in Davies *et al.* (2019).

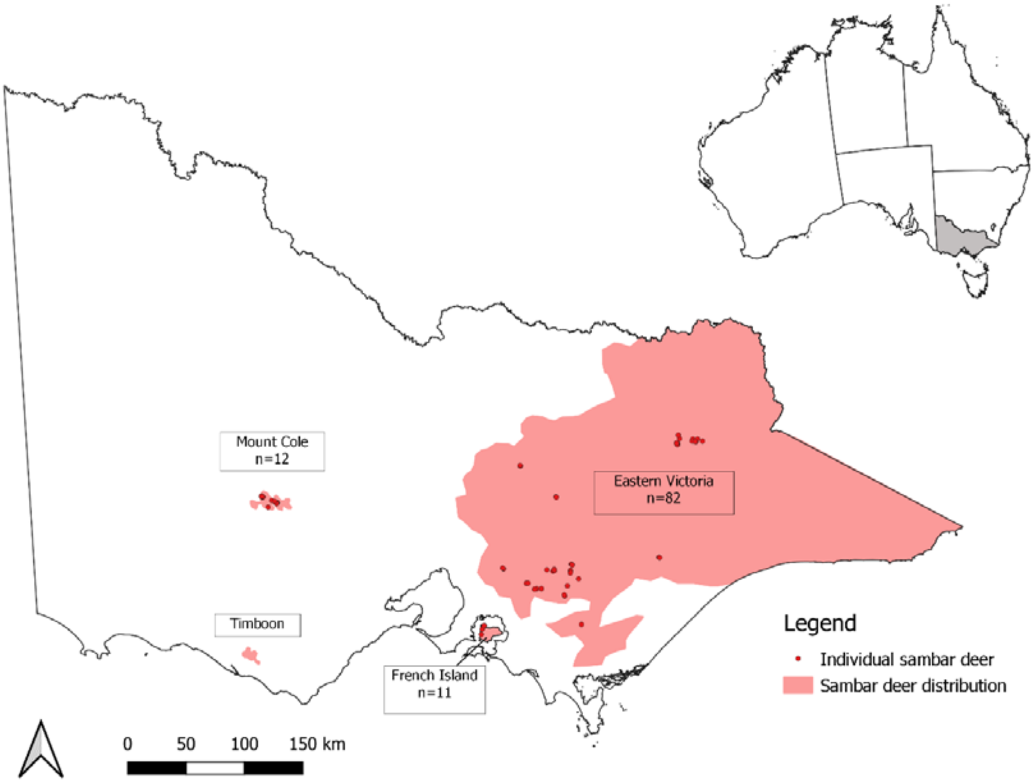

Fig. 3.1: Sampling locations of individual sambar deer genotypes (n=105) in south eastern Australia. The four 'reproductively isolated populations' suggested by Forsyth *et al.* (2015) are labelled (Eastern Victoria, French Island, Mount Cole and Timboon). Red shading indicates estimated current sambar deer distribution in Victoria.

3.3.2 Mitochondrial DNA amplification and sequencing

DNA isolates from EV (*n*=69), FI (*n*=5) and MC (*n*=11) were amplified for a ~600 base pair section of the mitochondrial (mtDNA) control region using primers CervCRH and CervtPro (Balakrishnan *et al.* 2003), following methods described in Davies *et al*. (2019). PCR products were sequenced using Sanger sequencing by the Australian Genome Research Facility (AGRF), in Melbourne, Australia. Sequences were trimmed using Sequence Scanner Software v2.0 (Applied Biosystems) and the software MEGA7 (Kumar *et al.* 2016) was used to align control region sequences using ClustalW. To understand the number of sambar deer lineages introduced to Victoria and to identify mitochondrial differences that may contribute to population structure, aligned sequences were used to produce a haplotype network via the R packages, *pegas* (Paradis 2010) and *ape* (Popescu *et al.* 2012). All new haplotypes were submitted to GenBank.

To gain information regarding the geographical origin of Victorian sambar deer we compared sequence data produced by this study with mtDNA sequence data reported by Gupta *et al.* (2015) and Martins *et al.* (2018) representing sambar deer from their native range in south east Asia. We compared a 139 bp section of mtDNA sequence in common between this study and those of Gupta *et al.* (2015) and Martins *et al.* (2018).

3.3.3 Microsatellite genotyping

DNA isolates from tissues (*n*=56) and scats (*n*=79) were genotyped using 11 fluorescently dye labelled cervid microsatellites: BL42, BM757, INRA121, IDVGA55, TGLA53, TGLA57 (Bonnet *et al.* 2002), Ca18, Ca43 (Gaur *et al.* 2003), CelJP38, OarFCB5 and RT7 (Pérez-Espona *et al.* 2008), previously described in Davies *et al.* (2019). Genotyping was carried out on the Applied Biosystems 3730 DNA analyser and GENEMAPPER 3.7 software (Applied Biosystems) by AGRF. All DNA isolates derived from scat samples were genotyped in triplicate as recommended by Davies *et al.* (2019). Consensus genotypes from replicates were generated using *ConGenR* (Lonsinger and Waits 2015). The R package *allelematch* (Galpern *et al.* 2012) was used to identify identical genotypes which were removed from the dataset. MICRO-CHECKER 2.2.3 (Van Oosterhout *et al.* 2004) was used to check for the presence of null alleles, stuttering and allelic dropout for each putative population (EV, FI and MC).

3.3.4 Population structure analysis

Genetic structuring was assessed using three methods. First, the Bayesian clustering approach in the program STRUCTURE (Pritchard *et al.* 2000) was used to identify the most likely number of genetic clusters. The software assigns individuals to clusters by minimising deviations from Hardy-Weinberg (HW) and Linkage Disequilibrium (LD) proportions. STRUCTURE was run with admixture and correlated allele frequencies with 4,000,000 Markov Chain Monte Carlo (MCMC) iterations and a burn-in of 1,000,000 iterations for K from 1–20. The most likely number of clusters was chosen based on the value of K with the lowest posterior probability from values that have plateaued (Pritchard *et al.* 2000).

Second, GENELAND which implements spatial Bayesian clustering methods (Guillot *et al.* 2005) was used to investigate fine scale population structure for a subset of samples with coordinate data from across Victoria (*n*=46) and EV (*n*=27). GENELAND analyses were performed using the spatial model and run with both correlated and uncorrelated allele frequencies. The correlated allele frequency model is more powerful at detecting subtle population structure, however it is more sensitive to departures from model assumptions, such as the presence of isolation by distance (Guillot 2008).

For both correlated and uncorrelated allele frequency models, the maximum number of populations (K) was set to 10 with 1,000,000 iterations and a burn in of 50,000. The thinning parameter was set to 1000 and 10 independent runs were conducted. The most likely number of clusters inferred by GENELAND was chosen according to the run with the highest posterior probability. Convergence was assessed in line with the GENELAND manual (Guillot *et al.* 2005).

Third, discriminant analysis of principal components (DAPC) was used to assess population structure using the R package *adegenet* version 2.0.2 (Jombart 2008). DAPC is based on genetic distances rather than minimisation of HW proportions (Jombart 2008). We used the function ‘find.cluster’ to determine the optimal number of clusters and retained all principal components.

3.3.5 Genetic statistics and isolation by distance analyses

GenAlEx version 6.5 (Peakall and Smouse 2006) was used to calculate the mean number of alleles (N_A), mean expected (H_E) and observed heterozygosity (H_O) and number of private alleles (P_A) across all loci for clusters assigned by STRUCTURE. Deviations from Hardy-Weinberg equilibrium (HWE) and genotypic disequilibrium for each cluster were calculated using GENEPOP 4.2 (Rousset 2008). The R package *diveRsity* (Keenan *et al.* 2013) was used to calculate allelic richness (A_R). We used both F_{ST} (Weir and Cockerham 1984) and D_{jost} (Jost 2008) to estimate genetic differentiation between clusters. Mantel tests were performed in GenAlEx to test for correlation between geographical and genetic distances for all samples with coordinate data in defined clusters. We also tested for evidence of fine scale isolation by distance by conducting spatial autocorrelation analyses in GenAlEx for all individuals of each cluster, for distances of 15-150 km.

3.4 Results

3.4.1 Quality control of DNA isolates

DNA from the outer surface of scats, as targeted in this study, can be removed and/or degraded by environmental conditions such as rain. As such, DNA isolates from all samples collected during this study were screened for DNA quality and quantity as outlined in Davies *et al.*

(2019). All DNA isolates from scats and tissue had DNA concentrations greater than 0.05 ng/µL and produced PCR products for the quality control amplification step, so were retained for genetic analysis, as recommended by Davies *et al.* (2019).

3.4.2 mtDNA sequencing

Mitochondrial control region sequencing of tissue (*n*=56) and scat samples (*n*=32) identified two different haplotypes (R.u1 and R.u2). The first haplotype (R.u1, GenBank accession number: MK473445) was found in individuals sampled from EV and FI and has been previously reported by Davies *et al.* (2019). The second haplotype (R.u2, GenBank accession number: MK473444) was unique to sambar deer from MC (Fig. 3.2). Comparison of mtDNA sequence (139 bp) from this study with sequences reported from across the native range of sambar deer, revealed that both R.u1 and R.u2 are likely to originate from Sri Lanka.

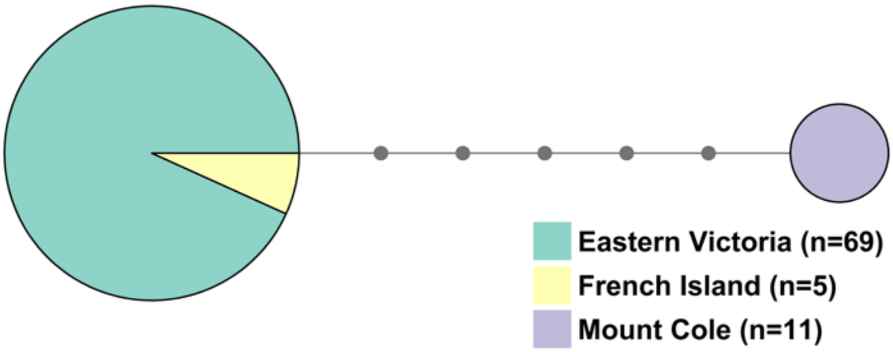

Fig. 3.2: Haplotype network based on the 536 bp partial mtDNA control region. Sampling locations included: Eastern Victoria, French Island and Mount Cole.

3.4.3 Microsatellite genotyping

When collecting DNA from discarded sources, multiple scats of the same individual can be inadvertently sampled. Matching of genotypic data derived from scat samples (*n*=79) revealed that 52 individual sambar deer had been sampled with 27 individuals being sampled more than once (four individuals in EV, twelve at FI and eleven at MC). After removal of matching genotypes, 105 individual sambar deer (*n*=52 originating from scats and *n*=53 originating from tissues) were identified from the regions sampled, i.e. EV (*n*=82), FI (*n*=11) and MC (*n*=12).

3.4.4 Population structure

Inspection of the mean log likelihood inferred by STRUCTURE indicated the most likely number of populations to be K=3 (Fig. 3.3). The three genetically distinct clusters inferred by STRUCTURE matched the reproductively separated populations proposed by Forsyth *et al.* (2015). STRUCTURE clustered most individuals strongly (Q>0.8) to their location of origin. Two individuals were identified as not originating from their sampled region, one individual sampled in EV was assigned to the MC cluster and one individual from FI was assigned to the EV cluster. Three individuals from EV also displayed some admixture with FI sambar deer.

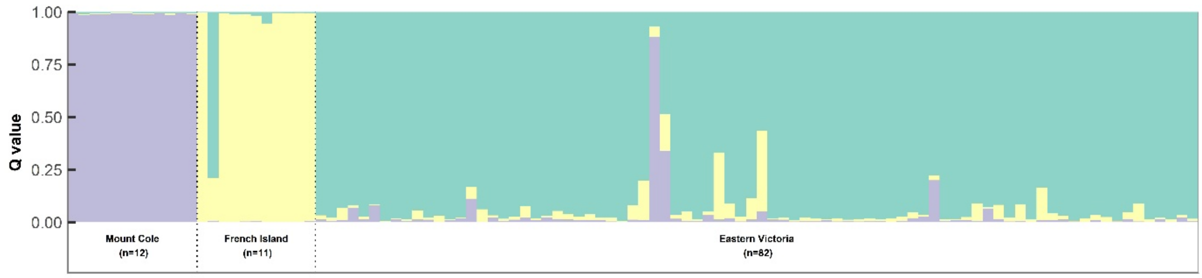

Fig. 3.3: Genetic structure and assignment of individual sambar deer using STRUCTURE. Each sambar deer is represented by a single vertical bar. Individuals are grouped according to their population of origin. Clusters are displayed as different colours and the fraction of each individual colour represents the probability of assignment to the cluster of that colour.

Using the uncorrelated allele frequency model across all regions GENELAND detected K=3, matching the results of STRUCTURE, whilst the correlated allele frequency model estimated K=4 identifying an extra cluster (Kanumbra) within the EV population (Fig. 3.4). Using eastern Victorian samples alone, GENELAND estimated K=1 using the uncorrelated allele frequency model and K=3 using the correlated allele frequency model (Supplementary material, S1), possibly suggesting further substructure of sambar deer present within the EV population.

DAPC inferred a total of five genetic clusters (FI, MC and three clusters in EV) with all individuals grouping to their sampling location except one individual from FI that was assigned to EV (Fig. 3.5).

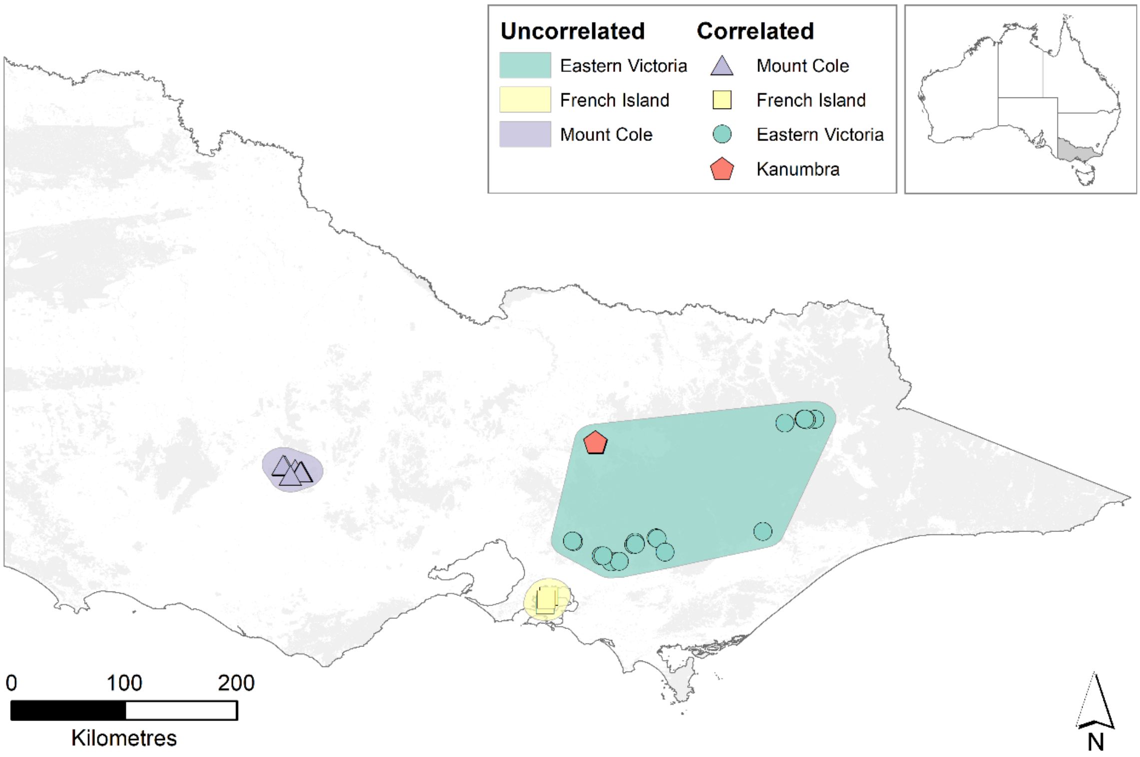

Fig. 3.4: Population structure of sambar deer in Victoria inferred using GENELAND with correlated and un-correlated allele frequency models. Points indicate clusters (K=4) inferred using correlated allele frequency model. Lines surround clusters (K=3) inferred using uncorrelated allele frequency model. Grey shading indicates forested areas.

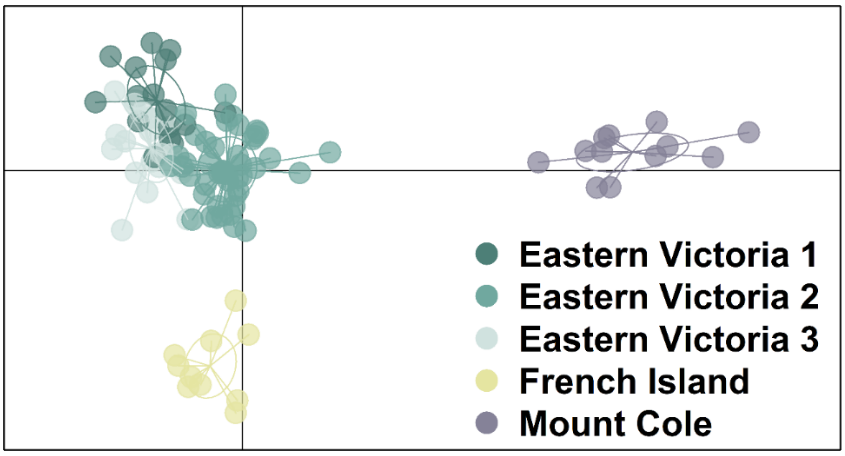

Fig. 3.5: Scatterplot of Discriminant Analysis of Principal Components (DAPC) based on five clusters inferred in *adegenet*. Circles represent individuals and colours correspond to assigned clusters.

3.4.5 Genetic statistics of inferred population clusters

MICRO-CHECKER indicated homozygote excess at loci BL42 in the EV region, consistent with the presence of null alleles or the Wahlund effect. These findings were not consistent across all regions sampled, therefore BL42 was retained for further analysis. Mean observed heterozygosity (H_O) and expected heterozygosity (H_E) across all regions was 0.42 and 0.43 respectively (Table 3.1). Private alleles within a sampled group can provide a measure of genetic distinctiveness, however estimates are dependent upon sample size. The EV cluster had the highest number of private alleles (*n*=15). Five private alleles were detected for the MC cluster, which is relatively high, considering the small number of individuals sampled (*n*=12), while in comparison, for FI, where a similar number of individuals were sampled (*n*=11), only one private allele was detected. Deviation from HWE ($P < 0.05$) was observed at loci RT7 within the EV cluster. Significant genotypic disequilibrium ($P < 0.05$) was detected between loci pairs: CelJP38 and IDVGA55, BL42 and INRA121 in the EV cluster, and between Ca18 and OarFCB5, BL42 and TGLA53 in the MC cluster. All three population clusters were significantly differentiated using F_{ST} and D_{jost} (Table 3.2). Genetic differentiation was high and ranged from 0.19 (FI – EV) to 0.44 (FI – MC) for D_{jost} and between 0.24 (FI – EV) and 0.48 (FI – MC) for F_{ST} (Table 3.2). Mantel tests showed no correlation between geographical and genetic distance within each individual region (EV: r = 0.068. P value = 0.220, FI: r = 0.006. P = 0.400, MC r = -0.002. P = 0.500).

Table 3.1: Genetic diversity metrics for the three sambar deer population clusters identified by STRUCTURE.

n, sample size, N_a, mean number of alleles, A_r, mean allelic richness, P_a, number of private alleles, H_O, observed heterozygosity and H_E, expected heterozygosity.

Cluster	***n***	**N_a**	**A_r**	**P_a**	**H_O**	**H_E**
Eastern Victoria	82	4.64	3.1 (2.7-3.5)	15	0.53	0.55
French Island	11	2.45	2.2 (1.6-2.5)	1	0.27	0.30
Mt Cole	12	2.36	2.3 (2.1-2.4)	5	0.47	0.43

Table 3.2: Genetic differentiation of clusters identified by STRUCTURE (D_{jost} below the diagonal and F_{ST} above the diagonal), Bootstrapped 95% confidence intervals shown in parentheses, F_{ST} Weir and Cockerham's fixation index estimator (Weir & Cockerham, 1984), D_{jost} Estimator of Jost's D (Jost, 2008).

	Eastern Victoria	French Island	Mt Cole
Eastern Victoria	-	0.24 (0.18 - 0.30)	0.28 (0.25 - 0.32)
French Island	0.19 (0.12 - 0.27)	-	0.48 (0.42 - 0.53)
Mt Cole	0.33 (0.25 - 0.42)	0.44 (0.35 - 0.55)	-

3.5 Discussion

Sambar deer have established themselves as a problematic invasive species in south eastern Australia and a lack of understanding of connectivity between populations has impeded their management. For the first time, we use microsatellite genotyping to investigate the population structure of sambar deer in Victoria and report three distinct population clusters (EV, FI and MC). Genetic clustering of individuals inferred in this study agree with previous assumptions made by Forsyth *et al.* (2015) who suggested that sambar deer from EV, FI and MC are reproductively isolated. The F_{ST} and D_{jost} values observed in this study show that all clusters displayed high genetic differentiation. The highest genetic differentiation between clusters was observed between FI and MC, and the lowest genetic differentiation between FI and EV. This is not surprising as the MC cluster exhibits a separate mitochondrial lineage (haplotype) while FI and EV shared a single haplotype. This suggests that either; 1) the FI sambar deer population was established from the same stock as other releases in EV or 2) the area was colonised by animals swimming to the island from Tooradin, as suggested by Forsyth *et al.* (2015).

3.5.1 Potential barriers to dispersal and movement

There are potential barriers to dispersal and movement between each population cluster identified in this study. These barriers are likely to have contributed to the high level of genetic differentiation and the continued separation of Victorian sambar deer populations despite the relatively long history of these populations in the State. Although sambar deer are recognised as strong swimmers (Leslie 2011), the waters of Westernport Bay are likely to impede movement of individuals between the EV and FI populations. Waterbodies have been found to impede dispersal and movement for other mammalian pests including common brush tail possums (*Trichosurus vulpecula*) in New Zealand (Adams *et al.* 2014). Expanses of cleared land also exist between the forested areas inhabited by sambar deer in eastern Victoria and

French Island and are likely to have impeded movement of individual animals, and hence gene flow, between these areas. Sambar deer commonly display preferences for areas of thick vegetation cover (Bentley 1957) so large areas of cleared land are likely to be perceived as a barrier by this species.

3.5.2 Sambar deer diversity in Victoria

Low to moderate microsatellite heterozygosity (Ho = 0.42) and allelic richness (Ar = 2.52) was observed in sambar deer during this study. We identified only three studies that used microsatellites to investigate the genetic diversity of sambar deer within their native or introduced ranges. Lin *et al.* (2014) used microsatellite genotyping to identify inbreeding in a domesticated sambar deer population (n=20) in Taiwan, reporting a mean heterozygosity of 0.31. Gupta (2014) investigated genetic diversity of Indian sambar deer (n=49) and reported mean expected and observed heterozygosity of 0.75 and 0.52, respectively. The lower microsatellite diversity observed in Victorian sambar deer (compared to the results of Gupta (2014)) may reflect the small founding population introduced to Victoria during the 1800s and an associated genetic bottleneck. However, further genotyping (with the same microsatellite markers) is required to make accurate comparisons in diversity measures. It is common for introduced species to display low genetic diversity as populations have often originated from a small number of founders (Shimatani *et al.* 2010). In some circumstances, however, populations of introduced species can have higher genetic variability than native populations, as is the case of European rabbits introduced into Australia (Zenger *et al.* 2003). This can occur due to rapid population expansion or due to breeding between individuals from separate introductions (from different areas of the native range).

3.5.3 Mitochondrial data provides insights into the origin of Victorian sambar deer

Sequencing of the mitochondrial control region revealed two sambar deer haplotypes (Fig. 3.2) providing evidence of at least two female founders of the Victorian sambar deer population. One haplotype was shared between deer from EV, FI and KOS (R.u1) with the second unique to sambar deer from MC (Ru.2). The presence of the different haplotypes between regions may have resulted from a range of different scenarios. One explanation is that the individual animals originally brought to Australia were from a single population (likely from Sri Lanka) containing a limited number of haplotypes and by chance only individuals with the R.u2 haplotype were released at MC.

3.5.4 Evidence of translocation or migration

Curiously, a single juvenile sambar deer sampled by hunters within the Alpine National Park (in the EV cluster) showed genetic characteristics indicating that a large proportion of its ancestry (Q=0.88) originated from the MC population. These areas are separated by hundreds of kilometres and wide expanses of cleared land. As such, the individual may represent a deer that has been translocated. Translocation of game animals for hunting has taken place in Australia since the 1900s (McKnight 1976) and is likely to have contributed to the spread of deer in Victoria (Moriarty 2004). Evidence of illegal translocation of feral pigs to supplement hunting stocks has previously been recorded in Western Australia (Spencer and Hampton 2005) but no previous studies have investigated the illegal translocation of deer species in Australia. Future research investigating the home range and dispersal ability of sambar deer in Victoria is required to assess whether long distance migration is a possible (or probable) explanation for the presence of this individual in the Alpine National Park. Furthermore, additional sampling will help distinguish if sambar deer with the MC (Ru.2) haplotype are present within other populations.

3.5.5 Opportunistic tissue sampling and non-invasive scat sampling

Sambar deer are inherently difficult to capture and sample tissue for genetic studies. Our approach, which combines opportunistic sampling of tissue, sourced from hunters or land management agencies (Parks Victoria) and targeted scat sampling has enabled the collection of DNA from 105 individual sambar deer across a wide geographical area. Using this approach, we were able to describe the population structure of sambar deer across south eastern Australia. DNA isolated from tissue (including blood) samples is typically higher in both yield and quality and data derived from such samples are less prone to errors compared to that generated from degraded DNA. Although scat samples are relatively easy to collect, they are more likely to contain degraded DNA.

While it is preferable to use tissue samples over scat samples for genetic studies, tissue sampling for deer can be limited to areas that allow hunting and/or locations where deer control efforts are undertaken. For example, such sampling would not usually be possible in National Parks (where hunting is prohibited) unless control operations are being undertaken, where deer pose the greatest threat to biodiversity. Scat collection offers a valid alternative to tissue sampling in protected areas and, as in this study, sampling can be focussed in areas in which deer activity has been detected. Furthermore, deer scats can be easily differentiated from the scats of native mammals so non-experts, volunteers and citizen scientists could be utilised to conduct field sampling and increase sample sizes. Since differentiation between scats of individuals of a single species can be problematic, DNA profile matching is necessary to ensure that individuals are not represented more than once in a data set.

3.5.6 Future directions

Increasing the sample size, geographical coverage of samples and number of microsatellite loci would help elucidate more subtle difference in population structure (Landguth *et al.* 2012), and possible clarify the different estimate of K observed using GENELAND. However, the differences in K estimates we observed in the EV sambar population using the correlated and uncorrelated allele frequency models in GENELAND could have arisen for a number of reasons. First, the additional clusters inferred by the correlated allele frequency model may accurately represent further population structuring within the geographically large EV population. This explanation would also help explain deviations from HWE and genotypic disequilibrium observed in the EV cluster. Alternatively, the additional clusters may have been identified due to gaps in the sampling distribution (large areas of the EV population were not sampled). Previous studies have reported that the effect of isolation by distance (IBD) can cause spurious results when using GENELAND and other spatially explicit Bayesian clustering techniques (Frantz *et al.* 2009). Despite no evidence of IBD in the EV population there may be other factors at play. For example, the large distribution and rapid expansion of sambar deer populations in Victoria may affect model assumptions.

During this study three of the four Victorian sambar deer populations identified by Forsyth *et al*. (2015) were sampled. Further collection of genetic data from the population not sampled for this study (Timboon, shown in Fig. 3.1) would help determine whether deer within this area also form a distinct management unit. Using genetic data to determine whether eradication, control or containment is feasible at Timboon is a priority for future research due to the potential for this population to spread into neighbouring areas, including the Great Otway National Park. Likewise, additional sampling and genetic analysis within the geographically large EV sambar population will help delineate fine scale population structure and may reveal discrete management units within this population.

Analyses of genetic data using a landscape genetic approach could be used to determine the influence of landscape features on gene flow and connectivity across Victoria. Such an approach could also identify leading edges of range expansion which could be targeted for prioritised eradication or control (Rollins *et al.* 2009). Additionally, further genetic analyses will allow estimates of effective population size for each of the clusters identified in this study. This will provide an indication of the size of each cluster and determine if each population is expanding or contracting. By collecting genetic information from scats before and after control operations, the effectiveness of the control operations can be assessed by estimating abundance of the target species using genetic mark recapture models (Berry *et al.* 2012) or through the detection of genetic bottlenecks (Hampton *et al.* 2004a; Rollins *et al.* 2006). Incorporating genetic approaches into sambar deer research will provide important information regarding distribution and population dynamics and is therefore recommended.

3.5.7 Implications for sambar deer management

Current management strategies of deer in Victoria are often *ad hoc* as little is known about the ecology of this invasive species (Davis *et al.* 2016). Sambar deer management interventions require more empirical data to help assign resources and improve management outcomes (Parliament of Victoria 2017). The findings of this study have important implications for sambar deer management in Victoria. We have identified three genetically isolated sambar deer populations in Victoria which should be applied as distinct management zones. Establishing deer management zones in Victoria will help guide where to conduct surveillance, monitoring, control and eradication efforts to meet localised deer management objectives (DEDJTR 2018).

The work of Hone *et al*. (2010) suggested that around 40% of sambar deer would require removal to stop annual population growth. If sambar deer formed a single homogenous population across Victoria removing this number of deer would be a daunting and unfeasible process, exacerbated by the rugged and inaccessible terrain preferred by the species. Here we provide evidence that multiple sambar deer populations exist in Victoria. With this information land managers can focus control and eradication efforts to the areas identified (EV, FI and MC) and make evidence based decisions regarding whether control and eradication efforts are feasible and cost effective. Further, if the EV sambar deer population displays additional substructure there is further scope for targeted eradication of management units within this large and geographically complex area.

Our results have immediate applicability for sambar deer management in Victoria by providing an indication of the likelihood of success of management actions. We show sambar deer on French Island are genetically isolated from deer on the mainland, suggesting that eradication of sambar deer from French Island is possible and control operations undertaken on the island have a low risk of reinvasion. In contrast, the observed connectivity between sambar deer across eastern Victoria suggests that eradication attempts performed at geographical scales smaller than the entire EV region are not feasible since reinvasion is likely to occur quickly. As such, an asset protection approach to deer impacts across EV that focuses on protecting vegetation communities assessed to be at the highest levels of risk (e.g. alpine peatlands and warm temperate rainforest communities) may be practical in the short term.

In addition, we identified that illegal translocation of sambar deer may be occurring in Victoria. The translocation of wild animals has potential to introduce the species to areas that were previously unoccupied and also poses a risk of spreading harmful pathogens (Höfle *et al*. 2004). Such activities would compound management efforts to reduce the negative impacts associated with the deer. Future research should further investigate the scale of illegal translocation of sambar deer in Victoria. This could be achieved by wide scale collection of scats as sources of DNA, microsatellite genotyping and the use of assignment tests to identify translocated individuals.

We suggest that incorporating a genetic approach into future studies will greatly improve the management of sambar deer in Victoria. The sampling methodology and genetic approach applied here could also be used to delineate management units for other introduced deer species in Victoria.

Recommendations from this study include:

1) Ongoing collection of deer tissue and scat samples for use in future genetic studies of sambar deer and other introduced deer species.
2) Further research into the potential for the eradication of sambar deer from French Island.
3) Managing the eastern Victorian sambar deer population as a large, connected population prioritising asset based protection of high value ecological communities threatened by sambar deer.
4) More thorough investigation of the rates of illegal translocation of sambar deer across Victoria.

3.6 References

Abdelkrim, J., Pascal, M., Calmet, C., and Samadi, S. (2005). Importance of assessing population genetic structure before eradication of invasive species: examples from insular Norway rat populations. *Conservation Biology* **19**, 1509-1518.

Adams, A. L., van Heezik, Y., Dickinson, K. J. M., and Robertson, B. C. (2014). Identifying eradication units in an invasive mammalian pest species. *Biological Invasions* **16**, 1481-1496.

Allendorf, F. W. and Lundquist, L. L. (2003). Introduction: Population biology, evolution, and control of invasive species. *Conservation Biology* **17**, 24-30.

Balakrishnan, C., Monfort, S., Gaur, A., Singh, L., and Sorenson, M. (2003). Phylogeography and conservation genetics of Eld's deer (*Cervus eldi*). *Molecular Ecology* **12**, 1-10.

Bayne, P., Harden, R., and Davies, I. (2004). Feral goats (*Capra hircus*) in the Macleay River gorge system, north-eastern New South Wales, Australia, impacts on soil erosion. *Wildlife Research* **31**, 519-525.

Bennett, A. and Coulson, G. (2011). The impacts of sambar (*Cervus unicolor*) on the threatened shiny nemtolepis (*Nematolepis wilsonii*). *Pacific Conservation Biology* **16**, 251.

Bentley, A. (1957). A brief account of the deer in Australia. *The Journal of Wildlife Management* **21**, 221-225.

Bentley, A. (1967) 'An introduction to the deer of Australia with special reference to Victoria.' (Kew, Victoria.)

Berry, O., Algar, D., Angus, J., Hamilton, N., Hilmer, S., and Sutherland, D. (2012). Genetic tagging reveals a significant impact of poison baiting on an invasive species. *Journal of Wildlife Management* **76**, 729-739.

Bilney, R. J. (2013). Antler rubbing of yellow-wood by sambar in East Gippsland, Victoria. *The Victorian Naturalist* **130**, 68-74.

Bonnet, A., Thévenon, S., Maudet, F., and Maillard, J. C. (2002). Efficiency of semi-automated fluorescent multiplex PCRs with 11 microsatellite markers for genetic studies of deer populations. *Animal Genetics* **33**, 343-350.

Cripps, J. K., Pacioni, C., Scroggie, M. P., Woolnough, A. P., and Ramsey, D. S. L. (2018). Introduced deer and their potential role in disease transmission to livestock in Australia. *Mammal Review* **49**, 60-77.

Davies, C., Hogan, F. E., Wedrowicz, F., and Wright, W. (2019). A DNA toolbox for non-invasive genetic studies of sambar deer (*Rusa unicolor*). *Australian Mammalogy*.

Davis, N. E., Bennett, A., Forsyth, D. M., Bowman, D. M. J. S., Lefroy, E. C., Wood, S. W., Woolnough, A. P., West, P., Hampton, J. O., and Johnson, C. N. (2016). A systematic review of the impacts and management of introduced deer (family Cervidae) in Australia. *Wildlife Research* **43**, 515-532.

DEDJTR (2018). Draft deer management strategy. Department of Economic Development, Jobs, Transport and Resources. (Melbourne, Victoria.)

Department of Environment (2015). National recovery plan for the alpine sphagnum bogs and associated fens ecological community. Department of Environment. (Canberra, Australia.)

Eyles, D. (2002) Sambar deer (*Cervus unicolor*) as a potential seed vector for the spread of the environmental weed Himalayan Honeysuckle (*Leycesteria formosa*) at Mount Buffalo National Park. BSc (Hons) Thesis, University of Melbourne, Parkville.

Forsyth, D. M. and Davis, N. E. (2011). Diets of non-native deer in Australia estimated by macroscopic versus microhistological rumen analysis. *The Journal of Wildlife Management* **75**, 1488-1497.

Forsyth, D. M., Stamation, K., and Woodford, L. (2015). Distributions of sambar deer, rusa deer and sika deer in Victoria. Arthur Rylah Institute. (Melbourne, Victoria.)

Frantz, A. C., Cellina, S., Krier, A., Schley, L., and Burke, T. (2009). Using spatial Bayesian methods to determine the genetic structure of a continuously distributed population: clusters or isolation by distance? *Journal of Applied Ecology* **46**, 493-505.

Fraser, E. J., Macdonald, D. W., Oliver, M. K., Piertney, S., and Lambin, X. (2013). Using population genetic structure of an invasive mammal to target control efforts – An example of the American mink in Scotland. *Biological Conservation* **167**, 35-42.

Galpern, P., Manseau, M., Hettinga, P., Smith, K., and Wilson, P. (2012). Allelematch: an R package for identifying unique multilocus genotypes where genotyping error and missing data may be present. *Molecular Ecology Resources* **12**, 771-778.

Gaur, A., Singh, A., Arunabala, V., Umapathy, G., Shailaja, K., and Singh, L. (2003). Development and characterization of 10 novel microsatellite markers from Chital deer (*Cervus axis*) and their cross-amplification in other related species. *Molecular Ecology Notes* **3**, 607-609.

Guillot, G. (2008). Inference of structure in subdivided populations at low levels of genetic differentiation—the correlated allele frequencies model revisited. *Bioinformatics* **24**, 2222-2228.

Guillot, G., Mortier, F., and Estoup, A. (2005). Geneland: a computer package for landscape genetics. *Molecular Ecology Notes* **5**, 712-715.

Gupta, S. K. (2014) Assessment of genetic variation in sambar deer *Rusa unicolor*. PhD Thesis, Saurashtra University, India.

Gupta, S. K., Kumar, A., Gaur, A., and Hussain, S. A. (2015). Detection of 40 bp insertion-deletion in mitochondrial control region among sambar (*Rusa unicolor*) populations in India. *BMC Research Notes* **8**, 581.

Hampton, J., Pluske, J. R., and Spencer, P. B. S. (2004a). A preliminary genetic study of the social biology of feral pigs in south-western Australia and the implications for management. *Wildlife Research* **31**, 375-381.

Hampton, J. O., Finch, N. A., Watter, K., Amos, M., Pople, T., Moriarty, A., Jacotine, A., Panther, D., McGhie, C., Davies, C., Mitchell, J., and Forsyth, D. M. (2018). A review of methods used to capture and restrain introduced wild deer in Australia. *Australian Mammalogy* **41**, 1-11.

Hampton, J. O., Spencer, P. B. S., Alpers, D. L., Twigg, L. E., Woolnough, A. P., Doust, J., Higgs, T., and Pluske, J. (2004b). Molecular techniques, wildlife management and the importance of genetic population structure and dispersal: a case study with feral pigs. *Journal of Applied Ecology* **41**, 735-743.

Hoffmann, B. D. and Broadhurst, L. M. (2016). The economic cost of managing invasive species in Australia. *NeoBiota* **31**, 1-18.

Höfle, U., Vicente, J., Nagore, D., Hurtado, A., Peña, A., Fuente, J. d. l., and Gortazar, C. (2004). The risks of translocating wildlife: Pathogenic infection with *Theileria* sp. and *Elaeophora elaphi* in an imported red deer. *Veterinary Parasitology* **126**, 387-395.

Hone, J., Duncan, R. P., and Forsyth, D. M. (2010). Estimates of maximum annual population growth rates (rm) of mammals and their application in wildlife management. *Journal of Applied Ecology* **47**, 507-514.

Jombart, T. (2008). adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* **24**, 1403-1405.

Jost, L. (2008). GST and its relatives do not measure differentiation. *Molecular Ecology* **17**, 4015-4026.

Keenan, K., McGinnity, P., Cross, T. F., Crozier, W. W., and Prodöhl, P. A. (2013). diveRsity: An R package for the estimation and exploration of population genetics parameters and their associated errors. *Methods in Ecology and Evolution* **4**, 782-788.

Kumar, S., Stecher, G., and Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution* **33**, 1870-1874.

Landguth, E. L., Fedy, B. C., Oyler-Mccance, S. J., Garey, A. L., Emel, S. L., Mumma, M., Wagner, H. H., Fortin, M.-J., and Cushman, S. A. (2012). Effects of sample size, number of markers, and allelic richness on the detection of spatial genetic pattern. *Molecular Ecology Resources* **12**, 276-284.

Leslie, D. M. (2011). *Rusa unicolor* (Artiodactyla: Cervidae). *Mammalian Species*, 1-30.

Lin, D. Y., Chiang, T. Y., Huang, C. C., Lin, H. D., Tzeng, S. J., Kang, S. R., Sung, H. M., and Wu, M. C. (2014). Polymorphic microsatellite loci isolated from *Cervus unicolor* (Cervidae) show inbreeding in a domesticated population of Taiwan sambar deer. *Genetics and Molecular Research* **13**, 3967-3971.

Lonsinger, R. and Waits, L. (2015). ConGenR: rapid determination of consensus genotypes and estimates of genotyping errors from replicated genetic samples. *Conservation Genetics Resources* **7**, 841-843.

Martins, R., Schmidt, A., Lenz, D., Wilting, A., and Fickel, J. (2018). Human-mediated introduction of introgressed deer across Wallace's line: Historical biogeography of *Rusa unicolor* and *Rusa timorensis*. *Ecology & Evolution* **8**, 1465-1479.

McKnight, T. (1976) 'Friendly vermin : a survey of feral livestock in Australia.' (University of California Press: Berkeley, California.)

Moloney, P. D. and Powell, Z. (2019). Estimates of the 2018 deer harvest in Victoria: results from surveys of Victorian Game Licence holders in 2018. Unpublished client report for the Game Management Authority, Arthur Rylah Institute for Environmental Research, Department of Environment, Land, Water and Planning, Heidelberg, Victoria.

Moloney, P. D. and Turnbull, J. D. (2018). Estimates of harvest for deer in Victoria: results from surveys of Victorian Game licence holders in 2016. Unpublished client report for the Game Management Authority, Arthur Rylah Institute for Environmental Research, Department of Environment, Land, Water and Planning, Heidelberg, Victoria.

Mora, M., Medina-Vogel, G., Sepúlveda, M. A., Noll, D., Álvarez-Varas, R., and Vianna, J. A. (2018). Genetic structure of introduced American mink (*Neovison vison*) in Patagonia: colonisation insights and implications for control and management strategies. *Wildlife Research* **45**, 344-356.

Moriarty, A. (2004). The liberation, distribution, abundance and management of wild deer in Australia. *Wildlife Research* **31**, 291-299.

Ng, J., Yang, R., Whiffin, V., Cox, P., and Ryan, U. (2011). Identification of zoonotic *Cryptosporidium* and *Giardia* genotypes infecting animals in Sydney's water catchments. *Experimental Parasitology* **128**, 138-144.

Paradis, E. (2010). pegas: an R package for population genetics with an integrated-modular approach. *Bioinformatics* **26**, 419-420.

Parliament of Victoria (2017). Inquiry into the control of invasive animals on Crown land. Environment Natural Resources and Regional Development Committee. (Victorian Government: Melbourne.)

Peakall, R. and Smouse, P. E. (2006). GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6**, 288-295.

Peel, B., Bilney, R. J., and Bilney, R. J. (2005). Observations of the ecological impacts of Sambar (*Cervus unicolor*) in East Gippsland, Victoria, with reference to destruction of rainforest communities. *The Victorian Naturalist* **122**, 189-200.

Pérez-Espona, S., Pérez-Barbería, F. J., McLeod, J. E., Jiggins, C. D., Gordon, I. J., and Pemberton, J. M. (2008). Landscape features affect gene flow of Scottish Highland red deer (*Cervus elaphus*). *Molecular Ecology* **17**, 981-996.

Perrings, C., Dalmazzone, S., and Williamson, M. H. (2000) 'The economics of biological invasions.' (Edward Elgar Publishing, United Kingdom).

Phillips, B. L., Brown, G. P., Greenlees, M., Webb, J. K., and Shine, R. (2007). Rapid expansion of the cane toad (*Bufo marinus*) invasion front in tropical Australia. *Austral Ecology* **32,** 169-176.

Pimentel, D., Zuniga, R., and Morrison, D. (2005). Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological* economics **52**, 273-288.

Popescu, A.-A., Huber, K., and Paradis, E. (2012). ape 3.0: New tools for distance-based phylogenetics and evolutionary analysis in R. *Bioinformatics* **28**, 1536-1537.

Pritchard, J. K., Stephens, M., and Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics* **155**, 945-959.

Robertson, B. C. and Gemmell, N. J. (2004). Defining eradication units to control invasive pests. *Journal of Applied Ecology* **41**, 1042-1048.

Rollins, L. A., Woolnough, A. P., and Sherwin, W. B. (2006). Population genetic tools for pest management: a review. *Wildlife Research* **33**, 251-261.

Rollins, L. E. E., Woolnough, A., Wilton, A., Sinclair, R. O. N., and Sherwin, W. (2009). Invasive species can't cover their tracks: using microsatellites to assist management of starling (*Sturnus vulgaris*) populations in Western Australia. *Molecular Ecology* **18**, 1560-1573.

Rousset, F. (2008). genepop’007: a complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources* **8**, 103-106.

Ryan, U. and Power, M. (2012). *Cryptosporidium* species in Australian wildlife and domestic animals. *Parasitology* **139**, 1673-1688.

Shimatani, Y., Fukue, Y., Kishimoto, R., and Masuda, R. (2010). Genetic variation and population structure of the feral American mink (*Neovison vison*) in Nagano, Japan, revealed by microsatellite analysis. *Mammal Study* **35**, 1-7.

Spencer, P. B. S. and Hampton, J. O. (2005). Illegal translocation and genetic structure of feral pigs in Western Australia. *Journal of Wildlife Management* **69**, 377-384.

Van Oosterhout, C., Hutchinson, W., Wills, D. P. M., and Shipley, P. (2004). micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* **4**, 535-538.

Veale, A. J., Edge, K. A., McMurtrie, P., Fewster, R. M., Clout, M. N., and Gleeson, D. M. (2013). Using genetic techniques to quantify reinvasion, survival and in situ breeding rates during control operations. *Molecular Ecology* **22**, 5071-5083.

Veale, A. J., Gleeson, D. M., and Clout, M. N. (2014). Measuring connectivity of invasive stoat populations to inform conservation management. *Wildlife Research* **41**, 395-406.

Weir, B. S. and Cockerham, C. C. (1984). Estimating F-Statistics for the analysis of population structure. *Evolution* **38**, 1358-1370.

Woinarski, J. C. Z., Burbidge, A., and Harrison, P. L. (2015). Ongoing unraveling of a continental fauna: Decline and extinction of Australian mammals since European settlement. *Proceedings of the National Academy of Sciences of the United States of America* **112**, 4531-4540.

Zenger, K. R., Richardson, B. J., and Vachot-Griffin, A. M. (2003). A rapid population expansion retains genetic diversity within European rabbits in Australia. *Molecular Ecology* **12**, 789-794.

Foreword to chapter 4

Deer impacts including browsing, wallowing and trampling pose direct threats to sensitive alpine and subalpine vegetation communities. Management interventions such as ground and aerial shooting are commonly used to mitigate the impacts of deer to sensitive areas. However, little information is available to guide the application of these control methods.

This chapter focusses on Baw Baw National Park (BBNP) a protected area located at the southern end of the Great Dividing Range. Camera traps are used to investigate the detectability and activity patterns of sambar deer within BBNP and establish information to guide control efforts.

This chapter presents novel and important information regarding sambar deer ecology that have important implications for their management in BBNP and more broadly, across Victoria. Camera traps were useful for providing ecological information for cryptic invasive sambar deer. The camera trap survey methodology described will be useful for future deer surveillance aimed at containing deer to the management units described in chapter three. Key recommendations for future research are also included.

Chapter 4 – Detectability and activity patterns of sambar deer (*Rusa unicolor*) in Baw Baw National Park, Victoria

Family group of sambar deer, subalpine heathland,
Baw Baw National Park, October 2016

Photo credit: Christopher Davies

Davies, C., Wright, W., Hogan, F. and Davies, H. (2020) Detectability and activity patterns of sambar deer (*Rusa unicolor*) in Baw Baw National Park, Victoria. *Australian Mammalogy*, https://doi.org/10.1071/AM19029

Chapter 4 – Detectability and activity patterns of sambar deer (*Rusa unicolor*) in Baw Baw National Park, Victoria

4.1 Abstract

Introduced sambar deer (*Rusa unicolor*) are increasing in abundance and distribution across much of south eastern Australia and causing damage to native ecosystems. However, the current paucity of knowledge surrounding many aspects of sambar deer ecology is limiting our capacity to make informed management decisions, and properly gauge the extent of deer impacts. Here we investigate correlates of sambar deer detectability and describe activity patterns of sambar deer in Baw Baw National Park (BBNP) to inform control operations. Camera traps were deployed in BBNP between October and December 2016. We used an occupancy modelling framework to investigate sambar deer detectability and camera trap record time stamps to determine sambar deer activity patterns. Sambar deer were found to be significantly more detectable near roads and in areas of sparse tree density and displayed strong crepuscular activity patterns. Control operations carried out along roads at dawn and dusk could be effective, at least in the short term. Likewise, aerial culling could be an effective control option for sambar deer populations in BBNP. This study highlights the utility of camera trap data to inform the application of control operations for cryptic invasive species.

4.2 Introduction

Invasive species are recognised globally as a leading threat to biodiversity, and pose a significant risk to ecosystem function (Butchart *et al.* 2010). In Australia, invasive predators have been implicated in the decline and extinction of numerous mammal, bird and reptile species (Abbott 2011; Nogales *et al.* 2013), however, introduced herbivores have also caused substantial environmental degradation. Introduced herbivores, including camels, goats, buffalo and deer can degrade ecosystems by grazing and trampling plants, resulting in changes to vegetation structure and increased erosion (Husheer *et al*. 2003; Bayne *et al*. 2004; Joys *et al*. 2004; Pellerin *et al.* 2006).

In Victoria, there are four species of deer with self-sustaining populations: sambar (*Rusa unicolor*), red (*Cervus elaphus*), fallow (*Dama dama*) and hog (*Axis porcinus*) (Moriarty 2004). Of these four species, sambar deer are currently the most abundant (Forsyth *et al.* 2015). Sambar deer were first introduced to Victoria during the 1800's to provide game for hunting (Bentley 1957). Initial release sites included areas around the Koo-Wee Rup swamp, Kinglake, French Island and Gembrook (Bentley 1957). The current distribution of sambar deer is estimated to encompass most forested areas of eastern Victoria, along the Great Dividing Range into New South Wales and the Australian Capital Territory (Forsyth *et al.* 2015; Gormley *et al.* 2011). Annual estimates of deer harvested by recreational hunting are increasing, with over 100,000 individuals taken in 2016 (Moloney and Turnbull 2017) rising to over 120,000 in 2018 (Moloney and Powell 2019).

Due to increases in the abundance and distribution of sambar deer, there is growing concern regarding their ecological impacts in south eastern Australia (Davis *et al.* 2016). Sambar deer damage native vegetation through browsing (Bennett 2008), antler rubbing (Bilney 2013) and wallowing (Phillipson *et al.* 2015). Furthermore, sambar deer have potential to spread environmental weeds (Eyles 2002: Forsyth and Davis 2011), to harbour parasites that are harmful to livestock (Cripps *et al.* 2018) and humans (Ng *et al.* 2011; Ryan and Power 2012) and are likely to become a significant vehicle collision risk, as demonstrated for other deer species in North America (Steiner *et al.* 2014). Sambar deer also present a substantial risk to ecological assets within many of Victoria's iconic National Parks. Baw Baw National Park (BBNP) is of particular interest because of its unique and fragile ecosystems, including subalpine peatlands which are often negatively impacted by sambar deer trampling and wallowing (Davis *et al.* 2016).

Despite their significant detrimental impact on native ecosystems, little research has been undertaken on sambar deer in Victoria (Davis *et al*. 2016). As such, most information regarding their abundance and distribution has relied on anecdotal reports and observations (Davis *et al.* 2016). The development and implementation of robust monitoring methods is needed to better understand the habitat preferences and activity patterns of sambar deer outside of their natural range. Such information will help prioritise the spatial and temporal application of management efforts (Bengsen *et al.* 2012). Additionally, the empirical data on sambar deer populations gained from monitoring programs are vital to allow the effectiveness of management interventions (such as population reduction activities) to be assessed. However, due in part to their cryptic nature, such data is challenging to obtain for exotic deer species, and currently represents a significant knowledge gap for wild deer in Australia.

Various methods are used by land managers to manage and control overabundant exotic herbivores. In Australia, ground shooting is the favoured method to reduce deer numbers and was successfully used to eradicate fallow deer from Kangaroo Island (Masters *et al.* 2018). Recently, aerial shooting from helicopters has been employed as a control strategy to reduce impacts associated with overabundant chital deer (*Axis axis*) in Queensland (Pople *et al.* 2017). Aerial shooting is also currently being undertaken in Victoria to reduce populations of sambar and fallow deer (Parks Victoria 2019). Exclusion fencing is another management tool that can be applied to exclude sambar deer from sensitive areas (Bennett and Coulson 2008) and to protect high value environmental assets at small scales. While fertility control methods to reduce the density of overabundant deer have been successfully trialled in the United States at small scales (Rutberg and Naugle 2008), this method is difficult to apply to large populations across wide geographic areas (Raiho *et al.* 2015) and are unlikely to be effective in the Australian context. Currently, there is little information available regarding the effectiveness of deer management interventions in Victoria and further research is required.

Data collected from camera trap surveys are likely to play an important role in informing deer management and have potential to underpin future monitoring of sambar deer in Victoria. Camera trapping has proved effective for monitoring many other terrestrial mammals (Tobler *et al*. 2008), and passive methods have advantages over other direct monitoring techniques such as live trapping and observer surveys (De Bondi *et al.* 2010; Meek *et al.* 2014). Past studies have used camera traps for invasive species surveillance (Caravaggi *et al*. 2016) and to help land managers determine the target, scale and effectiveness of control operations (Bengsen *et al*. 2011). However, the usefulness of this approach for the detection of cryptic deer species in Australia has received little attention. Here we use camera traps to investigate the correlates of sambar deer detectability and the activity patterns of sambar deer in BBNP.

4.3 Materials and methods

4.3.1 Study location

Baw Baw National Park (BBNP) is located approximately 130 km east of Melbourne, Victoria, Australia (Fig. 4.1). The park features a distinctive subalpine plateau and forms part of the Highlands Southern Fall; Victorian Alps bioregion (Parks Victoria 2005). At elevations over 1300 metres the plateau is dominated by a low overstorey of snow gum (*Eucalyptus pauciflora*) with an understorey of woody shrubs, grasses and herbs. Vegetation communities below the subalpine plateau include tall forest dominated by mountain ash (*Eucalyptus regnans*) and alpine ash (*Eucalyptus delegatensis*), with elements of subalpine rainforest. Lower elevations of BBNP (below 1000 metres) encompass expanses of shrubby dry forest, and damp forest (Parks Victoria 2005). A wide variety of fauna including threatened species such as the critically endangered Baw Baw frog (*Philoria frosti*) and Leadbeater’s possum (*Gymnobelideus leadbeateri*) occur in the park (Jean-Marc Hero 2004; Woinarski 2016). Highly restricted and rare floral communities including alpine bogs and cool temperate rainforest also occur within BBNP (Parks Victoria 2005). Total annual rainfall for the subalpine plateau of BBNP is over 1,500 mm with seasonal snow falls common during the winter months (June-September) at altitudes over 1000 metres (Australian Bureau of Meteorology 2018).

4.3.2 Study species

Sambar deer are Asia’s most widespread deer species. Their native range encompasses areas of India, Nepal, Sri Lanka, Thailand, China and Indonesia (Leslie 2011). Sambar deer are Australia’s largest introduced deer species, with mature females weighing up to 225 kg and males over 300 kg (Leslie 2011). Sambar deer are generalist browsers and consume a wide variety of grasses, shrubs and forbs (Forsyth and Davis 2011). Despite concerns about their ecological impacts sambar deer are regarded as a valuable game species and protected under Victorian State legislation (*Wildlife Act 1975*).

4.3.3 Data collection

Motion-triggered infrared camera traps were deployed at 30 sites across the BBNP for between 33 and 77 days during October, November and December, 2016 (Fig. 4.1). Sites were selected as follows: First, 30 random waypoints were plotted within the park boundary using ArcMap (ESRI, 2017). Plotted points were adjusted to ensure that ten waypoints were located across the subalpine plateau (elevation >1300m), 10 were in alpine ash dominated forest (elevations 1000-1300m) and 10 were located at lower elevations (<1000m) to allow stratification by elevation. Cameras were then deployed as close as possible to these waypoints, taking into consideration the accessibility of each site. At each site, a single Reconyx^{TM} RC55 camera trap (Reconyx Inc., Holmen, WI, USA) with infrared flash was secured to a tree at approximately one metre above the ground, using an elastic strap. Camera traps were installed to face areas with evidence of animal activity, including game trails, footprints and evidence of browsing. Where possible, cameras were positioned to face open areas in order to maximise the detection zone of each camera. Vegetation clearance, within 1 metre of each camera trap was performed to reduce the likelihood of false triggers and baits were not used. Camera traps were programmed to operate 24 hours a day and to take three photographs at one second intervals when triggered. The sensitivity of cameras was set to high. Reconyx Mapview^{TM} Professional Software was used to view and identify the species present in the first photograph of each trigger. Sambar deer detection histories for each camera trap site were generated by recording daily detections of the species (coding detections with ‘1’ and non-detections with ‘0’). Photographs with more than one individual deer present were counted as one detection for the species.

4.3.4 Site covariates

We compiled site covariates likely to influence occupancy and detectability of sambar deer across BBNP based on published literature (Table 4.1). Site covariates are described, and their inclusion in the study justified, in Table 4.1. Covariate data was extracted from raster layers using ArcGIS software (ESRI, 2017) at 500 metre resolution. All covariates were tested for correlation and highly correlated covariates removed from further analysis. Sealed and unsealed roads in the area were classified as roads. The road and track network of BBNP is displayed in Figure 4.1.

Table 4.1: Description and justification of the variables used in analyses to assess the correlates of sambar deer detectability within BBNP.

Variable	**Description and justification for inclusion in detectability model**
Aspect	A variable measuring the prevailing aspect (north, south, east, west) of terrain. Different aspects have differing vegetation classes and amounts of solar radiation which may influence deer occupancy.
Distance to roads	A variable measuring the Euclidean distance (m) from each camera site to the closest road. Roads included sealed and unsealed roads. Deer commonly avoid roads with high traffic density (D'Amico *et al*. 2016) however, can also be attracted to roadsides due to the availability of high quality forage (Meisinget *et al.* 2013).
Distance to water	A variable measuring the distance (m) from each camera site to closest permanent water body. This variable was included as water appears to be important for sambar deer (Yamada *et al*. 2003; Gormley *et al.* 2011) and the abundance of sambar deer faecal pellets declined with increasing distance to water (Forsyth *et al*. 2009).
Elevation	The elevation (m) for each camera site derived from a digital elevation layer. The abundance of sambar deer faecal pellets has been shown to decline with increasing elevation in the Upper Yarra ranges (Forsyth *et. al*. 2009), suggesting a possible preference for lower elevations.
Greeness	Remote sensed mean seasonal change in vegetation (2003–2013). Greeness can provide an indication of feed quality and has been used to model the distribution of large herbivores, including the eastern grey kangaroo (Visintin *et al.* 2016).
Tree density	Remote sensed tree coverage within 500 square metres in decimal percentage. Tree density influences the amount of solar radiation entering an area. Solar radiation was found to influence site occupancy by rusa deer in New Zealand (Allen *et al.* 2015).

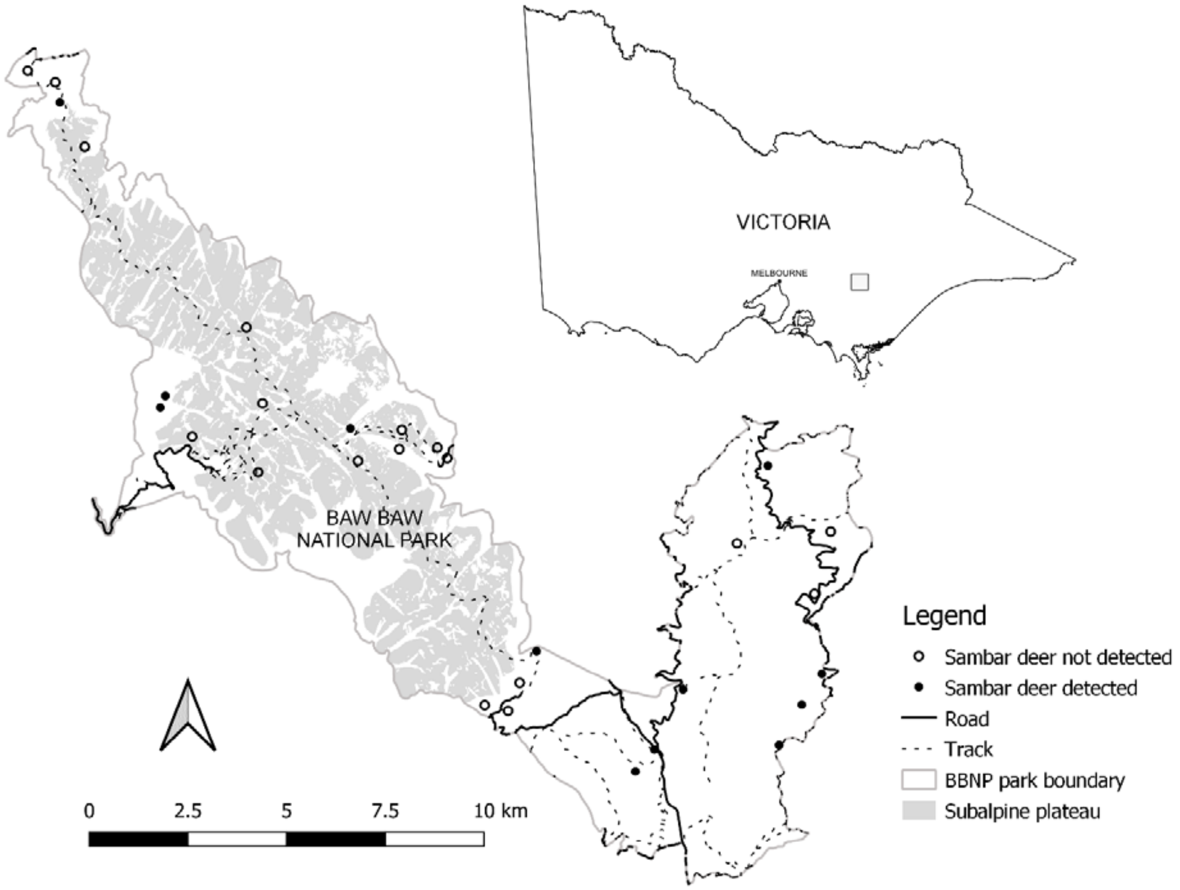

Fig. 4.1: The location of 30 camera trap sites surveyed across Baw Baw National Park. Sites where camera traps detected sambar deer are displayed as solid circles; sites where sambar deer were not detected are displayed as open circles. Roads are shown with solid black lines; tracks are shown with dotted black lines. The park boundary is represented by grey line and the subalpine plateau of BBNP is shown shaded grey. The location of BBNP relative to the state of Victoria is indicated by grey frame in the inset.

4.3.5 Correlates of sambar deer detectability

Single-season occupancy models were used to investigate the biophysical correlates of sambar deer detectability (MacKenzie *et al.* 2002). Prior to analysis, all covariates were centred and standardised by subtracting the variable's mean and dividing by the variable's standard deviation (Gelman and Hill 2006). Gormley *et al.* (2011) predicted constant sambar deer occupancy across BBNP, so we considered all six covariates as potential correlates of sambar deer detectability, and ran all combinations of these (64 models) with the assumption of constant occupancy. Where no single model was clearly superior (i.e. ΔAIC < 2), we used model averaging to estimate parameter coefficients (Burnham and Anderson 2002). All modelling was conducted in R (R Core Development Team, 2004) using the R-package 'Unmarked' (Fiske and Chandler 2011).

4.3.5 Activity patterns

We used the time stamp of the first image from all sambar deer camera trap triggers (from all 30 sites) to determine sambar deer activity patterns. We used the R package 'camtrapR' (Niedballa *et al.* 2016) to plot the kernel density of sambar deer activity over daily periods, considering 24 one hour intervals for each day.

4.4 Results

4.4.1 Camera images

Camera traps recorded 2,136 images from 712 triggers. We observed four native mammal species, including common brush-tail possum (*Trichosurus vulpecula*), short-beaked echidna (*Tachyglossus aculeatus*), swamp wallaby (*Wallabia bicolor*) and common wombat (*Vombatus ursinus*). Images of three introduced species; feral cat (*Felis catus*), red fox (*Vulpes vulpes*) and sambar deer were recorded. In total, camera traps were triggered by sambar deer 410 times, representing 58% of all camera triggers.

4.4.2 Correlates of sambar deer detectability

Sambar deer were detected at 12 of 30 sites, a naïve occupancy rate of 40%. The most parsimonious model suggested that the probability of detecting sambar deer at each camera site (consisting of a single camera) during one sampling occasion (i.e. a single day) was 0.03. Given the length of time each camera was deployed at each site (averaging 55 days), the overall probability of detecting sambar deer at each site was 0.85 (Fig. 4.2). The detectability of sambar deer was significantly and negatively associated with distance to roads and with tree density (Fig. 4.3). Sambar deer were more detectable closer to roads (Fig. 4.4a) and in areas with lower tree density (Fig. 4.4b).

4.4.3 Sambar deer activity patterns

Sambar deer activity patterns in BBNP were determined from the time stamp of the first captured image from each of the 410 sambar deer camera triggers. Sambar deer were most active during the early morning and late evening, displaying strong crepuscular activity patterns (Fig. 4.5). Sambar deer detections were obtained most frequently between 18:00 and 22:00.

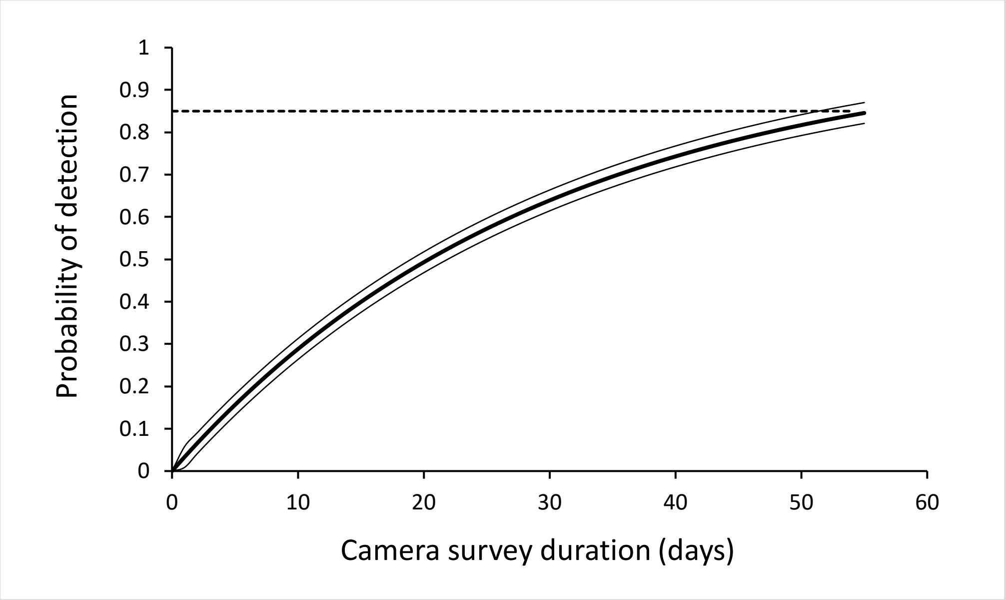

Fig. 4.2: The cumulative probability of detecting sambar deer as a result of the number of days camera traps were deployed at each site. Thin lines represent the 95% confidence interval. Dashed line indicates the minimum level of overall detection recommended for accurate occupancy estimation, i.e. 0.85 (Guillera-Arroita *et al.* 2014).

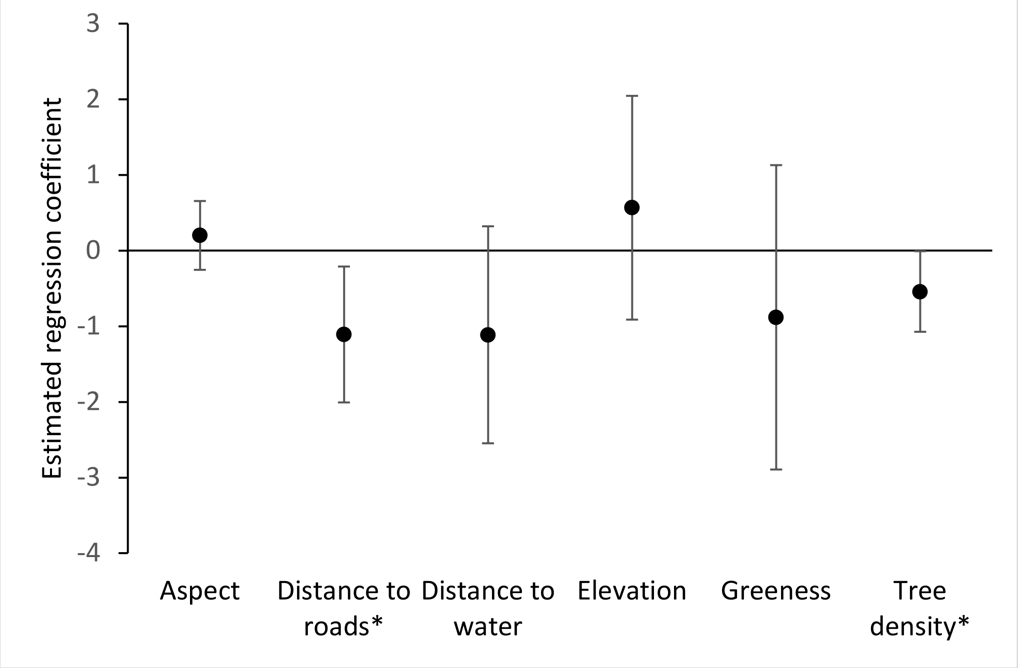

Fig. 4.3: Standardised regression coefficients for sambar deer detectability in BBNP. Error bars indicate 95% confidence intervals; asterisks indicate where they do not overlap zero (variables that display statistical significance for sambar deer detectability).

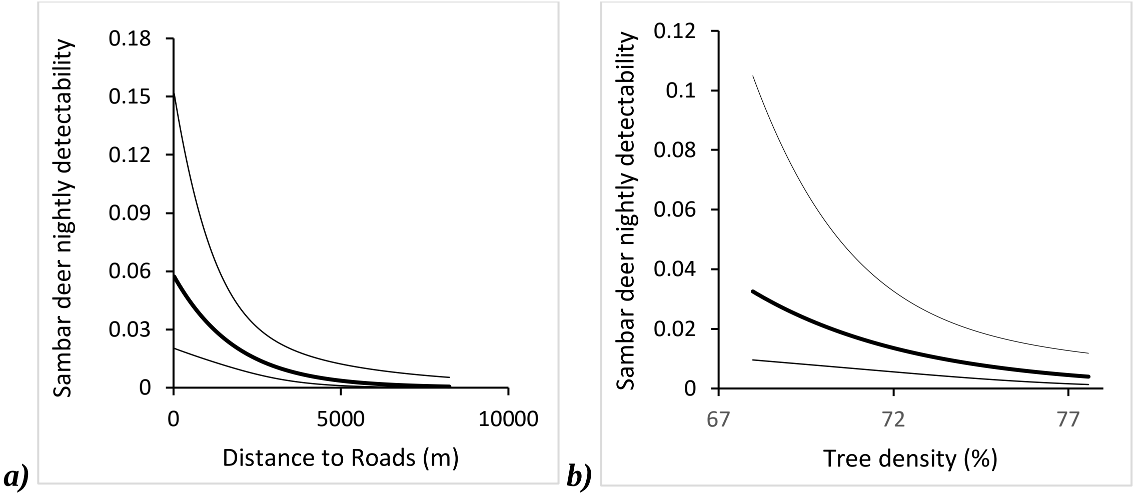

Fig. 4.4: Modelled relationship between (a) distance to roads (b) tree density on nightly sambar deer detectability in BBNP. Thin lines indicate 95% confidence intervals.

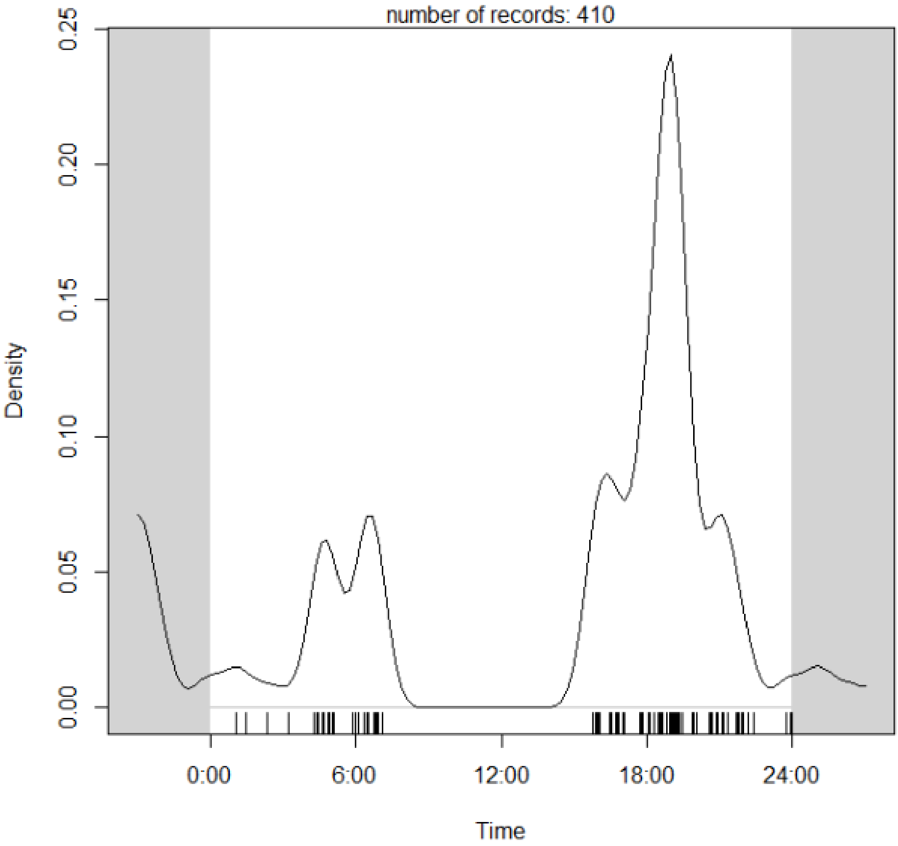

Fig. 4.5: Activity patterns of sambar deer in BBNP measured during October – December 2016. Sambar deer display crepuscular activity patterns with peak movements detected at dawn and dusk.

4.5 Discussion

Introduced sambar deer are increasing their distribution and abundance across much of south eastern Australia and causing significant damage to native ecosystems, including highly restricted, sensitive, subalpine bogs (Parliament of Victoria 2017, DEDJTR 2018). However, sambar deer are poorly studied and a lack of understanding of sambar deer ecology and impacts is constraining management decisions (Davis *et al.* 2016). To address this important knowledge gap, we investigated the correlates of sambar deer detectability and activity patterns of sambar deer in BBNP. Our results suggest that sambar deer are more detectable near roads, in areas of sparse tree density and display crepuscular activity patterns.

McCarthy *et al.* (2013) demonstrated that the detection rate of species generally increases with abundance. Our results showed that sambar deer were more detectable at camera trap sites close to roads. As such, we can assume that, in BBNP, sambar deer are more abundant, or display preferences for areas close to roads. Previous studies have demonstrated that deer usually avoid roads, particularly major roads with high traffic density (D’Amico *et al*. 2016; Prokopenko *et al*. 2017). However, it is possible that sambar deer in BBNP are taking advantage of low traffic density and using roads to avoid rugged terrain, similar to elk (*Cervus canadensis*) in North America (Killeen *et al.* 2014). Alternatively, or in addition, sambar deer may be attracted to roadside vegetation. Red deer (*Cervus elaphus*) in Norway exploit better feeding opportunities closer to roads at times of low traffic burden (Meisingset *et al.* 2013). Although not explored here, it is likely that increased light penetration and potentially greater water availability (due to run off) at roadsides could provide better conditions for growth of vegetation. Sambar deer are considered to be intermediate mixed feeders (Semiadil *et al.* 2009) and generalist browsers (Forsyth and Davis 2011). In Victoria, sambar deer have demonstrated a preference for shrubs and trees and shown to display increased grazing in autumn and browsing in spring (Forsyth and Davis 2011). As such, the vegetation along roadsides may be attracting sambar deer to these areas.

Much of BBNP consists of forests dominated by dense alpine ash (*Eucalyptus regnans*) and mountain ash (*Eucalyptus delegatensis*) (Parks Victoria 2005). Subalpine, snow-gum woodland is dominant at elevations over 1200 metres. Open areas of subalpine heathland and grassy subalpine shrub-land are also present at these elevations. Sambar deer were more detectable in areas of low tree density in BBNP, suggesting they have a preference for more open areas. This result is at odds with previous studies suggesting that sambar deer have a strong preference for thick vegetation cover (Moore 1994; Karanth 2016). A preference for areas of low tree density may indicate the influence of solar radiation. Yen *et al.* (2019) demonstrated sambar deer in Taiwan prefer areas with moderate solar radiation. Similarly, habitat suitability mapping in Lake Eildon National Park, in Victoria, Australia, showed that areas of higher potential solar radiation indicated higher suitability for habitation by sambar deer (Yamada *et al*. 2003). Allen *et al.* (2015) found that closely related rusa deer (*Rusa timorensis*) in New Zealand demonstrated a preference for areas with higher levels of solar radiation. Solar radiation contributes to primary production and influences the quality and quantity of available forage for deer. One explanation for the increased detectability of sambar deer in areas of lower tree density is that these areas provide forage that is preferred by sambar deer. Solar radiation may also contribute to sambar deer thermal regulation during cold weather. Further research is required to examine the effect of solar radiation on sambar deer occupancy and detectability and vegetation preferences in BBNP.

Camera trap data revealed that sambar deer exhibit crepuscular behaviour during spring and summer within BBNP, with peak activity times during the morning and evening. Crepuscular activity patterns of sambar deer have been previously observed in their native range (Leslie 2011), however this is the first study to empirically demonstrate this behaviour in Australia. The activity patterns we observed are consistent with those reported for many other Cervid species, including red deer (Kamler *et al.* 2007), red brocket deer (*Mazama americana*) and gray brocket deer (*Mazama gouazoubira*) (Ferreguetti *et al.* 2015). However, sambar deer have also demonstrated predominately nocturnal activity patterns in Borneo (Ross *et al.* 2013) and cathemeral activity patterns in Malaysia (Tan *et al.* 2018). The activity patterns of sambar deer, particularly in the subalpine areas of BBNP may be different during winter as they move from high altitudes to lower altitudes to avoid cold temperatures and find forage. Similar movement patterns have been observed in sika deer (*Cervus nippon*) in Japan, with the majority of deer studied moving from higher elevations to lower elevations during winter (Igota *et al*. 2004). Snow cover is thought to be major factor that influences deer population dynamics during winter as it impedes animal movements and prevents deer from accessing forage beneath snow cover (Patterson and Power 2002; White *et al.* 2009). Camera trap monitoring across the year in the BBNP is required to determine whether sambar activity patterns differ during the winter months and establish whether they leave the plateau at this time.

Based on the findings of Gormley *et al.* (2011) we constructed our models of detectability with the assumption of constant occupancy across the BBNP. However, while we recorded a high overall probability of detecting sambar deer (0.85), sambar deer were not detected at 60% of sites. Several factors could explain why sambar deer were not detected at all sites in BBNP. Sambar deer may exhibit fine scale habitat preferences and only occupy certain areas of BBNP. Previous studies have shown that sambar deer have distinct habitat preferences in their native (Yen *et al*. 2019) and introduced ranges (Gormley *et al*. 2011) which are likely to be influenced by food resource availability, temperature and life history traits (Jacques *et al*. 2009; Zeng *et al.* 2010). Steep, densely forested areas may have prevented sambar deer access to some areas of BBNP. As such, sambar deer may use distinct refuges in BBNP and utilise set routes into and out of the park. Identification of sambar deer refuges and movement routes in BBNP would allow these areas to be targeted during control operations.

While we have demonstrated the utility of camera traps for monitoring invasive deer in Australia, we can also suggest several improvements. Accurate estimates of site occupancy require an overall minimum detection probability of 0.85 (Guillera-Arroita *et al.* 2014; Shannon *et al.* 2014). To achieve the desired probability of overall detection for sambar deer in BBNP, 55 days of camera deployment was required. As demonstrated by O'Connor *et al.* (2017), the deployment of a second camera at each site (orientated in a different direction) would likely reduce the survey time required to achieve this level of detectability. Likewise, detectability can be improved by using multiple survey methods, such as searches for scats and deer sign. The use of multiple survey methods has been demonstrated to improve the probability of detection of deer (Gormley *et al.* 2011), thus improving the statistical power of occupancy models. Future studies should aim to determine the optimal number of cameras and survey duration for surveys targeting sambar deer. A better understanding of these trade-offs could be used to optimise the cost effectiveness of sambar deer monitoring programs.

4.5.1 Management implications

The proposition that sambar deer are more abundant or active close to roads is of concern, as deer-vehicle collisions represent a significant threat, with serious implications for both humans and animal welfare (Finder *et al.* 1999; Romin 1996). Future research should assess both the risk that sambar deer pose to motorists, as well as potential mitigation strategies including roadside fencing and reduction of speed limits during high risk times (Bissonette and Rosa 2012). An approach similar to Visintin *et al.* 2016 could be used to predict areas with high deer vehicle collision risk and identify collision hotspots. Our study showed that in BBNP sambar deer were frequently detected near roadsides. This may provide an opportunity for shooting operations carried out from vehicles. However, the effectiveness of this method could be limited, as deer rapidly learn to avoid areas where shooting interventions are performed (Lone *et al.* 2015; Gürtler *et al.* 2018).

Aerial shooting from helicopters has been used widely in attempts to reduce populations and impacts of terrestrial invasive species (Bayne *et al.* 2000; Edwards *et al.* 2016). Nugent *et al.* (1987) demonstrated that aerial culling reduced the density of introduced deer in New Zealand by 81 percent. However, the efficacy of aerial shooting has displayed mixed results in Australia. On Kangaroo Island in South Australia, aerial shooting contributed to the complete eradication of fallow deer (*Dama dama*) and feral goats (*Capra hircus*) from the island (Masters *et al.* 2018). In contrast, on mainland areas, aerial shooting has proven ineffective for long term control of feral pigs (Cowled *et al*. 2006). Recently, aerial shooting has been applied to reduce deer populations in Australia as it provides the best management outcomes in rugged and inaccessible terrain (Pople *et al*. 2017; Parks Victoria 2019). Visibility is a key factor for the success of aerial culling operations because shooters need to accurately identify the target species and ensure humane placement of shots (Baillie 2014). As such, open areas are commonly targeted for aerial shooting operations because they have improved efficacy and animal welfare outcomes (Hampton *et al*. 2014; Hampton *et al*. 2017). Our data suggest that sambar deer are more detectable in areas of low tree density which might indicate potential for aerial shooting operations to be conducted in BBNP. Controlling deer in subalpine environments, including those within BBNP, is a priority given our knowledge of sambar deer impacts and risks to ecological assets that occur in these regions.

Sambar deer populations, and their associated impacts, are predicted to increase in south eastern Australia (Davis *et al.* 2016). As such, land managers require more information regarding sambar deer distribution, abundance, impacts and the efficacy of control strategies to make timely, evidence based management decisions. Recommendations resulting from this study include:

1) Ongoing optimisation of sambar deer monitoring, including power analyses to detect trends in occupancy data such as by Guillera-Arroita and Lahoz-Monfort (2012).
2) Year round monitoring of subalpine peatlands within BBNP and across Victoria to understand spatial and temporal trends of deer impacts.
3) Undertaking control operations in BBNP to reduce sambar deer numbers and mitigate associated ecological impacts.

4) Conducting studies to quantify the risk to motorists posed by sambar deer populations across Victoria.

A lack of ecological information is contributing to the poor management of deer in south eastern Australia. This study has shown that sambar deer are more detectable and likely to be more abundant in proximity to roads and areas of low tree density in BBNP, a protected area encompassing important subalpine ecosystems. We also demonstrate that sambar deer display crepuscular activity patterns in BBNP. This information has important implications for the spatial and temporal application of management strategies such as ground and aerial shooting in BBNP and other protected areas occupied by sambar deer. More broadly, our study highlights the utility of camera trap data to inform the management of cryptic invasive

4.6 References

Abbott, I. (2011). The importation, release, establishment, spread, and early impact on prey animals of the red fox *Vulpes vulpes* in Victoria and adjoining parts of south-eastern Australia. *Australian Zoologist* **35**, 463-531.

Allen, R. B., Forsyth, D. M., Allen, R. K. J., Affeld, K., and MacKenzie, D. I. (2015). Solar radiation determines site occupancy of coexisting tropical and temperate deer species introduced to New Zealand forests. *PloS One* **10.**

Australian Bureau of Meteorology (2018). *Australian rainfall and temperature surface data*. Available at: http://www.bom.gov.au/climate/data-services/maps.shtml [accessed 30 October 2018].

Baillie, D. J. (2014) Ground truthing helicopter surveys: the relationship between habitat density and accuracy for a range of large vertebrate species. Masters Thesis, The University of Queensland, Gatton.

Bayne, P., Harden, B., Pines, K., and Taylor, U. (2000). Controlling feral goats by shooting from a helicopter with and without the assistance of ground-based spotters. *Wildlife Research* **27**, 517-523.

Bayne, P., Harden, R., and Davies, I. (2004). Feral goats (*Capra hircus*) in the Macleay River gorge system, north-eastern New South Wales, Australia, impacts on soil erosion. *Wildlife Research* **31**, 519-525.

Bengsen, A., Butler, J., and Masters, P. (2011). Estimating and indexing feral cat population abundances using camera traps. *Wildlife Research* **38**, 732-739.

Bengsen, A. J., Butler, J. A., and Masters, P. (2012). Applying home-range and landscape-use data to design effective feral-cat control programs. *Wildlife Research* **39**, 258-265.

Bennett, A. (2008) The impacts of sambar (*Cervus unicolor*) in the Yarra Ranges National Park. Ph.D. Thesis, The University of Melbourne, Melbourne.

Bennett, A. and Coulson, G. (2008). Evaluation of an exclusion plot design for determining the impacts of native and exotic herbivores on forest understoreys. *Australian Mammalogy* **30**, 83-87.

Bentley, A. (1957). A Brief Account of the Deer in Australia. *The Journal of Wildlife Management* **21**, 221-225.

Bilney, R. J. (2013). Antler rubbing of yellow-wood by sambar in East Gippsland, Victoria. *The Victorian Naturalist*, **130**, 68.

Bissonette, J. A. and Rosa, S. (2012). An evaluation of a mitigation strategy for deer-vehicle collisions. *Wildlife Biology* **18**, 414-423.

Burnham, K. P. and Anderson, D. R. (2002) 'Model selection and multimodel inference: A practical information-theoretic approach.' (Springer-Verlag: New York.)

Butchart, S. H. M., Butchart, S. H. M., Walpole, M., Collen, B., van Strien, A., Scharlemann, J. P. W., Almond, R. E. A., Baillie, J. E. M., Bomhard, B., Brown, C., Bruno, J., Carpenter, K. E., Carr, G. M., Chanson, J., Chenery, A. M., Csirke, J., Davidson, N. C., Dentener, F., Foster, M., Galli, A., Galloway, J. N., Genovesi, P., Gregory, R. D., Hockings, M., Kapos, V., Lamarque, J. F., Leverington, F., Loh, J., McGeoch, M. A., McRae, L., Minasyan, A., Morcillo, M. H., Oldfield, T. E. E., Pauly, D., Quader, S., Revenga, C., Sauer, J. R., Skolnik, B., Spear, D., Stanwell Smith, D., Stuart, S. N., Symes, A., Tierney, M., Tyrrell, T. D., Vie, J. C., and Watson, R. (2010). Global Biodiversity: Indicators of Recent Declines. *Science* **328**, 1164-1168.

Caravaggi, A., Zaccaroni, M., Riga, F., Schai-Braun, S. C., Dick, J. T. A., Montgomery, W. I., and Reid, N. (2016). An invasive-native mammalian species replacement process captured by camera trap survey random encounter models. *Remote Sensing in Ecology and Conservation* **2**, 45-58.

Cowled, B. D., Lapidge, S. J., Hampton, J. O., and Spencer, P. B. S. (2006). Measuring the demographic and genetic effects of pest control in a highly persecuted feral pig population. *The Journal of Wildlife Management* **70**, 1690-1697.

Cripps, J. K., Pacioni, C., Scroggie, M. P., Woolnough, A. P., and Ramsey, D. S. L. (2018). Introduced deer and their potential role in disease transmission to livestock in Australia. *Mammal Review* **49**, 60-77.

D'Amico, M., Périquet, S., Román, J., and Revilla, E. (2016). Road avoidance responses determine the impact of heterogeneous road networks at a regional scale. *Journal of Applied Ecology* **53**, 181-190.

Davis, N. E., Bennett, A., Forsyth, D. M., Bowman, D. M. J. S., Lefroy, E. C., Wood, S. W., Woolnough, A. P., West, P., Hampton, J. O., and Johnson, C. N. (2016). A systematic review of the impacts and management of introduced deer (family Cervidae) in Australia. *Wildlife Research* **43**, 515-532.

De Bondi, N., White, J., Stevens, M., and Cooke, R. (2010). A comparison of the effectiveness of camera trapping and live trapping for sampling terrestrial small-mammal communities. *Wildlife Research* **37**, 456-465.

DEDJTR (2018). Draft Deer Management Strategy. (Ed. DELWP): Melbourne, Victoria.)

Edwards, G., Digby, D., O'Leary, P., Rafferty, D., Jensen, M., Woolnough, A., Secomb, N., Williams, M., Schwartzkopff, K., and Bryan, R. (2016). Planning and conducting aerial culling operations for feral camels. *The Rangeland Journal* **38**, 153-162.

Eyles, D. (2002) Sambar deer (*Cervus unicolor*) as a potential seed vector for the spread of the environmental weed Himalayan Honeysuckle (*Leycesteria formosa*) at Mount Buffalo national Park. B.Sc (Hons) Thesis, The University of Melbourne, Parkville.

Ferreguetti, Á. C., Tomás, W. M., and Bergallo, H. G. (2015). Density, occupancy, and activity pattern of two sympatric deer (*Mazama*) in the Atlantic Forest, Brazil. *Journal of Mammalogy* **96**, 1245-1254.

Finder, R., Roseberry, J., and Woolf, A. (1999). Site and landscape conditions at white-tailed deer/vehicle collision locations in Illinois. *Landscape and urban planning* **44**, 77-85.

Fiske, I. J. and Chandler, R. B. (2011). Unmarked: An R package for fitting hierarchical models of wildlife occurrence and abundance. *Journal of Statistical Software* **43**, 1-23.

Forsyth, D. M. and Davis, N. E. (2011). Diets of non-native deer in Australia estimated by macroscopic versus microhistological rumen analysis. *The Journal of Wildlife Management* **75**, 1488-1497.

Forsyth, D. M., McLeod, S. R., Scroggie, M. P., and White, M. D. (2009). Modelling the abundance of wildlife using field surveys and GIS: non-native sambar deer (*Cervus unicolor*) in the Yarra Ranges, south-eastern Australia. *Wildlife Research* **36**, 231-241.

Forsyth, D. M., Stamation, K., and Woodford, L. (2015). Distributions of sambar deer, rusa deer and sika deer in Victoria. Arthur Rylah Institute, Melbourne, Victoria.

Gelman, A. and Hill, J. (2006). 'Data analysis using regression and mulitlevel/heirarchical models.' (Cambridge University Press).

Gormley, A. M., Forsyth, D. M., Griffioen, P., Lindeman, M., Ramsey, D. S. L., Scroggie, M. P., and Woodford, L. (2011). Using presence-only and presence–absence data to estimate the current and potential distributions of established invasive species. *Journal of Applied Ecology* **48**, 25-34.

Guillera-Arroita, G. and Lahoz-Monfort, J. J. (2012). Designing studies to detect differences in species occupancy: power analysis under imperfect detection. *Methods in Ecology and Evolution* **3**, 860-869.

Guillera-Arroita, G., Lahoz-Monfort, J., MacKenzie, D., Wintle, B., McCarthy, M., and White, E. (2014). Ignoring imperfect detection in biological surveys is dangerous: a response to 'fitting and interpreting occupancy models'. *PloS One* **9**.

Gürtler, R., Rodríguez Planes, L., Gil, G., Izquierdo, V. M., Cavicchia, M., and Maranta, A. (2018). Differential long-term impacts of a management control program of axis deer and wild boar in a protected area of north-eastern Argentina. *Biological Invasions* **20**, 1431-1447.

Hampton, J. O., Cowled, B. D., Perry, A. L., Miller, C. J., Jones, B., and Hart, Q. (2014). Quantitative analysis of animal-welfare outcomes in helicopter shooting: a case study with feral dromedary camels (*Camelus dromedarius*). *Wildlife Research* **41**, 127-135.

Hampton, J. O., Edwards, G. P., Cowled, B. D., Forsyth, D. M., Hyndman, T. H., Perry, A. L., Miller, C. J., Adams, P. J., and Collins, T. (2017). Assessment of animal welfare for helicopter shooting of feral horses. *Wildlife Research* **44**, 97-105.

Husheer, S. W., Coomes, D. A., and Robertson, A. W. (2003). Long-term influences of introduced deer on the composition and structure of New Zealand Nothofagus forests. *Forest Ecology and Management* **181**, 99-117.

Igota, H., Sakuragi, M., Uno, H., Kaji, K., Kaneko, M., Akamatsu, R., and Maekawa, K. (2004). Seasonal migration patterns of female sika deer in eastern Hokkaido, Japan. *Ecological Research* **19**, 169-178.

Jacques, C. N., Jenks, J. A., and Klaver, R. W. (2009). Seasonal movements and home-range use by female pronghorns in sagebrush-steppe communities of western South Dakota. *Journal of Mammalogy* **90**, 433-441.

Jean-Marc Hero, G. G., Peter Robertson, Murray Littlejohn, Frank Lemckert (2004). *Philoria frosti*. The IUCN Red List of Threatened Species 2004. Available at: https://www.iucnredlist.org/species/16997/6695616 [Accessed 30 October 2018].

Joys, A. C., Fuller, R. J., and Dolman, P. M. (2004). Influences of deer browsing, coppice history, and standard trees on the growth and development of vegetation structure in coppiced woods in lowland England. *Forest Ecology and Management* **202**, 23-37.

Kamler, J., Jędrzejewska, B., and Jędrzejewski, W. (2007). Activity patterns of red deer in białowieża National park, Poland. *Journal of Mammalogy* **88**, 508-514.

Karanth, K. (2016). Wildlife in the matrix: Spatio-temporal patterns of herbivore occurrence in Karnataka, India. *Environmental Management* **57**, 189-206.

Killeen, J., Thurfjell, H., Ciuti, S., Paton, D., Musiani, M., and Boyce, M. (2014). Habitat selection during ungulate dispersal and exploratory movement at broad and fine scale with implications for conservation management. *Movement Ecology* **2**.

Leslie, D. M. (2011). *Rusa unicolor* (Artiodactyla: Cervidae). *Mammalian Species*, 1-30.

Lone, K., Loe, L. E., Meisingset, E. L., Stamnes, I., and Mysterud, A. (2015). An adaptive behavioural response to hunting: surviving male red deer shift habitat at the onset of the hunting season. *Animal Behaviour* **102**, 127-138.

MacKenzie, D., Nichols, J., Lachman, G., Droege, S., Andrew Royle, J., and Langtimm, C. (2002). Estimating site occupancy rates when detection probabilities are less than one. *Ecology* **83**, 2248-2255.

Masters, P., Markopoulos, N., Florance, B., and Southgate, R. (2018). The eradication of fallow deer (*Dama dama*) and feral goats (*Capra hircus*) from Kangaroo Island, South Australia. *Australasian Journal of Environmental Management* **25**, 86-98.

McCarthy, M., Moore, J., Morris, W., Parris, K., Garrard, G., Vesk, P., Rumpff, L., Giljohann, K., Camac, J., Bau, S. S., Friend, T., Harrison, B., and Yue, B. (2013). The influence of abundance on detectability. *Oikos* **122**, 717-726.

Meek, P. D., Ballard, G., Claridge, A., Kays, R., Moseby, K., O'Brien, T., O'Connell, A., Sanderson, J., Swann, D. E., Tobler, M., and Townsend, S. (2014). Recommended guiding principles for reporting on camera trapping research. *Biodiversity and Conservation* **23**, 2321-2343.

Meisingset, E. L., Loe, L. E., Brekkum, Ø., Van Moorter, B., and Mysterud, A. (2013). Red deer habitat selection and movements in relation to roads. *The Journal of Wildlife Management* **77**, 181-191.

Moloney, P. D. and Powell, Z. (2019). Estimates of the 2018 deer harvest in Victoria: results from surveys of Victorian Game Licence holders in 2018. Unpublished client report for the Game Management Authority, Arthur Rylah Institute for Environmental Research, Department of Environment, Land, Water and Planning, Heidelberg, Victoria.

Moloney, P. D. and Turnbull, J. D. (2017). Estimates of harvest for deer in Victoria: results from surveys of Victorian Game licence holders in 2016. Unpublished client report for the Game Management Authority, Arthur Rylah Institute for Environmental Research, Department of Environment, Land, Water and Planning, Heidelberg, Victoria.

Moore, I. A. (1994) Habitat use and activity patterns of sambar (*Cervus unicolor*) in the Bunyip sambar enclosure. Masters Thesis, The University of Melbourne, Parkville.

Moriarty, A. (2004). The liberation, distribution, abundance and management of wild deer in Australia. *Wildlife Research* **31**, 291-299.

Ng, J., Yang, R., Whiffin, V., Cox, P., and Ryan, U. (2011). Identification of zoonotic *Cryptosporidium* and *Giardia* genotypes infecting animals in Sydney's water catchments. *Experimental Parasitology* **128**, 138-144.

Niedballa, J., Sollmann, R., Courtiol, A., and Wilting, A. (2016). camtrapR: an R package for efficient camera trap data management. *Methods in Ecology and Evolution* **7**, 1457-1462.

Nogales, M., Vidal, E., Medina, F. M., Bonnaud, E., Tershy, B. R., Campbell, K. J., and Zavaleta, E. S. (2013). Feral cats and biodiversity conservation: the urgent prioritization of island management. *BioScience* **63**, 804-810.

Nugent, G., Parkes, J., and G. Tustin, K. (1987). Changes in the density and distribution of red deer and wapiti in northern Fiordland. *New Zealand Journal of Ecology* **10**, 11-21.

O'Connor, K. M., Oconnor, K., Nathan, L., Liberati, M., Tingley, M., Vokoun, J., Rittenhouse, T. A. G., and Bonter, D. N. (2017). Camera trap arrays improve detection probability of wildlife: Investigating study design considerations using an empirical dataset. *PloS one* **12**.

Parks Victoria (2005). Baw Baw National Park management plan. Melbourne, Victoria.

Parks Victoria (2019). 'Deer control program - Parks Victoria trialling aerial deer shooting program.' (Parks Victoria, Melbourne, Victoria.) Available at: https://parkweb.vic.gov.au/explore/parks/mount-buffalo-national-park/plans-and-projects/deer-control-program [Accessed 30 July 2019].

Parliament of Victoria (2017). Inquiry into the control of invasive animals on Crown land. Victorian Government, Melbourne.

Patterson, B. R. and Power, V.A. (2002). Contributions of forage competition, harvest, and climate fluctuation to changes in population growth of northern white-tailed deer. *Oecologia* **130**, 62-71.

Pellerin, S., Huot, J., and Ct, S. (2006). Long-term effects of deer browsing and trampling on the vegetation of peatlands. *Biological Conservation* **128**, 316-326.

Phillipson, S., Spencer, K., and Hill, A. (2015). Monitoring changes in the levels of deer abundance and impacts in the Alpine National Park. ETHOS NRM Pty Ltd, Bairnsdale, Victoria.

Pople, T., Brennan, M., Amos, M., Kearns, B., McBride, K., and Blokland, A. (2017) Management of an expanding chital deer population in North Queensland. In 'Proceedings of the 17th Australasian Vertebrate Pest Conference.' (Ed. Buckmaster, T.) pp. 88-89. (Invasive Species Cooperative Research Centre: Canberra.)

Prokopenko, C. M., Boyce, M. S., and Avgar, T. (2017). Extent-dependent habitat selection in a migratory large herbivore: road avoidance across scales. *Landscape Ecology* **32**, 313-325.

R Core Development Team. (2004). R: A language and environment for statistical computing.

Raiho, A., Hooten, M., Bates, S., Hobbs, N. T., and Umapathy, G. (2015). Forecasting the effects of fertility control on overabundant ungulates: white-tailed deer in the National capital region. *PloS One* **10**.

Romin, L. A. (1996). Deer-vehicle collisions: Status of state monitoring activities and mitigation efforts. *Wildlife Society Bulletin* **24**, 276-283.

Ross, J., Hearn, A. J., Johnson, P. J., and Macdonald, D. W. (2013). Activity patterns and temporal avoidance by prey in response to Sunda clouded leopard predation risk. *Journal of Zoology* **290**, 96-106.

Rutberg, A. and Naugle, R. E. (2008). Population-level effects of immunocontraception in white-tailed deer (*Odocoileus virginianus*). *Wildlife Research* **35**, 494-501.

Ryan, U. and Power, M. (2012). Cryptosporidium species in Australian wildlife and domestic animals. *Parasitology* **139**, 1673-1688.

Semiadil, G., Barry, T. N., Muir, P. D., and Hodgson, J. (2009). Dietary preferences of sambar (*Cervus unicolor*) and red deer (*Cervus elaphus*) offered browse, forage legume and grass species. *The Journal of Agricultural Science* **125**, 99-107.

Shannon, G., Lewis, J. S., and Gerber, B. D. (2014). Recommended survey designs for occupancy modelling using motion-activated cameras: Insights from empirical wildlife data. *PeerJ* .

Steiner, W., Leisch, F., and Hackländer, K. (2014). A review on the temporal pattern of deer-vehicle accidents: Impact of seasonal, diurnal and lunar effects in cervids. *Accident Analysis and Prevention* **66**, 168-181.

Tan, W. S. (2018). Observations of occurrence and daily activity patterns of ungulates in the Endau Rompin Landscape, Peninsular Malaysia. *Journal of Threatened Taxa* **10**, 11245-11253.

Tobler, M. W., Carrillo-Percastegui, S. E., Leite Pitman, R., Mares, R., and Powell, G. (2008). An evaluation of camera traps for inventorying large- and medium-sized terrestrial rainforest mammals. Animal Conservation **11**, 169-178.

Visintin, C., van der Ree, R., and McCarthy, M. (2016). A simple framework for a complex problem? Predicting wildlife-vehicle collisions. *Ecology & Evolution* **6**, 6409-6421.

White, K., Pendleton, G., and Hood, E. (2009). Effects of snow on sitka black-tailed deer browse availability and nutritional carrying capacity in southeastern Alaska. *Journal of Wildlife Management* **73**, 481-487.

Woinarski, J. B., A.A (2016). *Gymnobelideus leadbeateri*. The IUCN Red List of Threatened Species 2016. Available at: https://www.iucnredlist.org/species/9564/21959976 [Accessed 30 November 2018].

Yamada, K., Elith, J., McCarthy, M., and Zerger, A. (2003). Eliciting and integrating expert knowledge for wildlife habitat modelling. *Ecological Modelling* **165**, 251-264.

Yen, S. C., Yen, S.-C., Wang, Y., Yu, P.-H., Kuan, Y.-P., Liao, Y.-C., Chen, K.-H., and Weng, G.-J. (2019). Seasonal space use and habitat selection of sambar in Taiwan. *Journal of Wildlife Management* **83**, 22-31.

Zeng, Z.G., Beck, P. S. A., Wang, T.J., Skidmore, A. K., Song, Y.L., Gong, H.S., and Prins, H. H. T. (2010). Effects of plant phenology and solar radiation on seasonal movement of golden takin in the Qinling Mountains, China. *Journal of Mammalogy* **91**, 92-100.

Foreword to chapter 5

Deer-vehicle collisions (DVCs) represent the most serious economic impact of deer in the United States (US) and Europe. Each year, in the US and Europe, DVCs result in substantial damage to vehicles and road networks. Collisions involving deer also result in human injury and death. In Australia, DVCs are becoming increasingly common, especially in the south eastern States which are seeing rapid increases in sambar and fallow deer populations. With deer populations forecast to continue their expansion in Victoria it is critical to identify high risk areas so mitigation strategies can be implemented.

Previous studies in south east Australia have investigated the spatial and temporal trends in wildlife vehicle collisions involving native species, however no previous studies have investigated collisions involving introduced deer.

This chapter explores an emerging area of concern relating to invasive deer populations in Victoria; the risk wild deer pose to motorists. Chapter five provides important information for Victoria’s road network managers and outlines the location of three areas with increased risk of DVCs. The information will guide the implementation of management strategies to mitigate collisions risk including deer fencing, speed limit reduction or the application of control.

Chapter 5 – Predicting deer-vehicle collision risk across Victoria, Australia

Sambar deer killed by vehicle collision, Templestowe, Victoria

April 19, 2018

Photo credit: Chris Watson

Davies, C., Wright, W., Hogan, F., and Visintin, C. (2019) Predicting deer-vehicle collision risk across Victoria, Australia. *Australian Mammalogy*, doi: https://doi.org/10.1071/AM19042

Chapter 5 – Predicting deer-vehicle collision risk across Victoria, Australia

5.1 Abstract

The risk of deer vehicle collisions (DVC) is increasing in south east Australia as populations of introduced deer expand rapidly. There are no investigations of the spatial and temporal patterns of DVC or predictions of where such collisions are most likely to occur. Here, we use an analytical framework to model deer distribution and vehicle movements in order to predict DVC risk across the State of Victoria. We modelled the occurrence of deer using existing occurrence records and geographic climatic variables. We estimated patterns of vehicular movements from records of average annual daily traffic and speeds. Given the low number of DVCs reported in Victoria, we used a generalised linear regression model fitted to DVCs in California, USA. The fitted model coefficients suggested high collision risk on road segments with high predicted deer occurrence, moderate traffic volume and high traffic speed. We used the California deer model to predict collision risk on Victorian roads and validated the predictions with two independent datasets of DVC records from Victoria. The California deer model performed well when comparing predictions of collision risk to the independent DVC datasets and generated plausible DVC risk predictions across the State of Victoria.

5.2 Introduction

Globally, wildlife-vehicle collisions (WVC) cause significant social, economic and ecological impacts (van der Ree *et al.* 2011). Each year, WVC are responsible for a number of human deaths and serious injuries as well as millions of dollars' worth of damage to vehicles and road infrastructure (Huijser *et al.* 2007). Additionally, billions of vertebrate animals are thought to be killed each year on global transportation networks, however accurate estimates of wildlife deaths are difficult to ascertain (Seiler and Helldin 2006). This can cause substantial negative ecological impacts, particularly where native or rare and endangered species are affected (Clements *et al.* 2014). In Australia, WVC are common and involve a range of native and introduced animals.

Each year thousands of vehicle collisions involving wildlife are reported in Australia (Rowden *et al.* 2008). The five native mammal species most commonly killed on Australian road networks are: eastern grey kangaroo (*Macropus giganteus*), common wombat (*Vombastus ursinus*), black wallaby (*Wallabia bicolor*), koala (*Phascolarctos cinereus*) and brushtail possum (*Trichosurus vulpecula*) (Dique *et al.* 2003; Visintin *et al.* 2017). Collisions can significantly impact native animal populations and may also result in costly insurance claims, medical expenses and in some cases the loss of human life (Huijser *et al.* 2007). A recent study in Victoria demonstrated that in the last decade 152 major traumatic injuries resulted from vehicle collisions with animals; and collision rates are likely to increase in coming years (Ang *et al.* 2019).

In the United States and Europe, deer are involved in the majority of WVC and present the greatest risk to motorists due to their large body size and crepuscular activity patterns (Conover 1995; Hothorn *et al.* 2012; Huijser *et al.* 2007). The latter often coincide with periods of commuter traffic, especially during winter (Kämmerle *et al.* 2017). Many studies clearly relate areas of high deer density with the greatest risks of collision (Joyce and Mahoney 2001; Langbein *et al.* 2011). Where deer are commonly involved in collisions a number of mitigation strategies can be put in place. Commonly applied strategies to mitigate the risk of deer-vehicle collisions (DVC) include the construction of exclusion fencing (Bissonette and Rosa 2012), reduction of speed limits (Romin 1996) and the erection of warning signs (Huijser *et al.* 2007).

Populations of the four wild deer species present in Victoria have undergone rapid increases in the last decade (Moloney and Turnbull 2017) and recognition that they present a substantial risk to motorists, especially in peri-urban areas, is growing (Parliament of Victoria 2017;

DEDJTR 2018). Individuals of Victoria's largest and most abundant deer species, sambar deer (*Rusa unicolor*), can weigh up to 300 kilograms. Such a large-bodied animal has the potential to cause catastrophic damage during a high speed collision. While deer-vehicle collisions have been reported in Australia (Ramp *et al.* 2006), few studies have investigated the spatial and temporal patterns of DVC or attempted to identify deer vehicle collision risk across the Victorian road network. The Royal Automobile Club of Victoria (RACV) identifies deer as the fourth most common animal involved in insurance claims - after kangaroos, wombats and dogs - and reports 89 insurance claims for DVC in Victoria during 2014-15 and 76 in 2015-16 (Keogh 2016).

The current draft deer management strategy for Victoria calls for an improvement of deer management in peri urban areas, including identifying hot spots and trialling mitigation strategies for DVC (DEDJTR 2018). However, the literature regarding DVC in Victoria is severely under-represented, thus constraining management decisions. Modelling DVC in Australia is important to clearly identify high risk areas and to help optimise mitigation strategies. Once areas of high risk have been identified, mitigation strategies to reduce the risk of DVC can be assessed and implemented. High risk areas can be targeted for deer eradication programs, the construction of fencing, and signage or speed limit reductions.

Statistical modelling has been used to effectively quantify the risk of wildlife vehicle collisions in Australia and elsewhere (Visintin *et al.* 2016; Yang *et al*. 2019). Models incorporating Poisson (Ye *et al*. 2018), negative binomial (Zou *et al*. 2015), Poisson-lognormal (Murphy and Xia 2016) and Gamma regression (Oh *et al*. 2006) have been used by researchers to investigate spatial and temporal trends in vehicle collision data. Australian studies involving native species including wombats, kangaroos and wallabies have been undertaken (Ramp *et al.* 2005) but there is little research about the risks associated with collisions involving wild deer. Despite increases in deer abundance and distribution no previous studies have predicted spatial and temporal patterns of DVC in Victoria.

The source of data for wildlife collision studies can influence the accuracy of results and identification of collision hotspots (Yang *et al.* 2019). A number of studies have used carcass records of individual species as data for collision modelling (Knapp *et al*. 2007; Stevens and Dennis 2013; Santos *et al*. 2018). Carcass records can be underreported as carcasses may be difficult to detect and may not be reported to management agencies (Santos *et al.* 2016). Collision data can also be collected directly from motorists during insurance claims, accident investigations and self-reporting databases (Visintin *et al*. 2017). Collision data directly from motorists is also commonly underreported as collisions are only reported if damage is over a certain threshold (Yang *et al*. 2019).

This study employs an existing conceptual risk model to predict where in Victoria DVC are likely to take place. Predictions are tested by comparing predicted DVC risk with patterns of actual DVC events. Determining priority areas where accidents are most likely to occur will help support decision making to reduce the risks and identify where control mechanisms can be implemented.

5.3 Materials and methods

5.3.1 Study area

The entire State of Victoria (227,819 square kilometres - Australian Bureau of Statistics, 2018), located in south east Australia was used as our study area (Fig. 5.1). Our study analysed deer collision risk across approximately 147,970 km of sealed roads. The roads were divided into segments of 500 m and less, which were used as the modelling units for collision risk. We overlaid a spatial grid of 500 m x 500 m (25 ha) cells on the study area and used each grid cell as a modelling unit for deer occurrence.

5.3.2 Study species

Four species of introduced deer have established wild populations in Victoria, these include; sambar deer (*Rusa unicolor*), fallow deer (*Dama dama*), red deer (*Cervus elaphus*) and hog deer (*Axis porcinus*). Wild deer are distributed widely across Victoria and have varied habitat preferences. Harvest rates from recreational hunters suggest that deer numbers are increasing (Moloney and Turnbull 2017), leading to concern regarding ecological impacts and risks posed to motorists. We obtained occurrence records for all deer species from the Victorian Biodiversity Atlas (DELWP, 2018), and collision records from Vicroads and Wildlife Victoria. Deer collision records were not species specific and, therefore, represented collisions involving either sambar, fallow, red or hog deer.

5.3.3 Model framework

We used a quantitative risk model framework (Visintin *et al.* 2016) to predict DVC risk on the road network of Victoria based on traffic volume, traffic speed and modelled occurrence of deer. The model framework predicts collision risk by modelling hazard (presence and movement of vehicles) and exposure (the occurrence of animals) across geographical space. We developed a species distribution model (SDM) (pooling presence records for all Victorian deer species: fallow, red, hog and sambar) to predict the occurrence of deer across the study area. Traffic volume and traffic speed for all road segments were predicted from a model that regressed annual average daily traffic (AADT) counts and speed limit data on anthropogenic variables - detailed methods are provided in Visintin *et al*. 2016. All statistical analyses were conducted using the open source software package ‘R’ version 3.4.1 (R core team, 2016).

5.3.4 Deer occurrence modelling

We obtained deer observation records made between the years of 2000 and 2018, inclusive, from the Victorian Biodiversity Atlas (VBA). Only occurrences with spatial uncertainty equal to or below 500 metres were considered. Presence of deer across the study area was determined by selecting all grid cells that contained at least one occurrence record; grid cells with multiple occurrences were treated as single records. As deer absence data were not available, we randomly selected 10,000 grid cells as background data. Our choice of 10,000 grid cells is based on a common practice in SDM work, however, we acknowledge that in some cases more background cells may improve model performance (Warton and Shepherd, 2010). In our case, this choice balanced adequately capturing environmental variation – given the scale of our landscape - with computational efficiency.

We selected environmental variables which the published literature suggested were influential on the ecology of deer species (Table 5.1). Each predictor variable was represented as a 500 m x 500 m (25ha) raster grid to match the grid cells used to model deer occurrence. Because environmental variables exhibit spatial gradients, and species surveying is subject to spatial biases, we included grid cell coordinates – X (Easting) and Y (Northing) - as predictor variables in the species occurrence model to reduce the effects of spatial autocorrelation and survey bias. All variables used in the species occurrence model exhibited variable inflation factors (VIF) between one and six, indicating low effects of multicollinearity (James *et al.* 2013). However, our chosen modelling method is less influenced by these effects than generalised linear regression models (Dormann *et al.* 2013). We fitted Boosted Regression Tree (BRT) models to deer presence/background data. This is a machine learning method which uses classification to produce a null model of binary splits based on the predictor variables and then iteratively regresses the residuals of each fit on the predictor variables using internal cross-validation to prevent over-fitting (Elith *et al.* 2008). A tree complexity of five, a learning rate of 0.005 and bag fraction of 0.5 were used in our BRT model.

Table 5.1: Predictor variables used in deer occurrence model, including descriptions, and justification for the inclusion in the model, for each variable. The spatial variables X and Y were set to zero when predicting from the species occurrence model.

Variable	Description and justification for inclusion	Units	Mean; Range
ELEV	Elevation of terrain in meters above sea level. Elevation has been shown to be influential for the occurrence of many deer species, including sambar (Forsyth *et al*. 2009) and red deer (Debeljak *et al*. 2001).	m	248.16; 0.00-949.63
GREEN	Remote-sensed mean seasonal change in greenness (2003-2013) in vegetation. This variable has been shown to be influential for predicting the occurrence of mule deer in California (Visintin 2017).	-	0.22; 0.62-0.63
LIGHT	Remote-sensed relative artificial light intensity. Artificial light has been negatively correlated with the probability of occurrence of roe deer (*Capreolus capreolus*) in urban landscapes (Ciach and Fröhlich 2019).	-	0.19; 0.00-54.69
SLOPE	Slope of terrain in decimal percent rise. Slope was found to be the most influential non-spatial predictor for mule deer occurrence in California (Visintin 2017).	%	2.13; 0.00-28.48
TREE DENSITY	Tree canopy coverage in decimal percentage. Deer in Victoria, especially sambar deer are thought to display preferences for areas with high levels of vegetation coverage as protection from predators, hunters and adverse weather conditions (Moore 1994).	decimal %	23.81; 0.00-81.01
X	X spatial coordinate of intersecting 500 m^2 grid centroid, in metres.	m	-
Y	Y spatial coordinate of intersecting 500 m^2 grid centroid, in metres.	m	-

5.3.5 Collision model and validation

GPS coordinates of spatially unique deer collision/carcass records (*n*=9) involving all deer species were provided by the Victorian State Government's road management agency, VicRoads. All road segments that intersected with a reported collision/carcass were coded "1" and remaining road segments coded "0", to represent background data. For each road segment, we sampled species occurrence predictions based on the values in grid cells that intersected with the road segment. For cases where road segments were contained entirely within grid cells, the segments were assigned the cell values of deer occurrence. Otherwise, road segments spanning multiple grid cells were allocated weighted averages of deer occurrence based on the proportions of segment length intersecting each grid cell. Traffic volume and speed data for each road segment were provided from previous modelling (Visintin *et al.* 2016).

Due to the low number of deer collision/carcass records we were unable to properly train a collision model for Victoria. Instead, we predicted collision risk for all road segments within our study area using our predictor variables (deer occurrence, traffic volume and traffic speed) and the coefficients of an existing model developed by Visintin *et al.* (unpublished data) for mule deer (*Odocoileus hemionus*) in a 146,478 square kilometre section of central California. The latter model regressed reported collisions on variables of deer occurrence, traffic volume and traffic speed using a complementary log-log (cloglog) link. An offset term was included to account for variation in road lengths. The model, with fitted coefficients, is expressed as:

$$\mathrm{cloglog}(p_i) = -55.43 + 0.83 * \log(O_i) + 4.68 * \log(V_i) - 0.26 * (\log(V_i))^2 + 7.52 * \log(S_i) + \log(\mathrm{L_i})$$

where ($p_i = \Pr(Y_i=1)$) is the relative likelihood of a collision occurring on a road segment i, O_i is species occurrence, V_i traffic volume, S_i traffic speed and $\mathrm{L_i}$ is an offset for road segment length.

We validated the collision model predictions using the dataset of deer collision/carcass records obtained from VicRoads. We tested the calibration strength and discrimination ability of the model predictions with the collision/carcass data. To assess calibration strength, we regressed the collision/carcass observations on predictions made using the collision/carcass observations in the trained California model expressed as:

$$\mathrm{cloglog}(\mathrm{p_i}) = \beta_0 + \beta_1 \mathrm{P_i}$$

where ($p_i = \Pr(Y_i{=}1)$) is the relative likelihood of a collision occurring on a road segment *i* and P_i is the predicted relative collision rate for each segment on the link scale (complementary log-log). An intercept coefficient of 'zero' and slope coefficient of 'one' indicates a perfectly calibrated model (Miller *et al*. 1991). To measure the ability of the model to discriminate between true positive and false positive predictions we used a receiver operating characteristic (ROC) score - a score of one indicating perfect discrimination ability while 0.5 suggesting a performance no better than random (Metz, 1978).

Given the small sample size of the data from VicRoads, we performed sensitivity analysis on the validation outputs to determine whether our results were from a collision risk signal produced by the model or by random chance. For each of 1000 iterations, we randomly shuffled the locations of the nine recorded collisions on the road network, made new predictions, and then compared the observations with the predictions to calculate a slope coefficient and ROC score.

We also validated collision model predictions using a second dataset obtained from the Wildlife Victoria database, comprising reported deer collisions between the years of 2010 and 2018, inclusive (n=254). Because the spatial accuracy of these records was resolved only to town level, we could not use the same validation methods as for the VicRoads collision/carcass data. We therefore summed collision records by town and calculated the expected number of collisions within each town boundary based on the predicted values of collision risk for all road segments within the boundary. To assess calibration strength, we regressed the total reported collisions on the expected total collisions based on the predictions made from the trained California model. Due to the response data being positive integers, we used the Poisson link:

$$\log(C_j) = \beta_0 + \beta_1 \log(E_j)$$

where C_j is the count of reported collisions, E_j is the expected count of collisions, in a town j. Once again, an intercept coefficient of 'zero' and slope coefficient of 'one' indicates a perfectly calibrated model.

5.4 Results

5.4.1 Deer occurrence modelling

The deer occurrence model produced plausible predictions of deer occurrence across Victoria (Fig. 5.1). The deviance reduced by the model was 35.1% and the mean cross-validated ROC score was 0.93.

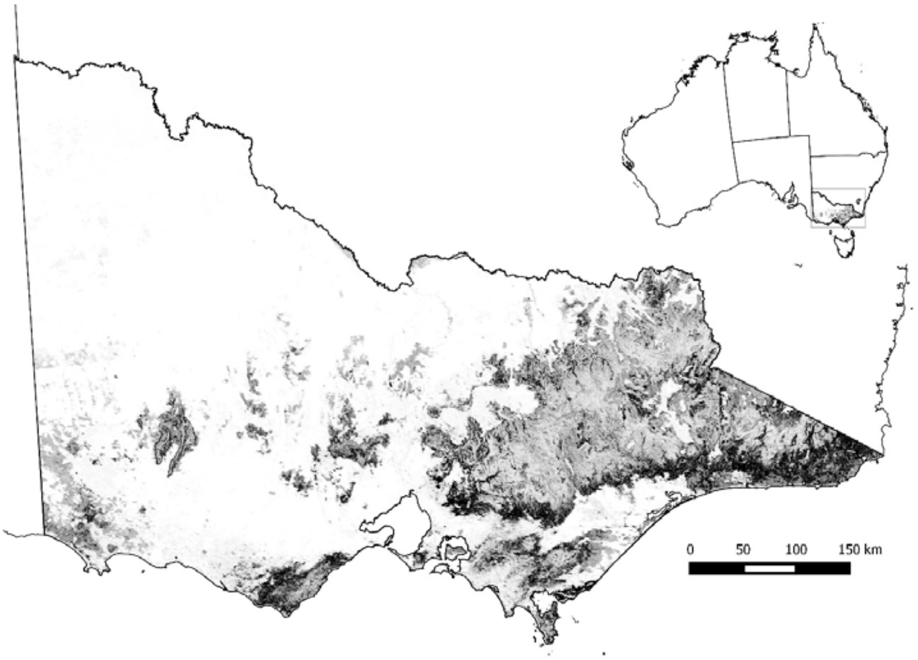

Fig. 5.1: Predicted relative likelihood of deer occurrence (all species) in Victoria. Darker shading indicates higher relative likelihood of deer occurrence. The location of the study area, the State of Victoria, in south east Australia is shown top right.

5.4.2 Collision model performance

The collision model performed well when validated with independent data. Using VicRoads data, the model was able to discriminate between true positives and false positives (ROC value of 0.91). Regressing the VicRoads collision observations on the predictions made by the collision model resulted in a slope coefficient of 0.63 whilst regressing the Wildlife Victoria observations on the predictions resulted in a slope coefficient of 0.83. All regression coefficients were highly significant (p<0.001).

Although the VicRoads data contained few observations, they were useful for validation. Both the slope coefficient (0.63) and ROC value (0.91) were outside the ranges of values obtained through simulating variations in the collision observations: slope coefficient (-0.68–0.49), ROC (0.50–0.85).

5.4.3 Effect of predictor variables

Deer collision risk increased with the likelihood of deer occurrence (Fig. 5.2a) and with higher traffic speeds (Fig. 5.2b). We observed a quadratic shape in the response to traffic volume (Fig. 5.2c) indicating a possible threshold effect, where deer may avoid areas of high traffic volume due to the disturbance of traffic noise (Forman and Alexander 1998).

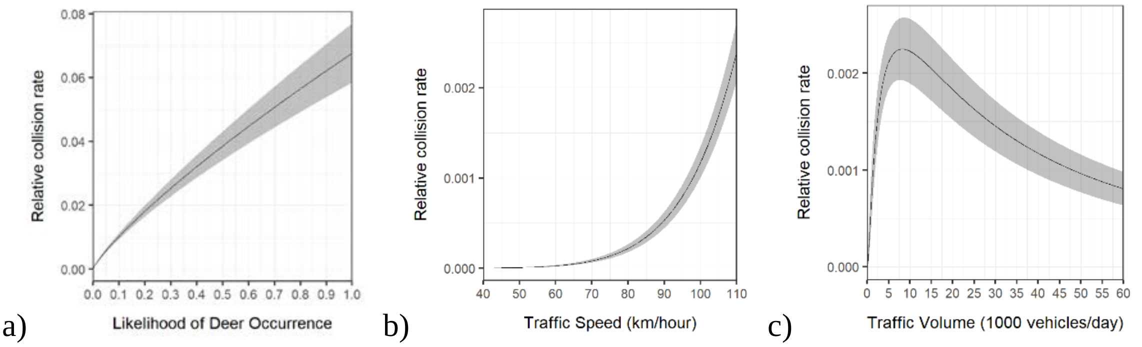

Fig. 5.2: Effects of predictor variables on relative likelihood of collision. Note shapes are based on the fitted model coefficients for California mule deer A) Relative likelihood of deer occurrence, B) Traffic speed and C) Traffic volume. Shaded regions indicate error bounds (95% confidence) on coefficient estimates.

5.4.4 Model predictions

The map of predicted deer collision risk identifies road segments where deer collisions are most likely to occur in Victoria. Visual inspection of predicted collision risk across the Victorian road network indicated three regions with increased deer vehicle collision risk (Fig. 5.3). Roads with high speed limits and high traffic volumes which border forested areas appear to exhibit the highest overall collision risk. In particular, three main areas of Victoria exhibited increased deer collision risk. The first location was an area of the Western Freeway near Gordon (Fig. 5.3A). This appears to be one of the few areas of the Western Highway between Ballarat and Melbourne that has remnant vegetation in close proximity to the highway. The second (Fig. 5.3B) occurs to the east of Melbourne, including Wellington Rd near Lysterfield Park and roads surrounding Emerald and Gembrook, including the Gembrook - Launching Place road. The third location of predicted increased DVC risk is an area incorporating many of the roads in West Gippsland (Fig. 5.3C), including segments of the Strzelecki Highway between Mirboo North and Driffield and roads north of Moe including the Moe – Rawson road and Tyers – Walhalla road.

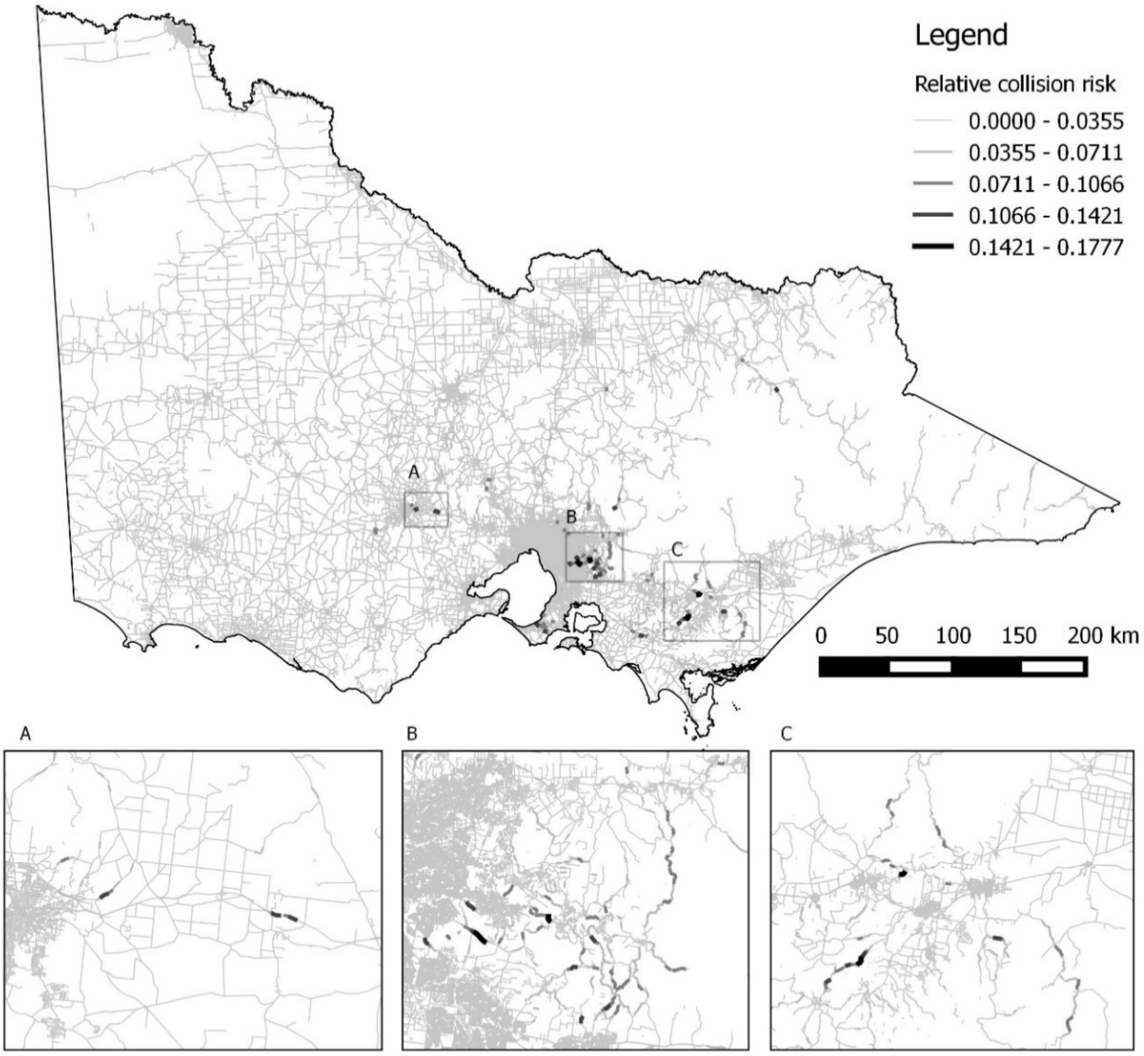

Fig. 5.3: Map of deer collision risk per road segment. Darker line segments indicate higher relative risk of deer-vehicle collisions. Visual inspection of predicted collision risk across the Victorian road network indicated three regions with increased deer vehicle collision risk; Gordon, Lysterfield and West Gippsland (labelled on map by A, B and C).

5.5 Discussion

Our study successfully applied a collision risk framework in south eastern Australia and made plausible predictions of DVC risk across the entire Victorian road network. We highlight three regions of heightened DVC risk in Victoria. This is the first study to specifically model DVC risk in Victoria and was performed at a large spatial scale. Other studies have used similar modelling approaches to assess wildlife collision risk in Australia but have focused on native species including kangaroos, common wombats, koalas and wallabies (Dique *et al.* 2003; Klocker *et al.* 2006; Ramp and Ben-Ami 2006).

Monitoring changes in the distribution of wild deer in Victoria was established as a research priority by the comprehensive review of deer impacts and management conducted by Davis *et* *al*. (2016). While the focus of this paper is to help establish DVC risk in Victoria, it also provides useful information on the current modelled distribution of deer in Victoria. Our results show that wild deer are likely to occur within most forested areas across the State and confirm anecdotal reports of deer in the Great Otway and Grampians National Parks (Fig. 5.1). This is concerning as without proper management deer can cause significant ecological damage to these important and biodiverse protected areas. As deer numbers increase across Victoria so will the risk they pose to motorists.

Our results suggest that deer vehicle collision risk is greatest in three main regions in Victoria (Fig. 5.3), the first, on the Western Freeway near Ballarat, the second to the east of Melbourne near Lysterfield and the third in West Gippsland. All these regions are known to be occupied by wild deer, particularly sambar and fallow deer (Forsyth *et al*. 2015, Forsyth *et al*. 2016). Despite many anecdotal reports of deer collisions between Orbost and the NSW border on the Princes Freeway in Eastern Victoria (C. Davies, personal observation), road sectors in this area did not show high predicted risk of DVC. This section of the Princes Freeway has a speed limit of 110 km per hour and is located in known deer habitat (Gormley *et al.* 2011). The low risk of DVC predicted in Eastern Victoria may have been influenced by relatively low daily traffic volumes. The three main areas that exhibited higher deer vehicle collision risk could be considered as collision hotspots, a term commonly used in road ecology. However, collision hotspots are commonly identified using Kernel Density Estimation (Ramp *et al*. 2005; Snow *et al*. 2014), a method that was not applied during this study.

This study was constrained by a lack of accurate deer collision data from Victoria to train models, we therefore used a model trained for mule deer in California as a surrogate to make predictions. Our results assume that the four deer species in Victoria share similar ecological and behavioural traits to mule deer. This assumption is supported by ecological data: Mule deer and the deer species present in Victoria are all medium to large, ungulate herbivores and are likely to share similar ecological requirements (Keegan *et al*. 2011, Leslie 2011; Forsyth *et al*. 2015). Deer often display overlap in dietary preferences in areas where multiple species coexist, as observed with white tailed deer and mule deer (Berry *et al*. 2019) and white tailed deer and sika deer (Kalb *et al*. 2018) in the US. It is likely that if mule deer were introduced to Victoria their dietary preferences would be similar to the deer species already established. Previous modelling has also shown similarities between the habitat preferences of mule deer and deer species present in Victoria. Russell *et al*. 2015 showed elevation, vegetation cover and distance to roads are important in determining habitat selection for mule deer during winter and summer in the USA. Forest cover was also found to be an important determinant of mule deer habitat use in Oregon, USA (Coe *et al*. 2018). Similarly, sambar deer display preferences for dense cover (Moore 1994) and have demonstrated seasonal altitudinal movements in their native range in Taiwan (Yen *et al*. 2019).

Our model also assumes that Victorian roads have similar characteristics to roads in California. With the exception of a few 12+ lane freeway segments in the central California study region, the characteristics of the road network were similar to Victoria. Both used classification systems that categorised roads into local, collector, sub-arterial, arterial, highway, and freeway/interstate types with similar implications for traffic planning. The traffic models - parameterised and fitted using the same covariates - were used to predict traffic volume and speed for Victoria and central California, respectively. In Victoria, predicted traffic volumes were between 195 and 130,000 vehicles per day whilst the central California traffic volumes were between 765 and 189,000 vehicles per day. Traffic speeds were predicted between 42 and 101 kilometres per hour in Victoria and between 36 and 112 kilometres per hour in central California. We visually inspected the distributions of traffic volume and speed between the two areas and discovered similar patterns, however, we acknowledge that the two road networks have notable differences. Despite these assumptions and limitations, the predictions made by the model may be useful to authorities with responsibility for road safety to target areas for further investigation and greater data collection.

There are potential reporting biases involving deer vehicle collisions because deer are an introduced species in Victoria and not a priority for wildlife rescues or reporting. As such, accurate records describing vehicle collisions involving deer were difficult to obtain. In accordance with recommendations from the Victorian Draft Deer Management Strategy (DEDJTR, 2018), we advocate that a database registry for vehicle collisions involving deer is initiated. This concept is considered a priority to improve the extent and accuracy of deer collision data, increase the statistical power of modelling studies such as this one, and provide better information to management authorities.

Many collision records did not identify deer to the species level so it was not possible to separately model the collision risks posed by each of the four species of deer found in Victoria. The database registry suggested above should make use of improved techniques for recording data relating to deer-vehicle collisions, including the time and date of the accident as well as information regarding the species of deer involved. Accurate and more extensive collision data for each individual deer species (sambar, red, fallow and hog) will allow the risk of collisions to be estimated for each species. This is important due to differences - such as the behaviour and size of the animal - which could influence the likelihood or severity of a collision.

Temporal variation in animal activity can be a useful predictor of collision risk. Deer often display crepuscular activity patterns and are therefore more likely to be involved in collisions with transport during the early morning and evening, as demonstrated by Steiner *et al.* (2014). The inclusion of temporal data would help identify high risk times of day. Additionally, deer may display migratory patterns such as moving from higher elevations to lower elevations during winter. Collisions risk may therefore be increased on roads at higher elevations during early winter. If deer collision risk is found to be higher during particular times of the day and the year, warning signs or a reduction in speed limits during high risk times could be used to help reduce deer collision risk.

A number of mitigation strategies could be applied high risk areas. Fencing to prevent deer from accessing road networks was proved effective for mule deer in the USA (Bissonette and Rosa 2012). The removal of roadside vegetation may also reduce collision risk in some areas, especially where such vegetation is known to attract ungulates (Rea 2003). Furthermore, signage could be improved in high risk areas to make motorists aware of deer collision risk, particularly during high risk times of day. However, there is little evidence that warning signs as a primary mitigation strategy actually reduce the rate of wildlife collisions (Bond and Jones 2013). Culling deer through ground and aerial shooting is the primary control method used to reduce the negative impacts associated with deer in Australia (Pople *et al*. 2017; DEDJTR 2018). As shown by DeNicola and Williams (2008), targeted culling of deer populations in high risk areas can reduce the risk of DVC. Currently in Victoria, deer culling programs aim to improve ecological outcomes rather than reduce the risk of DVC. As such, future culling operations could be coordinated to achieve dual outcomes; reduction of DVC risk as well as favourable ecological outcomes. However, performing deer control operations that incorporate shooting is unfeasible in peri-urban areas. As such, other mitigation strategies such as fencing along high risk roads should be prioritised in these areas.

In conclusion, we have used a modelling approach to identify road sectors within Victoria’s road network that are likely to be of increased risk for deer-vehicle collisions. As more deer collision data is collected, the modelling and analyses can be updated and improved.

Recommendations from this study include:

1) The development of a deer collision register to provide more detailed data for further investigation including subsequent modelling and analysis.
2) Future modelling to further delineate areas of Victoria’s road network with high risk of DVC.
3) Applying mitigation strategies to high risk areas.

5.6 References

Ang, J. Y., Gabbe, B., Cameron, P., and Beck, B. (2019). Animal–vehicle collisions in Victoria, Australia: An under-recognised cause of road traffic crashes. *Emergency Medicine Australasia* **31**, 851-855.

Australian Bureau of Statistics (2018). ‘Census data’. Available at http://abs.gov.au/ [accessed 15th July 2018].

Berry, S. L., Shipley, L. A., Long, R. A., and Loggers, C. (2019). Differences in dietary niche and foraging behavior of sympatric mule and white-tailed deer. *Ecosphere* **10**, e02815.

Bissonette, J. A. and Rosa, S. (2012). An evaluation of a mitigation strategy for deer-vehicle collisions. *Wildlife Biology* **18**, 414-423.

Bond, A. R. F. and Jones, D. N. (2013). Wildlife warning signs: Public assessment of components, placement and designs to optimize driver response. *Animals* **3**, 1142-1161.

Ciach, M. and Fröhlich, A. (2019). Ungulates in the city: light pollution and open habitats predict the probability of roe deer occurring in an urban environment. *Urban Ecosystems* **22**, 513-523.

Clements, G. R., Lynam, A. J., Gaveau, D., Yap, W. L., Lhota, S., Goosem, M., Laurance, S., and Laurance, W. F. (2014). Where and how are roads endangering mammals in Southeast Asia's forests? *PloS one* **9**.

Coe, P. K., Clark, D. A., Nielson, R. M., Gregory, S. C., Cupples, J. B., Hedrick, M. J., Johnson, B. K., and Jackson, D. H. (2018). Multiscale models of habitat use by mule deer in winter. *The Journal of Wildlife Management* **82**, 1285-1299.

Conover, M. R. (1995). Review of human injuries, illnesses, and economic losses caused by wildlife in the United States. *Wildlife Society Bulletin* **23**, 407-414.

Davis, N. E., Bennett, A., Forsyth, D. M., Bowman, D. M. J. S., Lefroy, E. C., Wood, S. W., Woolnough, A. P., West, P., Hampton, J. O., and Johnson, C. N. (2016). A systematic review of the impacts and management of introduced deer (family Cervidae) in Australia. *Wildlife Research* **43**, 515-532.

Debeljak, M., Džeroski, S., Jerina, K., Kobler, A., and Adamič, M. (2001). Habitat suitability modelling for red deer (*Cervus elaphus*) in South-central Slovenia with classification trees. *Ecological Modelling* **138**, 321-330.

DeNicola, A. J. and Williams, S. C. (2008). Sharpshooting suburban white-tailed deer reduces deer–vehicle collisions. *Human-Wildlife Conflicts* **2**, 28-33.

Department of Economic Development Jobs Transport and Resources (DEDJTR) (2018). Draft deer management strategy. Melbourne, Victoria.

Department of Environment Land Water and Planning (DELWP). (2018) Victorian Biodiversity Atlas fauna records (unrestricted) for sites with high spatial accuracy.

Dique, D., Thompson, J., Preece, H., Penfold, G., de Villiers, D., and Leslie, R. S. (2003). Koala mortality on roads in south-east Queensland: the koala speed-zone trial. *Wildlife Research* **30**, 419-426.

Dormann, C. F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G., and Münkemüller, T. (2013). Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. *Ecography*, **36**, 27-46.

Elith, J., Leathwick, J. R., and Hastie, T. (2008). A working guide to boosted regression trees. *Journal of Animal Ecology* **77**, 802-813.

Forman, R. and Alexander, L. E. (1998). Roads and their major ecological effects. *Annual Review of Ecology and Systematics* **29**, 207-231.

Forsyth, D. M., McLeod, S. R., Scroggie, M. P., and White, M. D. (2009). Modelling the abundance of wildlife using field surveys and GIS: non-native sambar deer (*Cervus unicolor*) in the Yarra Ranges, south-eastern Australia. *Wildlife Research* **36**, 231-241.

Forsyth, D. M., Stamation, K., and Woodford, L. (2015). Distributions of sambar deer, rusa deer and sika deer in Victoria. Arthur Rylah Institute, Heidelberg, Victoria.

Forsyth, D. M., Stamation, K., and Woodford, L. (2016). Distributions of fallow deer, red deer, hog deer and chital deer in Victoria. Arthur Rylah Institute for Environmental Research, Unpublished Client Report for the Biosecurity Branch, Heidelberg, Victoria.

Gormley, A. M., Forsyth, D. M., Griffioen, P., Lindeman, M., Ramsey, D. S. L., Scroggie, M. P., and Woodford, L. (2011). Using presence-only and presence–absence data to estimate the current and potential distributions of established invasive species. *Journal of Applied Ecology* **48**, 25-34.

Hothorn, T., Brandl, R., and Müller, J. (2012). Large-scale model-based assessment of deer-vehicle collision risk. *PloS one* **7**.

Huijser, M. P., McGowen, P. T., Fuller, J., Hardy, A., and Kociolek, A. (2007). Wildlife-vehicle collision reduction study: report to Congress. Western Transportation Institute. Bozeman, Montana, USA.

James, G., Witten, D., Hastie, T., and Tibshirani, R. (2013). An introduction to statistical learning. Springer, New York

Joyce, T. L. and Mahoney, S. P. (2001). Spatial and temporal distributions of moose-vehicle collisions in Newfoundland. *Wildlife Society Bulletin* **29**, 281-291.

Kalb, D., Bowman, J., and DeYoung, R. W. (2018). Dietary resource use and competition between white-tailed deer and introduced sika deer. *Wildlife Research* **45**, 457-472.

Kämmerle, J.-L., Brieger, F., Kröschel, M., Hagen, R., Storch, I., and Suchant, R. (2017). Temporal patterns in road crossing behaviour in roe deer (*Capreolus capreolus*) at sites with wildlife warning reflectors. *PloS one* **12**.

Keegan, T. W., Ackerman, B. B., Aoude, A. N., Bender, L. C., Boudreau, T., Carpenter, L. H., Compton, B. B., Elmer, M., Heffelfinger, J. R., Lutz, D. W., Trindle, B. D., Wakeling, B. F., and Watkins, B. E. (2011). Methods for monitoring mule deer populations, Mule Deer Working Group, USA.

Keogh, L. (2016). 'Risk of animal collisions increases warns RACV'. Available at https://www.racv.com.au/about-racv/our-business/media-releases/risk-of-animal-collisions-increases-warns-racv.html [accessed 16th November 2018].

Klocker, U., Croft, D., and Ramp, D. (2006). Frequency and causes of kangaroo-vehicle collisions on an Australian outback highway. *Wildlife Research* **33**, 5-15.

Knapp, K. K., Lyon, C., Witte, A., and Kienert, C. (2007). Crash or carcass data: Critical definition and evaluation choice. *Transportation Research Record* **2019**, 189-196.

Langbein, J., Putman, R. J., and Pokorny, B. (2011). Traffic collisions involving deer and other ungulates in Europe. In ‘Ungulate Management in Europe: Problems and Practices’ (Eds R. Putman.) Pp. 215-259. (Cambridge University Press: New York.)

Leslie, D. M. (2011). *Rusa unicolor* (Artiodactyla: Cervidae). *Mammalian Species*, 1-30.

Metz, C. E. (1978). Basic principles of ROC analysis. *Seminars in Nuclear Medicine* **8**, 283-298.

Miller, M. E., Hui, S. L. & Tierney, W. M. (1991) Validation techniques for logistic regression models. *Statistics in Medicine* **10**, 1213-1226.

Moloney, P. D. and Turnbull, J. D. (2017). Estimates of harvest for deer in Victoria: results from surveys of Victorian game licence holders in 2017. Game Management Authority, Victoria.

Moore, I. A. (1994) Habitat use and activity patterns of sambar (*Cervus unicolor*) in the Bunyip sambar enclosure. M.Sc. Thesis, The University of Melbourne, Melbourne.

Murphy, A. and Xia, J. (2016). Risk analysis of animal–vehicle crashes: a hierarchical Bayesian approach to spatial modelling. *International Journal of Crashworthiness* **21**, 1-13.

Oh, J., Washington, S. P., and Nam, D. (2006). Accident prediction model for railway-highway interfaces. *Accident Analysis & Prevention* **38**, 346-356.

Parliament of Victoria (2017). Inquiry into the control of invasive animals on Crown land. Victorian Government, Melbourne.

Pople, T., Brennan, M., Amos, M., Kearns, B., McBride, K., and Blokland, A. (2017) Management of an expanding chital deer population in North Queensland. In ‘Proceedings of the 17th Australasian Vertebrate Pest Conference, Canberra, Australia, 1-4 May 2017’. Available at: https://www.pestsmart.org.au/avpc-2017-proceedings/

R Core Team (2016). R: a language and environment for statistical computing. (R Foundation for Statistical Computing: Vienna, Austria.)

Ramp, D. and Ben-Ami, D. (2006). The effect of road-based fatalities on the viability of a peri-urban swamp wallaby population. *Journal of Wildlife Management* **70**, 1615-1624.

Ramp, D., Caldwell, J., Edwards, K. A., Warton, D., and Croft, D. B. (2005). Modelling of wildlife fatality hotspots along the Snowy Mountain Highway in New South Wales, Australia. *Biological Conservation* **126**, 474-490.

Ramp, D., Wilson, V., and Croft, D. (2006). Assessing the impacts of roads in peri-urban reserves: Road-based fatalities and road usage by wildlife in the Royal National Park, New South Wales, Australia. *Biological Conservation* **129**, 348-359.

Rea, R. V. (2003). Modifying roadside vegetation management practices to reduce vehicular collisions with moose *Alces alces*. *BIOONE* **9**, 81-91.

Romin, L. A. (1996). Deer-vehicle collisions: Status of state monitoring activities and mitigation efforts. *Wildlife Society Bulletin* **24**, 276-283.

Rowden, P., Steinhardt, D., & Sheehan, M. (2008). Road crashes involving animals in Australia. *Accident Analysis & Prevention* **40**, 1865-1871.

Russell, R., Gude, J., Anderson, N., and Ramsey, J. M. (2015). Identifying priority chronic wasting disease surveillance areas for mule deer in Montana. *Journal of Wildlife Management* **79**, 989-997.

Santos, R. A. L., Mota-Ferreira, M., Aguiar, L. M. S., and Ascensão, F. (2018). Predicting wildlife road-crossing probability from roadkill data using occupancy-detection models. *Science of The Total Environment* **642**, 629-637.

Santos, R. A. L., Santos, S. M., Santos-Reis, M., Picanço de Figueiredo, A., Bager, A., Aguiar, L. M. S., and Ascensão, F. (2016). Carcass persistence and detectability: Reducing the uncertainty surrounding wildlife-vehicle collision surveys. *PloS one* **11**, e0165608.

Seiler, A. and Helldin, J. O. (2006). Mortality in wildlife due to transportation. In 'The ecology of transportation: managing mobility for the environment'. (Eds J. Davenport and J.L Davenport) Pp. 165-189. (Springer: Netherlands.)

Snow, N., Williams, D., and Porter, W. F. (2014). A landscape-based approach for delineating hotspots of wildlife-vehicle collisions. *Landscape Ecology* **29**, 817-829.

Steiner, W., Leisch, F., and Hackländer, K. (2014). A review on the temporal pattern of deer-vehicle accidents: Impact of seasonal, diurnal and lunar effects in cervids. *Accident Analysis and Prevention* **66**, 168-181.

Stevens, B. and Dennis, B. (2013). Wildlife mortality from infrastructure collisions: statistical modeling of count data from carcass surveys. *Ecology* **94**, 2087-2096.

van der Ree, R., van der Ree, R., Jaeger, J. A. G., van der Grift, E. A., and Clevenger, A. (2011). Effects of roads and traffic on wildlife populations and landscape function: Road ecology is moving toward larger scales. *Ecology and society* **16**, 48.

Visintin, C. (2017). Modelling and predicting collision risks between wildlife and moving vehicles across time and space. PhD Thesis, The University of Melbourne, Melbourne.

Visintin, C., van der Ree, R., and McCarthy, M. (2016). A simple framework for a complex problem? Predicting wildlife-vehicle collisions. *Ecology & Evolution* **6**, 6409-6421.

Visintin, C., van der Ree, R., and McCarthy, M. A. (2017). Consistent patterns of vehicle collision risk for six mammal species. *Journal of Environmental Management* **201,** 397-406.

Warton, D. I., and Shepherd, L. C. (2010). Poisson point process models solve the “pseudo-absence problem” for presence-only data in ecology. *The Annals of Applied Statistics*, **4**, 1383-1402.

Yang, X., Zou, Y., Wu, L., Zhong, X., Wang, Y., Ijaz, M., and Peng, Y. (2019). Comparative analysis of the reported animal-vehicle collisions data and carcass removal data for hotspot identification. *Journal of Advanced Transportation* **2019**, 13.

Ye, X., Wang, K., Zou, Y., and Lord, D. (2018). A semi-nonparametric Poisson regression model for analyzing motor vehicle crash data. *PloS one* **13**, e0197338.

Yen, S. C., Yen, S.-C., Wang, Y., Yu, P.-H., Kuan, Y.-P., Liao, Y.-C., Chen, K.-H., and Weng, G.-J. (2019). Seasonal space use and habitat selection of sambar in Taiwan. *Journal of Wildlife Management* **83**, 22-31.

Zou, Y., Wu, L., and Lord, D. (2015). Modeling over-dispersed crash data with a long tail: Examining the accuracy of the dispersion parameter in Negative Binomial models. *Analytic Methods in Accident Research* **5**, 1-16.

Chapter 6 – General discussion

Sambar stag, Hill End, Victoria

April 12, 2016

Photo credit: Christopher Davies

Chapter 6 – General discussion

Wild deer have emerged as a serious threat to native biodiversity in south east Australia. Evidence suggests that deer numbers are increasing, particularly populations of sambar and fallow deer. As deer populations increase so do deer impacts. Deer browsing, antler rubbing, trampling and wallowing is damaging threatened native ecosystems as well as agricultural crops. Deer are also increasingly being involved in collisions with vehicles and so pose a growing risk to motorists. Currently, many knowledge gaps exist in regards to deer ecology, impacts and management systems. This lack of understanding is constraining wild deer management in south east Australia.

The aim of this thesis was to provide tools and information to improve the management of wild deer in south east Australia. In chapter one (Introduction) I discussed the current issues associated with introduced deer in south east Australia. This highlighted the research gaps that currently constrain the effective management of invasive deer populations. In this chapter I synthesise the contribution of my research and discuss implications for managing deer in south east Australia. I also identify limitations of my research and suggest future research directions. The tools and information I present in this thesis will help address several pressing ecological knowledge gaps regarding deer, resulting in better informed and more effective management of invasive deer populations, in particular sambar deer, in south east Australia.

6.2 Genetics and its potential to guide applied deer management

Land managers in Victoria require more information about the ecology and population dynamics of introduced deer in order to guide the application, and assess the effectiveness of management interventions. In particular, methods to accurately estimate deer abundance and distribution are required to establish best practice management strategies. Genetic approaches can be applied to generate such information, but to date these approaches have had a minimal role in the contemporary management of invasive deer species.

6.2.1 Population genetics and invasive species management

Population genetics is a field whereby genetic data is used to investigate the ecology of wild animals and plants (Broquet and Petit 2009; Lande 1988). Genetic data can provide insights into the abundance and density of the target species (Brinkman *et al.* 2011; Poole *et al.* 2011), determine the structure and connectivity of populations (Wedrowicz *et al.* 2018) and inform investigations of dispersal (Manel *et al.* 2003). Such information is commonly used to help manage species for conservation purposes (Frankham *et al.* 2014). For example, Pacioni *et al.* (2011) investigated the population structure and connectivity of endangered brush-tailed bettongs (*Bettongia penicillata ogilbyi*) in Western Australia and detailed the effect of habitat fragmentation on the species.

Population genetics also has wide applications for invasive species management (Rollins *et al.* 2006). Past studies have used population genetics to identify fallow deer management units in Tasmania (Webley *et al.* 2007), to assess genetic diversity and occurrence of hybridisation in Victorian hog deer (Hill *et al.* 2019) and to identify genetic bottlenecks in rusa deer (*Rusa timorensis*) in New South Wales (Webley *et al.* 2004). No previous peer reviewed studies have used population genetics to guide sambar deer management in Victoria.

The 'genetic toolbox' I developed in chapter two (Davies *et al.* 2019a) facilitates the collection of good quality DNA from wild deer scats. This toolbox has wide applications for collecting information to fulfil research priorities established by Davis *et al.* (2016), DEDJTR (2018) and Parliament of Victoria (2017), particularly regarding monitoring the abundance and distribution of wild deer. The toolbox described in chapter two (Davies *et al.* 2019a) can be applied to extract high quality DNA from deer scat collected non-invasively. From this you can identify the species from which scats originated and provide a unique individual sambar deer genotype. The method was particularly valuable for collecting genetic data non-invasively from cryptic deer species in challenging environments. The ways in which genetic data can contribute to deer management in Australia is discussed below.

6.2.2 Using genetics to monitor deer distribution

Understanding the distribution of invasive species is critical to direct and evaluate management options (Gormley *et al.* 2011) and determine the scale of control operations (Myers *et al.* 2000). Studies that estimate distribution require occurrence data, often sourced from observer surveys (Pennino *et al.* 2019), camera traps (Kafley *et al.* 2019), or the presence of animal signs including footprints, rubs and wallows (Claridge 2016). Discarded biological material (such as scats) can also be used to confirm the presence of a species in a particular location. Scats are often assigned to a species through morphological identification, however, it can be difficult to distinguish between the scats of closely related species. Scats from Victoria's four established deer species are difficult to distinguish as the size and form of scats can vary both within and between each deer species, due to differences in diet and age class (Claridge 2016). In chapter two (Davies *et al.* 2019a) I show that DNA isolated from scats can be used to confirm deer identity through the amplification of mitochondrial DNA. As such, the identification of deer species from scat DNA could be combined with the location the sample was collected, to obtain a robust prediction of deer distribution.

Species presence, determined from scat DNA has been used to establish the distribution of endangered ungulates, including the small red brocket deer (*Mazama Bororo*) in Brazil (Duarte *et al.* 2017). Similarly, Litvaitis *et al.* (2006) used genetically confirmed scats collected at a landscape level to determine the distribution of New England cottontails (*Sylvilagus transitionalis*) in north east USA. These studies highlight how scat DNA can be used to bolster our understanding of species distributions. As scat DNA permits an understanding of species distribution without the need for direct observation, this method it likely to be extremely useful when establishing the distribution of cryptic species such as deer. By unequivocally identifying the species of deer from which a scat at a particular location came from, the tools I established in chapter two (Davies *et al*. 2019a) could contribute to the understanding of species-specific deer distributions across Victoria. Submitting the location of genetically confirmed scats to wildlife databases such as the Victorian Biodiversity Atlas (VBA) will increase the amount of data available to perform future analyses, including species distribution modelling allowing more accurate and up to date estimates of deer distributions. This will improve our understanding of the rate and location of deer spread, which was a priority of the Victorian Draft Deer Management Strategy (DEDJTR 2018).

6.2.3 Estimating deer abundance using genetics

Genetic data (multilocus genotypes) can be used to provide estimates of effective population size (N_e) and abundance (N), for conservation (Frankham *et al.* 2014) and invasive species management purposes (Rollins *et al.* 2006). As N_e is an estimate of the number of breeding individuals in a population during each generation (Luikart *et al.* 2010), it is related to the total population abundance (N). As such, estimates of N_e and N are useful for investigating the viability of populations (Frankham *et al.* 2014) and assessing the efficacy of invasive species management strategies (Hampton *et al.* 2004; Mora *et al.* 2018). However, the accurate estimation of N_e relies on a number of assumptions, including stable population size, no immigration, mutation or selection (Luikart *et al*. 2010).

Genetic mark recapture (GMR) is a common method used to estimate the abundance (N) of wildlife populations (Goode *et al.* 2014; Mills *et al.* 2000). GMR uses multiple captures of the same individual, often generated from non-invasive samples including scats and hair, to estimate abundance (De Barba *et al.* 2010). GMR has been applied widely to estimate the abundance of terrestrial ungulates using genetic profiles isolated from scats (Brazeal *et al.* 2017; Brinkman *et al.* 2011; Harris *et al.* 2010). In Australia, GMR has been applied to determine the effect of poison baiting on red fox abundance (Berry *et al.* 2012; Piggott *et al.* 2008). Both Berry *et al*. (2012) and Piggott *et al*. (2008) describe the value of using scats as a source of DNA for GMR studies. As GMR can provide more accurate estimates of deer abundance than alternative methods such as scat counts (Brinkman *et al*. 2013) this approach is recommended.

Scat count surveys, where researchers sample plots and count individual deer scats and scat groups is a commonly used protocol to provide estimates of deer abundance and density (Forsyth *et al.* 2007; Forsyth *et al.* 2009). In Australia, previous studies have used scat surveys to investigate the effect of fire on sambar deer abundance (Forsyth *et al.* 2012), to provide baseline estimates of sambar deer abundance in newly established areas (Mulvaney *et al.* 2017) and to determine the efficacy of sambar deer control operations (Bennett *et al.* 2015). Despite being cost effective and relatively easy to undertake, scat surveys have a major limitation; it is difficult and in most cases impossible to differentiate the scats of sympatric deer species (Costa *et al.* 2017). Therefore, in areas where multiple deer species occur, misidentification is a significant issue and can lead to erroneous estimations of abundance and density for individual deer species (Spitzer *et al.* 2019). The rapid increase in the abundance and distribution of fallow and sambar deer compounds this problem as many areas of south east Australia are now occupied by both deer species.

The ‘genetic toolbox’ I developed in chapter two (Davies *et al*. 2019a) allows the identification of individual sambar deer from DNA sourced from scats. As such, genetic profiles collected using the methodology described could be useful to estimate sambar deer abundance using GMR and provide more robust estimates of abundance than scat count surveys, which are commonly used to estimate deer abundance.

6.2.4 Defining sambar deer management units

Invasive vertebrate pests are commonly targeted for eradication to reduce and/or remove the detrimental impacts to their host ecosystems. To be successful, eradication efforts need to be well planned, with clearly defined target populations. Many successful eradication programs have been undertaken on isolated island ecosystems focussing on pests including ship rats (*Rattus norvegicus*) (Poncet *et al.* 2011), feral cats (*Felis catus*) (Nogales *et al.* 2004) and larger ungulate mammals, such as goats and deer (Campbell *et al.* 2004; Masters *et al.* 2018). The methods commonly used to eradicate these species include; poisoning, trapping and ground shooting with the assistance of detector dogs. A combination of these methods is often applied (Myers *et al.* 2000). There are fewer examples of successful eradication of invasive species on mainland areas, where pest species populations are highly connected and likely to reinvade quickly after eradication efforts. As such, in mainland areas, where large homogenous populations of pest species occur, controlling the target species and minimising their detrimental effects are the most relevant management options (Spencer *et al.* 2016).

The genetic toolbox I developed in chapter two (Davies *et al*. 2019a), was applied in chapter three and demonstrated that sambar deer in Victoria display pronounced population structure. I identified three separate management units and potential sub-structure in the eastern Victorian (EV) population. The existence of distinct sambar deer populations in Victoria is consistent with the proposition of Forsyth *et al.* (2015) who suggested distinct populations are likely to be geographically isolated due to the presence of barriers to dispersal, including roads, water bodies and wide expanses of cleared land. The population structure information provided in chapter three has clear implications for the management of sambar deer in Victoria.

In chapter three I demonstrated that sambar deer form a distinct cluster on French Island, suggesting that Westernport Bay is acting as an effective barrier to dispersal. This is despite sambar deer being recognised as strong swimmers (Leslie 2011). This suggests that the eradication of sambar deer from French Island is possible. However, eradicating deer from French Island would likely require significant resources, as deer are likely to rapidly display avoidance behaviour and move into areas of dense vegetation. If eradication is feasible and in line with community values, it could be guided by the work of Masters *et al*. (2018) which successfully eradicated goats and fallow deer from Kangaroo Island, Australia’s third largest island (4400km^2). Eradication efforts could apply methods including sustained ground and aerial shooting, coupled with ongoing monitoring using camera traps and helicopter surveys to achieve favourable management outcomes (Masters *et al*. 2018).

The high habitat connectivity and the large geographical scale of the eastern Victoria sambar deer population suggests a high likelihood of failure for eradication efforts, due to rapid reinvasion. In addition, the eastern Victorian population of sambar deer is considered the most valuable population in terms of a game resource (Finch *et al.* 2014) and eradication efforts are likely to be an unpopular management option. An asset protection approach could incorporate ground and aerial shooting operations in national parks, as well as exclusion fencing around high value ecological assets, such as alpine and subalpine bogs and remnant areas of littoral rainforest (Department of Environment 2015; Peel *et al.* 2005).

6.2.5 Limitations of genetic data

Microsatellites are the genetic markers of choice for studies involving low quality and degraded DNA samples (Alacs *et al.* 2010; Lampa *et al.* 2013). Due to the reliability of this method, many studies (including this one) use microsatellites with capillary electrophoresis (CE) and genotype samples based on size polymorphisms. However, the use of CE as a genotyping method has a number of constraints regarding the transferability of data between platforms and low throughput (De Barba *et al.* 2017). Alternative genotyping methods including high throughput sequencing (HTS) using Next Generation Sequencing (NGS) has advantages over CE, as they provide direct access to the sequence read. Data generated using NGS is therefore comparable between research groups using different instruments, while data from CE analysis is not. NGS also allows for thousands of samples to be analysed simultaneously, for a similar cost to hundreds of samples using CE technology. Therefore, establishing a high throughput suite of markers for genotyping sambar deer and other deer species, similar to those established for brown bears (*Ursus arctos*) by De Barba *et al*. (2017) would be valuable. Such an approach would improve genotyping success, accuracy and cost efficiency, allowing genetic methods to be used routinely in the monitoring and management of deer.

6.2.6 Future genetic applications to deer management

Obtaining a robust estimate of sambar deer abundance both before and after management interventions is vital to determine if culling programs have been effective. The methodology I developed in chapter two (Davies *et al*. 2019a) could be applied to collect genetic profiles from scats, systematically before and after control operations, thus allowing robust estimates of deer abundance (using GMR) to determine if control operations have been effective. Additionally, abundance estimates, sourced from genetic data can be used to determine control targets. The work of Hone *et al.* (2010) suggested that 40% of a sambar deer population should be removed each year in order to stop population growth. Therefore, accurate estimates of abundance established from GMR studies for each Victorian sambar deer population could help identify these targets.

New techniques that use environmental DNA (eDNA) have recently been developed. These techniques can simultaneously identify the presence of multiple species from cells collected from environmental sources, such as creeks and streams (Deiner *et al.* 2017). This has wide applications for defining the distribution of invasive species (Deiner *et al.* 2017). eDNA has improved the management of aquatic invasive species (Keskin 2014; Klymus *et al.* 2015), as well as terrestrial invasive species such as feral pigs (Williams *et al.* 2018). Developing an eDNA survey that can detect the presence of multiple deer species from water sources could be useful for future deer management and provide evidence of deer presence or absence, at a landscape level.

The methodology I describe in chapter two (Davies *et al*. 2019a) is directly applicable to the other three species of introduced deer in Victoria (fallow, red and hog). Therefore, the genetic tools described could be used in future genetic studies of these species to assess population structure, diversity and dispersal. However, a pilot study to assess cross species amplification and polymorphism in these species is recommended.

6.3 Camera trapping and its application to applied deer management

Camera traps will play a critical role in the monitoring of deer populations in Victoria. They can be used to collect data over long periods of time, measure abundance and density, and provide insights into the behaviour and activity patterns of deer. This information is vital to guide the spatial and temporal application of management efforts and assess if they have been effective.

6.3.1 Camera traps and their application to invasive species management

Camera traps have rapidly established as a valuable monitoring tool for scientists and land managers. A major advantage of camera traps is the ability to collect data non-invasively over long periods of time, allowing insights into animal behaviour without disturbance (Meek *et al.* 2014). Camera traps have been used widely to monitor invasive terrestrial mammals in Australia, including feral pigs (Bengsen *et al.* 2011b), foxes (Towerton *et al.* 2011) and feral cats (Bengsen *et al.* 2011a). They have also been used to provide occurrence data for modelling the distribution of sambar deer in Victoria (Gormley *et al.* 2011), investigate the scavenging behaviour of carnivores on deer carcasses (Forsyth *et al.* 2014) and determine changes in sambar deer occupancy after fire (Forsyth *et al.* 2012). Recently, camera traps were used to investigate the effect of homesteads, water and dingoes on the relative abundance of chital deer (*Axis axis*) in Queensland, Australia (Forsyth *et al.* 2019). This study found that chital deer relative abundance was higher near permanent water sources and in proximity to homesteads, with the authors suggesting that management efforts focus control mechanisms on these areas (Forsyth *et al.* 2019). Thus highlighting the utility of camera traps to inform deer management.

6.3.2 Monitoring the occurrence of deer in sensitive areas

Wild deer, in particular sambar, are thought to be impacting many of Victoria’s most sensitive ecosystems, including alpine peatlands (Department of Environment 2015) and littoral rainforest (Peel *et al*. 2005). Identifying new, and enhancing existing monitoring strategies to detect deer, and their impacts, is an established priority to improve deer management and protect ecosystems most at risk of the impacts of wild deer (DEDJTR 2018). In chapter four I outline a camera trap survey that was not only effective at detecting deer, but also provided valuable insight into the ecology and behaviour of sambar deer in Baw Baw National Park (BBNP). These insights can be used to guide control efforts.

One of the most interesting findings from my work in chapter four was the dominance of sambar deer detections compared to native species. From a total of 712 camera trap triggers, 410 triggers were from sambar deer, representing 58% of all triggers. In addition, sambar deer were detected in a range of different habitats, including subalpine peatlands and lowland forests. These findings suggest that sambar deer are widely established in BBNP and occupy a range of habitat types. Sambar deer have previously been sighted in BBNP and have been recorded in databases including the Victorian Biodiversity Atlas (VBA). However, few published studies have reported their presence in BBNP or in other subalpine ecosystems. The high number of sambar deer detected within BBNP suggests threatened subalpine peatlands in the area may be at risk from deer impacts, including browsing and wallowing (Department of Environment 2015). To mitigate the impacts of deer to subalpine peatlands in the area, the construction of exclusion fencing could be considered. Alternatively, aerial culling programs could focus on these areas.

6.3.3 Camera traps for deer surveillance

Surveillance is a critical element of invasive species management, regardless of whether the strategy aims to eliminate the species or instigate sustained control (Davis *et al.* 2018). A common surveillance approach for invasive species is to deploy cameras in areas of high habitat suitability and check for incursions at set intervals (Davis *et al*. 2018). If incursions are detected, shooting, trapping and baiting operations aimed to remove the problem species can be undertaken quickly to eradicate the species before it becomes established. Camera traps have proven useful for the surveillance of invasive species including feral pig (Davis *et al.* 2018), red fox (Thompson *et al.* 2019), feral cat (Bengsen *et al.* 2011a), deer (Masters *et al.* 2018) and other terrestrial mammals (Meek *et al.* 2015).

6.3.4 Using information from camera traps to guide deer control operations

In chapter four my analysis showed that sambar deer were more detectable in proximity to roads and in areas of sparse tree density. As detectability often increases with abundance (McCarthy *et al.* 2013), areas close to roads and with sparse tree density in BBNP could support a greater number of sambar deer. This information provides insights into the habitat use of sambar deer within BBNP during the summer months. Furthermore, establishing that sambar deer were more detectable close to roads, highlights the potential of sambar deer as a risk to motorists.

The increased detectability of sambar deer in areas of sparse canopy cover suggests a preference for these areas in BBNP. This information highlights the potential for conducting aerial shooting from helicopters in BBNP. Aerial shooting from helicopters has been shown to be effective for reducing populations of wild deer in New Zealand (Nugent *et al.* 1987). However, uncertainty exists regarding how much control effort is required to reduce deer numbers when conducting aerial shooting (Forsyth *et al.* 2013). Furthermore, aerial shooting is an expensive management option (Edwards *et al.* 2016). As deer rapidly learn to avoid areas associated with hunting pressure (Lone *et al.* 2015), culling rates can fall rapidly as operations are rolled out, complicating management efforts.

The effectiveness of eradication and control efforts can be improved with a deeper understanding of the ecology and behaviour of the target species (Allendorf and Lundquist 2003; Courchamp *et al.* 2003). In chapter four, I showed that sambar deer within BBNP display crepuscular activity patterns, similar to other cervid species in their native ranges

(Ferreguetti *et al.* 2015; Ikeda *et al.* 2016). These findings were the first to empirically demonstrate the activity patterns of sambar deer in Victoria. Although demonstrated at a relatively small scale (within BBNP), the crepuscular activity patterns are consistent with reports from recreational hunters (L. Treptow pers. comm.) and are likely to reflect the activity patterns of sambar deer at a landscape scale.

Previous studies have shown that sambar deer display crepuscular activity patterns in their native range (Leslie 2011). However, in contrast, outside of Australia, sambar deer have also demonstrated alternative activity patterns, including nocturnal (Ross *et al.* 2013) and cathemeral (Tan 2018). Interactions with predators including tigers (*Panthera tigris*) and Sunda clouded leopards (*Neofelis diardi*) could be driving the differences in activity patterns observed in the native range of sambar deer (Ross *et al.* 2013). The activity pattern data established by chapter four will help land managers improve control operations by targeting mornings and evenings, to increase encounters with sambar deer. As control operations can alter the behaviour of the target species (Brook *et al.* 2012), shooting operations can become less effective the longer they are applied. This challenge could be minimised by dedicating maximum resources to remove as many deer, as fast as possible before they start displaying avoidance behaviour to helicopters, vehicle noise and gun shots.

6.3.5 Limitations of camera traps

One limitation of chapter four was the timing of the study, which prevented addressing questions about the seasonal movement patterns of sambar deer. Camera traps were deployed only during spring/summer and it was therefore not possible to determine whether sambar deer leave the area in winter and migrate to lower elevations. Other cervid species demonstrate seasonal movement patterns (Igota *et al.* 2004; Martin *et al.* 2018), especially in areas where snowfall impedes deer movement and access to forage (Luccarini *et al.* 2006). If sambar deer also display seasonal movement patterns this information could be used to guide the timing of control programs. For example, shooting operations in BBNP could be planned to occur after snow melt on the subalpine plateau to coincide with arrival of sambar from lower elevations.

6.3.6 Future application of camera traps to guide deer management

As the camera trap survey was found to effectively detect sambar deer at a level suitable for occupancy studies, the survey methodology could be applied for future occupancy studies focussed on estimating sambar deer abundance. Abundance can be estimated from camera trap survey counts when detection is imperfect (Royle *et al.* 2005). The camera trap survey could be deployed across the subalpine plateau to monitor sambar deer occupancy in subalpine areas. Sambar deer occurrence data from camera traps could also be combined with wallow and browse surveys to investigate the impact sambar deer are having to subalpine peatlands (Department of Environment 2015). Future work should focus on optimising the camera trap survey for maximum cost effectiveness and power to detect trends in occupancy data (Southwell *et al.* 2019).

6.4 Spatial modelling and its application to deer management

Our understanding of the distribution, rate and location of the spread of deer populations in Victoria is relatively poor. Furthermore, one of the most pressing issues related to increasing deer abundance, which has largely been overlooked, is vehicle collision risk. Despite evidence of deer populations increasing in their abundance and distribution across Victoria, few resources have been allocated to mitigate this potentially fatal human wildlife conflict issue. Spatial modelling can be applied to further our understanding of these ecological parameters, to help determine where impacts are likely to occur and to guide where management efforts are likely to be the most effective.

6.4.1 Spatial modelling

Spatial modelling can be applied to investigate a range of complex ecological processes, including; distribution (Elith and Leathwick 2009), habitat suitability (Gormley *et al.* 2011) and, when combined with genetic data, dispersal (Duckett *et al.* 2013; Olah *et al.* 2017). Such information is critical to inform the conservation of threatened species (Liu *et al.* 2013; Marcer *et al.* 2013), as well as guide invasive species management (Mainali *et al.* 2015). Spatial modelling can also be used to model the impacts associated with the presence and activity of animals and guide where management interventions can have the greatest positive effect (Lesser *et al.* 2019).

The current and potential distribution of sambar deer in south east Australia has previously been investigated by Gormley *et al.* (2011). They highlighted that sambar deer occur in most forested areas of Victoria with the exception of several discrete areas, which are currently unoccupied by sambar deer. These areas of potential sambar deer distribution were prioritised for surveillance in order to help rapidly respond to deer incursions (Gormley *et al.* 2011). The current and potential distribution of wild deer was also investigated by Davis *et al*. (2016) using the Climatch algorithm (Invasive Animals CRC 2011). Both studies had similar predictions of current sambar deer distribution in Victoria. Additionally, areas predicted to have a high likelihood of deer occurrence (all species) in chapter five (Davies *et al*. 2019b) were similar to the areas identified by Gormley *et al*. (2011) and Davis *et al.* (2016).

6.4.2 Habitat preferences and movement patterns

Spatial modelling can be used to investigate the habitat preferences of wild animals to improve management outcomes. Understanding the habitat preference of invasive species can help identify areas to focus control efforts (Gormley *et al.* 2011) and help understand interactions between invasive and native species (Drygala and Zoller 2013). Telemetry studies are often employed to investigate habitat preferences. Such studies involve the capture of the target species and the attachment of equipment that transmits location data. Many studies have used GPS telemetry to investigate the habitat preferences and movement patterns of deer, in both their native (Grovenburg *et al.* 2014; Karns *et al.* 2011; Meisingset *et al.* 2013) and introduced ranges (Amos *et al.* 2014). Such studies are difficult and expensive to undertake (Matthews *et al.* 2013), but can provide important ecological data about introduced deer that could be utilised to improve their management, including seasonal movement patterns, home range and habitat preferences. One of the most concerning emerging threats of increasing deer populations in Victoria is the risk posed to motorists.

6.4.3 Spatial modelling to investigate collision risk in Victoria

My work in chapter five (Davies *et al*. 2019b) represents the first investigation of deer-vehicle collisions (DVC) conducted in Victoria. I identified three areas of increased DVC risk. The first on the Western Highway near Gordon, the second, to the east of Melbourne on roads surrounding Lysterfield Park, Emerald and Gembrook and third, roads including Strzelecki Highway and Moe-Rawson Road in West Gippsland. The three areas of increased DVC risk I identified in chapter five could be targeted for mitigation efforts, including speed limit reduction, signage, fencing or targeted deer control efforts. These mitigation strategies have had mixed results when applied in USA. The construction of fencing alongside high risk areas of road has been found to work well and significantly reduce the rate of DVC in the USA

(Bissonette and Rosa 2012). However, in contrast, there is little evidence that the installation of warning signs can effectively reduce the rate of wildlife collisions (Bond and Jones 2013).

6.4.4 Limitations of spatial modelling

All modelling approaches are constrained by the accuracy and reliability of the data they incorporate as well as chosen model specifications (Visintin 2017). Due to limitations in data available to train models in chapter five (Davies *et al.* 2019b), a model trained for DVC data from California, USA was used. Limited species specific deer collision data impeded the modelling of species specific collision risk across the Victorian road network. The establishment of a deer collision register would be useful to provide more data for subsequent modelling, including predicting deer collision risk for each of Victoria's introduced deer species. This is important because differences in the size, behaviour and abundance of each deer species can contribute to collision risk (Langley *et al.* 2006).

6.4.5 Future applications for spatial modelling

Incorporating spatial data with genetic data can help delineate fine scale population structure (Guillot *et al.* 2005; Manel *et al.* 2003). This approach is often referred to as 'landscape genetics' and has been used widely for both conservation and invasive species management (Bowman *et al.* 2016). By applying a landscape genetics approach researchers can identify conduits of dispersal for invasive species. This can help identify landscape features that impede or encourage the dispersal of invasive species and better understand invasion and recolonization scenarios (Zalewski *et al.* 2009). Dispersal conduits can be targeted for control, as demonstrated by Sacks *et al.* (2016) who identified topographically flat areas of California most effective for red fox control. Future studies investigating conduits of dispersal for sambar deer and other introduced deer species would be useful for identifying landscape features that encourage dispersal in the Victorian landscape. These areas could be targeted for the priority application of culling or other impact mitigation efforts including exclusion fencing.

Species distribution modelling can also be used to predict range shifts under different climatic scenarios (Duckett *et al.* 2013; McCallum *et al.* 2014). This approach has been used to forecast areas at risk of cane toad (*Rhinella marina*) invasion in response to changes in temperature and rainfall due to climate change (Phillips *et al.* 2008). This is useful for forecasting range shifts of species with specific habitat requirements. However, deer have demonstrated their adaptability in Australia and shown to tolerate habitats with wide temperature gradients. As such, forecasting range shifts in wild deer may provide little meaningful information because small temperature changes are unlikely to affect how they move, survive and reproduce. In contrast, fire has been shown to affect the abundance and occupancy of sambar deer in Victoria (Forsyth *et al.* 2012). Therefore, more severe fires driven by climate change may drive deer into new areas and compound management problems.

Recently, non-invasive genetic approaches have been used to investigate home range and habitat use of wild animals (Bischof *et al.* 2016; Yang *et al.* 2019), offering an alternative to expensive GPS and/or radio telemetry studies. To achieve this, genetic samples are collected from the environment and genotyped to identify individuals. Geographic Information Systems (GIS) can be used to draw polygons around repeated captures of the same individual and calculate range size (Yang *et al.* 2019). This approach has great capacity for investigating the home range of sambar deer in Victoria without the need to physically capture this difficult and elusive species. However, this would be reliant on the collection of potentially thousands of scat samples that would require DNA profiling and therefore would also be expensive. The development of a HTS genotyping assay for deer, similar to De Barba *et al.* (2017) could be applied in this circumstance to considerably reduce genotyping costs.

6.5 Conclusion

Wild deer, particularly sambar deer represent significant threats to social, economic and ecological values in south east Australia. To reduce the impacts of invasive wild deer species in the region, management agencies must better understand their ecology and impacts as well as identify robust methods to collect empirical data.

This thesis has established tools to increase our understanding of wild deer populations in south east Australia, provided new insights into sambar deer population structure and activity patterns and used a spatial modelling approach to investigate DVC risk across the Victorian road network. The work described in this thesis will improve the ability of management agencies to strategically plan deer control operations, determine whether such operations have been successful and monitor deer populations into the future.

The information outlined in this thesis addresses several of the established priorities for deer research in south east Australia and will help improve the effectiveness of deer management. This will hopefully result in positive outcomes for native biodiversity. Similar approaches could be applied to underpin the management of other invasive species throughout Australia.

6.6 References

Alacs, E. A., Georges, A., FitzSimmons, N. N., and Robertson, J. (2010). DNA detective: a review of molecular approaches to wildlife forensics. *Forensic Science, Medicine and Pathology* **6**, 180-194.

Allendorf, F. W. and Lundquist, L. L. (2003). Introduction: Population biology, evolution, and control of invasive species. *Conservation Biology* **17**, 24-30.

Amos, M., Baxter, G., Finch, N., and Murray, P. (2014). At home in a new range: wild red deer in south-eastern Queensland. *Wildlife Research* **41**, 258-265.

Bengsen, A., Butler, J., and Masters, P. (2011a). Estimating and indexing feral cat population abundances using camera traps. *Wildlife Research* **38**, 732-739.

Bengsen, A., Leung, L. K. P., Lapidge, S., and Gordon, I. (2011b). Using a general index approach to analyze camera-trap abundance indices. *Journal of Wildlife Management* **75**, 1222-1227.

Bennett, A., Haydon, S., Stevens, M., and Coulson, G. (2015). Culling reduces fecal pellet deposition by introduced sambar (*Rusa unicolor*) in a protected water catchment. *Wildlife Society Bulletin* **39**, 268-275.

Berry, O., Algar, D., Angus, J., Hamilton, N., Hilmer, S., and Sutherland, D. (2012). Genetic tagging reveals a significant impact of poison baiting on an invasive species. *Journal of Wildlife Management* **76**, 729-739.

Bischof, R., Gregersen, E. R., Brøseth, H., Ellegren, H., and Flagstad, Ø. (2016). Noninvasive genetic sampling reveals intrasex territoriality in wolverines. *Ecology and Evolution* **6**, 1527-1536.

Bissonette, J. A. and Rosa, S. (2012). An evaluation of a mitigation strategy for deer-vehicle collisions. *Wildlife Biology* **18**, 414-423, 410.

Bond, A. R. F. and Jones, D. N. (2013). Wildlife warning signs: Public assessment of components, placement and designs to optimise driver response. *Animals* **3**, 1142-1161.

Bowman, J., Greenhorn, J., Marrotte, R., McKay, M., Morris, K., Prentice, M., and Wehtje, M. (2016). On applications of landscape genetics. *Conservation Genetics* **17**, 753-760.

Brazeal, J., Weist, T., and Sacks, B. (2017). Noninvasive genetic spatial capture-recapture for estimating deer population abundance. *Journal of Wildlife Management* **81**, 629-640.

Brinkman, T. J., Person, D. K., Chapin, F. S., Smith, W., and Hundertmark, K. J. (2011). Estimating abundance of Sitka black-tailed deer using DNA from fecal pellets. *The Journal of Wildlife Management* **75**, 232-242.

Brinkman, T. J., Person, D. K., Smith, W., Chapin, F. S., McCoy, K., Leonawicz, M., and Hundertmark, K. J. (2013). Using DNA to test the utility of pellet-group counts as an index of deer counts. *Wildlife Society Bulletin* **37**, 444-450.

Brook, L. A., Johnson, C. N., and Ritchie, E. G. (2012). Effects of predator control on behaviour of an apex predator and indirect consequences for mesopredator suppression. *Journal of Applied Ecology* **49**, 1278-1286.

Broquet, T. and Petit, E. J. (2009). Molecular estimation of dispersal for ecology and population genetics. *Annual Review of Ecology, Evolution, and Systematics* **40**, 193-216.

Campbell, K., Donlan, C. J., Cruz, F., and Carrion, V. (2004). Eradication of feral goats *Capra* hircus from Pinta Island, Galápagos, Ecuador. *Oryx* **38**, 328-333.

Claridge, A. (2016). Introduced deer field identification guide for the Australian Alps. Office of Environment and Heritage, NSW National Parks and Wildlife Service. Queanbeyan, New South Wales.

Costa, E. B. V., de Oliveira, M., Peres, P. H. d. F., Grotta Neto, F., Vogliotti, A., Piovezan, U., and Duarte, J. M. B. (2017). Low accuracy of identifying Neotropical deer species by scat morphology. *Studies on neotropical fauna and environment* **52**, 37-42.

Courchamp, F., Chapuis, J.-L., and Pascal, M. (2003). Mammal invaders on islands: impact, control and control impact. *Biological Reviews* **78**, 347-383.

Davies, C., Wright, W., Wedrowicz, F. and Hogan, F. (2019a) A DNA toolbox for non-invasive genetic studies of sambar deer (*Rusa unicolor). Australian Mammalogy*.

Davies, C., Wright, W., Hogan, F., and Visintin, C. (2019b) Predicting deer-vehicle collision risk across Victoria, Australia. *Australian Mammalogy*.

Davis, A. J., McCreary, R., Psiropoulos, J., Brennan, G., Cox, T., Partin, A., and Pepin, K. M. (2018). Quantifying site-level usage and certainty of absence for an invasive species through occupancy analysis of camera-trap data. *Biological Invasions* **20**, 877-890.

Davis, N. E., Bennett, A., Forsyth, D. M., Bowman, D. M. J. S., Lefroy, E. C., Wood, S. W., Woolnough, A. P., West, P., Hampton, J. O., and Johnson, C. N. (2016). A systematic review of the impacts and management of introduced deer (family Cervidae) in Australia. *Wildlife Research* **43**, 515-532.

De Barba, M., Miquel, C., Lobréaux, S., Quenette, P. Y., Swenson, J. E., and Taberlet, P. (2017). High-throughput microsatellite genotyping in ecology: improved accuracy, efficiency, standardization and success with low-quantity and degraded DNA. *Molecular Ecology Resources* **17**, 492-507.

De Barba, M., Waits, L. P., Genovesi, P., Randi, E., Chirichella, R., and Cetto, E. (2010). Comparing opportunistic and systematic sampling methods for non-invasive genetic monitoring of a small translocated brown bear population. *Journal of Applied Ecology* **47**, 172-181.

Department of Economic Development Jobs Transport and Resources (DEDJTR) (2018). Draft deer management strategy. Melbourne, Victoria.

Deiner, K., Bik, H., Mächler, E., Seymour, M., LacoursièreRoussel, A., Altermatt, F., Creer, S., Bista, I., Lodge, D., Vere, N., Pfrender, M., and Bernatchez, L. (2017). Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Molecular Ecology* **26**, 5872-5895.

Department of Environment (2015). National recovery plan for the alpine sphagnum bogs and associated fens ecological community. Canberra, ACT.

Drygala, F. and Zoller, H. (2013). Spatial use and interaction of the invasive raccoon dog and the native red fox in Central Europe: Competition or coexistence? *European Journal of Wildlife Research* **59**.

Duarte, J. M. B., Talarico, Â., Vogliotti, A., Garcia, J., Oliveira, M., Maldonado, J., and González, S. (2017). Scat detection dogs, DNA and species distribution modelling reveal a diminutive geographical range for the vulnerable small red brocket deer *Mazama bororo*. *Oryx* **51**, 656-664.

Duckett, P., Wilson, P., Stow, A., and Burridge, C. (2013). Keeping up with the neighbours: using a genetic measurement of dispersal and species distribution modelling to assess the impact of climate change on an Australian arid zone gecko (*Gehyra variegata*). *Diversity and Distributions* **19**, 964-976.

Edwards, G., Digby, D., O'Leary, P., Rafferty, D., Jensen, M., Woolnough, A., Secomb, N., Williams, M., Schwartzkopff, K., and Bryan, R. (2016). Planning and conducting aerial culling operations for feral camels. *The Rangeland Journal* **38**, 153-162.

Elith, J. and Leathwick, J. (2009). Species distribution models: Ecological explanation and prediction across space and time. *Annual Review of Ecology, Evolution and Systematics* **40**, 677-697.

Ferreguetti, Á. C., Tomás, W. M., and Bergallo, H. G. (2015). Density, occupancy, and activity pattern of two sympatric deer (*Mazama*) in the Atlantic Forest, Brazil. *Journal of Mammalogy* **96**, 1245-1254.

Finch, N., Murray, P., Hoy, J., and Baxter, G. (2014). Expenditure and motivation of Australian recreational hunters. *Wildlife Research* **41**, 76-83.

Forsyth, D. M., Barker, R. J., Morriss, G., and Scroggie, M. P. (2007). Modeling the relationship between fecal pellet indices and deer density. *Journal of Wildlife Management* **71**, 964-970.

Forsyth, D. M., Gormley, A. M., Woodford, L., and Fitzgerald, T. (2012). Effects of large-scale high-severity fire on occupancy and abundances of an invasive large mammal in south-eastern Australia. *Wildlife Research* **39**, 555-564.

Forsyth, D. M., McLeod, S. R., Scroggie, M. P., and White, M. D. (2009). Modelling the abundance of wildlife using field surveys and GIS: non-native sambar deer (*Cervus unicolor*) in the Yarra Ranges, south-eastern Australia. *Wildlife Research* **36**, 231-241.

Forsyth, D. M., Pople, A., Woodford, L., Brennan, M., Amos, M., Moloney, P. D., Fanson, B., and Story, G. (2019). Landscape-scale effects of homesteads, water, and dingoes on invading chital deer in Australia’s dry tropics. *Journal of Mammalogy*.

Forsyth, D. M., Ramsey, D. S. L., Veltman, C. J., Allen, R. B., Allen, W. J., Barker, R. J., Jacobson, C. L., Nicol, S. J., Richardson, S. J., and Todd, C. R. (2013). When deer must die: large uncertainty surrounds changes in deer abundance achieved by helicopter- and ground-based hunting in New Zealand forests. *Wildlife Research* **40**, 447-458.

Forsyth, D. M., Stamation, K., and Woodford, L. (2015). Distributions of sambar deer, rusa deer and sika deer in Victoria. Arthur Rylah Institute. Melbourne, Victoria.

Forsyth, D. M., Woodford, L., Moloney, P. D., Hampton, J. O., Woolnough, A. P., and Tucker, M. (2014). How does a carnivore guild utilise a substantial but unpredictable anthropogenic food source? Scavenging on hunter-shot ungulate carcasses by wild dogs/dingoes, red foxes and feral cats in South-Eastern Australia revealed by camera traps. *PloS one* **9**.

Frankham, R., Bradshaw, C. J. A., and Brook, B. W. (2014). Genetics in conservation management: Revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. *Biological Conservation* **170**, 56-63.

Goode, M. J., Beaver, J. T., Muller, L. I., Clark, J. D., Manen, F. T. v., Harper, C. A., and Basinger, P. S. (2014). Capture—recapture of white-tailed deer using DNA from fecal pellet groups. *Wildlife Biology* **20**, 270-278.

Gormley, A. M., Forsyth, D. M., Griffioen, P., Lindeman, M., Ramsey, D. S. L., Scroggie, M. P., and Woodford, L. (2011). Using presence-only and presence–absence data to estimate the current and potential distributions of established invasive species. *Journal of Applied Ecology* **48**, 25-34.

Gormley, A. M. and Turnbull, J. D. (2010). Estimates of harvest for deer, duck and quail in Victoria: results from surveys of Victorian game licence holders in 2010. Arthur Rylah Institute for Environmental Research. Melbourne, Victoria.

Grovenburg, T. W., Klaver, R. W., Jacques, C. N., Brinkman, T. J., Swanson, C. C., DePerno, C. S., Monteith, K. L., Sievers, J. D., Bleich, V. C., Kie, J. G., and Jenks, J. A. (2014). Influence of landscape characteristics on retention of expandable radiocollars on young ungulates. *Wildlife Society Bulletin* **38**, 89-95.

Guillot, G., Mortier, F., and Estoup, A. (2005). Geneland: a computer package for landscape genetics. *Molecular Ecology Notes* **5**, 712-715.

Hampton, J. O., Spencer, P. B. S., Alpers, D. L., Twigg, L. E., Woolnough, A. P., Doust, J., Higgs, T., and Pluske, J. (2004). Molecular techniques, wildlife management and the importance of genetic population structure and dispersal: a case study with feral pigs. *Journal of Applied Ecology* **41**, 735-743.

Harris, R. B., Winnie Jr, J., Amish, S. J., Beja-Pereira, A., Godinho, R., Costa, V., and Luikart, G. (2010). Argali abundance in the Afghan pamir using capture recapture modeling from fecal DNA. *Journal of Wildlife Management* **74**, 668-677.

Hill, E., Linacre, A., Toop, S., Murphy, N., and Strugnell, J. (2019). Widespread hybridization in the introduced hog deer population of Victoria, Australia, and its implications for conservation. *Ecology and Evolution* **9**, 10828-10842.

Hone, J., Duncan, R. P., and Forsyth, D. M. (2010). Estimates of maximum annual population growth rates (rm) of mammals and their application in wildlife management. *Journal of Applied Ecology* **47**, 507-514.

Igota, H., Sakuragi, M., Uno, H., Kaji, K., Kaneko, M., Akamatsu, R., and Maekawa, K. (2004). Seasonal migration patterns of female sika deer in eastern Hokkaido, Japan. *Ecological Research* **19**, 169-178.

Ikeda, T., Uchida, K., Matsuura, Y., Takahashi, H., Yoshida, T., Kaji, K., Koizumi, I., and Yamazaki, S. (2016). Seasonal and diel activity patterns of eight sympatric mammals in northern Japan revealed by an intensive camera-trap survey. *PloS one* **11**, e0163602.

Invasive Animals CRC (2011). Climatch v1.0. (Australian Bureau of Agricultural and Resource Economics and Sciences: Canberra). Available at: https://climatch.cp1.agriculture.gov.au/

Kafley, H., Lamichhane, B., Maharjan, R., Thapaliya, B., Bhattarai, N., Khadka, M., and Gompper, M. E. (2019). Estimating prey abundance and distribution from camera trap data using binomial mixture models. *European Journal of Wildlife Research* **65**.

Karns, G. R., Lancia, R. A., DePerno, C. S., and Conner, M. C. (2011). Investigation of adult male white-tailed deer excursions outside their home range. *Southeastern Naturalist* **10**, 39-52.

Keskin, E. (2014). Detection of invasive freshwater fish species using environmental DNA survey. *Biochemical Systematics and Ecology* **56**, 68-74.

Klymus, K. E., Richter, C. A., Chapman, D. C., and Paukert, C. (2015). Quantification of eDNA shedding rates from invasive bighead carp *Hypophthalmichthys nobilis* and silver carp *Hypophthalmichthys molitrix*. *Biological Conservation* **183**, 77-84.

Lampa, S., Henle, K., Klenke, R., Hoehn, M., and Gruber, B. (2013). How to overcome genotyping errors in non-invasive genetic mark-recapture population size estimation-A review of available methods illustrated by a case study. *Journal of Wildlife Management* **77**, 1490-1511.

Lande, R. (1988). Genetics and demography in biological conservation. *Science* **241**, 1455-1460.

Langley, R. L., Higgins, S. A., and Herrin, K. B. (2006). Risk factors associated with fatal animal-vehicle collisions in the United States, 1995–2004. *Wilderness & Environmental Medicine* **17**, 229-239.

Leslie, D. M. (2011). *Rusa unicolor* (Artiodactyla: Cervidae). *Mammalian Species*, 1-30.

Lesser, M. R., Dovciak, M., Wheat, R., Curtis, P., Smallidge, P., Hurst, J., Kramer, D., Roberts, M., and Frair, J. (2019). Modelling white-tailed deer impacts on forest regeneration to inform deer management options at landscape scales. *Forest Ecology and Management* **448**, 395-408.

Litvaitis, J. A., Tash, J. P., Litvaitis, M. K., Marchand, M. N., Kovach, A. I., and Innes, R. (2006). A range-wide survey to determine the current distribution of New England cottontails. *Wildlife Society Bulletin* **34**, 1190-1197.

Liu, C., White, M., Newell, G., and Griffioen, P. (2013). Species distribution modelling for conservation planning in Victoria, Australia. *Ecological Modelling* **249**, 68-74.

Lone, K., Loe, L. E., Meisingset, E. L., Stamnes, I., and Mysterud, A. (2015). An adaptive behavioural response to hunting: surviving male red deer shift habitat at the onset of the hunting season. *Animal Behaviour* **102**, 127-138.

Luccarini, S., Mauri, L., Ciuti, S., Lamberti, P., and Apollonio, M. (2006). Red deer (*Cervus elaphus*) spatial use in the Italian Alps: home range patterns, seasonal migrations, and effects of snow and winter feeding. *Ethology Ecology & Evolution* **18**, 127-145.

Luikart, G., Ryman, N., Tallmon, D., Schwartz, M., and Allendorf, F. (2010). Estimation of census and effective population sizes: the increasing usefulness of DNA-based approaches. *Conservation Genetics* **11**, 355-373.

Mainali, K., Warren, D., Dhileepan, K., McConnachie, A., Strathie, L., Hassan, G., Karki, D., Shrestha, B., and Parmesan, C. (2015). Projecting future expansion of invasive species: comparing and improving methodologies for species distribution modeling. *Global Change Biology* **21**, 4464-4480.

Manel, S., Schwartz, M. K., Luikart, G., and Taberlet, P. (2003). Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology & Evolution* **18**, 189-197.

Marcer, A., Sáez, L., Molowny-Horas, R., Pons, X., and Pino, J. (2013). Using species distribution modelling to disentangle realised versus potential distributions for rare species conservation. *Biological Conservation* **166**, 221-230.

Martin, J., Tolon, V., Morellet, N., Santin-Janin, H., Licoppe, A., Fischer, C., Bombois, J., Patthey, P., Pesenti, E., Chenesseau, D., and Saïd, S. (2018). Common drivers of seasonal movements on the migration – residency behavior continuum in a large herbivore. *Scientific Reports* **8**, 7631.

Masters, P., Markopoulos, N., Florance, B., and Southgate, R. (2018). The eradication of fallow deer (*Dama dama*) and feral goats (*Capra hircus*) from Kangaroo Island, South Australia. *Australasian Journal of Environmental Management* **25**, 86-98.

Matthews, A., Ruykys, L., Ellis, B., FitzGibbon, S., Lunney, D., Crowther, M. S., Glen, A. S., Purcell, B., Moseby, K., Stott, J., Fletcher, D., Wimpenny, C., Allen, B. L., Van Bommel, L., Roberts, M., Davies, N., Green, K., Newsome, T., Ballard, G., Fleming, P., Dickman, C. R., Eberhart, A., Troy, S., McMahon, C., and Wiggins, N. (2013). The success of GPS collar deployments on mammals in Australia. *Australian Mammalogy* **35**, 65.

McCallum, K., Guerin, G., Breed, M., and Lowe, A. J. (2014). Combining population genetics, species distribution modelling and field assessments to understand a species vulnerability to climate change. *Austral Ecology* **39**, 17-28.

McCarthy, M., Moore, J., Morris, W., Parris, K., Garrard, G., Vesk, P., Rumpff, L., Giljohann, K., Camac, J., Bau, S. S., Friend, T., Harrison, B., and Yue, B. (2013). The influence of abundance on detectability. *Oikos* **122**, 717-726.

Meek, P. D., Ballard, G., Claridge, A., Kays, R., Moseby, K., O'Brien, T., O'Connell, A., Sanderson, J., Swann, D. E., Tobler, M., and Townsend, S. (2014). Recommended guiding principles for reporting on camera trapping research. *Biodiversity and Conservation* **23**, 2321-2343.

Meek, P. D., Ballard, G. A., and Fleming, P. J. S. (2015). The pitfalls of wildlife camera trapping as a survey tool in Australia. *Australian Mammalogy* **37**, 13-22.

Meisingset, E. L., Loe, L. E., Brekkum, Ø., Van Moorter, B., and Mysterud, A. (2013). Red deer habitat selection and movements in relation to roads. *The Journal of Wildlife Management* **77**, 181-191.

Mills, L. S., Citta, J. J., Lair, K. P., Schwartz, M. K., and Tallmon, D. A. (2000). Estimating animal abundance using noninvasive DNA sampling: promise and pitfalls. *Ecological Applications* **10**, 283-294.

Mora, M., Medina-Vogel, G., Sepúlveda, M. A., Noll, D., Álvarez-Varas, R., and Vianna, J. A. (2018). Genetic structure of introduced American mink (*Neovison vison*) in Patagonia: colonisation insights and implications for control and management strategies. *Wildlife Research* **45**, 344-356.

Mulvaney, J., Seddon, J., and Orgill, O. (2017). Monitoring impacts of sambar deer (*Rusa unicolor*) on forests in the cotter catchment, ACT: Monitoring design and initial findings. Canberra, ACT.

Myers, J. H., Simberloff, D., Kuris, A. M., and Carey, J. R. (2000). Eradication revisited: dealing with exotic species. *Trends in Ecology & Evolution* **15**, 316-320.

Nogales, M., Martín, A., Tershy, B. R., Donlan, C. J., Veitch, D., Puerta, N., Wood, B., and Alonso, J. (2004). A review of feral cat eradication on islands. *Conservation Biology* **18**, 310-319.

Nugent, G., Parkes, J., and G. Tustin, K. (1987). Changes in the density and distribution of red deer and wapiti in northern Fiordland. *New Zealand Journal of Ecology* **10**, 11-21.

Olah, G., Smith, A. L., Asner, G. P., Brightsmith, D. J., Heinsohn, R. G., and Peakall, R. (2017). Exploring dispersal barriers using landscape genetic resistance modelling in scarlet macaws of the Peruvian Amazon. *Landscape Ecology* **32**, 445-456.

Pacioni, C., Wayne, A. F., and Spencer, P. B. S. (2011). Effects of habitat fragmentation on population structure and long-distance gene flow in an endangered marsupial: the woylie. *Journal of Zoology* **283**, 98-107.

Parliament of Victoria (2017). Inquiry into the control of invasive animals on Crown land. Victorian Government, Melbourne.

Peel, B., Bilney, R. J., and Bilney, R. J. (2005). Observations of the ecological impacts of sambar (*Cervus unicolor*) in East Gippsland, Victoria, with reference to destruction of rainforest communities. *The Victorian Naturalist* **122**, 189-200.

Pennino, M. G., Paradinas, I., Illian, J. B., Muñoz, F., Bellido, J. M., López-Quílez, A., and Conesa, D. (2019). Accounting for preferential sampling in species distribution models. *Ecology and Evolution* **9**, 653-663.

Phillips, B. L., Chipperfield, J. D., and Kearney, M. R. (2008). The toad ahead: challenges of modelling the range and spread of an invasive species. *Wildlife Research* **35**, 222-234.

Piggott, M., Wilson, R., Banks, S., Marks, C., Gigliotti, F., and Taylor, A. (2008). Evaluating exotic predator control programs using non-invasive genetic tagging. *Wildlife Research* **35**, 617-624.

Poncet, S., Poncet, L., Poncet, D., Christie, D., Dockrill, C., and Brown, D. (2011). Introduced mammal eradications in the Falkland Islands and South Georgia. *Island invasives: eradication and management*, 332-336.

Poole, K. G., Reynolds, D. M., Mowat, G., and Paetkau, D. (2011). Estimating mountain goat abundance using DNA from fecal pellets. *Journal of Wildlife Management* **75**, 1527-1534.

Rollins, L. A., Woolnough, A. P., and Sherwin, W. B. (2006). Population genetic tools for pest management: a review. *Wildlife Research* **33**, 251-261.

Ross, J., Hearn, A. J., Johnson, P. J., and Macdonald, D. W. (2013). Activity patterns and temporal avoidance by prey in response to Sunda clouded leopard predation risk. *Journal of Zoology* **290**, 96-106.

Royle, J. A., Nichols, J., and Kéry, M. (2005). Modelling occurrence and abundance of species when detection is imperfect. *Oikos* **110**, 353-359.

Sacks, B., Brazeal, J., and Lewis, J. C. (2016). Landscape genetics of the nonnative red fox of California. *Ecology and Evolution* **6**, 4775-4791.

Southwell, D., Einoder, L., LahozMonfort, J., Fisher, A., Gillespie, G., and Wintle, B. A. (2019). Spatially explicit power analysis for detecting occupancy trends for multiple species. *Ecological Applications* **29**.

Spencer, P. B. S., Spencer, P. B. S., Yurchenko, A., David, V., Scott, R., Koepfli, K.-P., Driscoll, C., Obrien, S., and Menotti Raymond, M. (2016). The population origins and expansion of feral cats in Australia. *Journal of Heredity* **107**, 104-114.

Spitzer, R., Churski, M., Felton, A., Heurich, M., Kuijper, D. P. J., Landman, M., Rodriguez, E., Singh, N. J., Taberlet, P., van Beeck Calkoen, S. T. S., Widemo, F., and Cromsigt, J. P. G. M. (2019). Doubting dung: eDNA reveals high rates of misidentification in diverse European ungulate communities. *European Journal of Wildlife Research* **65**, 28.

Tan, W. S. (2018). Observations of occurrence and daily activity patterns of ungulates in the Endau Rompin Landscape, Peninsular Malaysia. *Journal of Threatened Taxa* **10**, 11245-11253.

Thompson, G. G., Thompson, S. A., and Bengsen, A. (2019). The value of camera traps in monitoring a feral-cat and fox reduction program. *Wildlife Research* **46**, 599-609.

Towerton, A., Penman, T., Kavanagh, R., and Dickman, C. R. (2011). Detecting pest and prey responses to fox control across the landscape using remote cameras. *Wildlife Research* **38**, 208-220.

Visintin, C. (2017) Modelling and predicting collision risks between wildlife and moving vehicles across space and time. Ph.D Thesis, The University of Melbourne.

Webley, L., Zenger, K., Hall, G., and Cooper, D. (2007). Genetic structure of introduced European fallow deer (*Dama dama*) in Tasmania, Australia. *European Journal of Wildlife Research* **53**, 40-46.

Webley, L. S., Zenger, K. R., English, A. W., and Cooper, D. W. (2004). Low levels of genetic variation within introduced Javan rusa deer (*Cervus timorensis russa*) in Australia. *European Journal of Wildlife Research* **50**, 137-140.

Wedrowicz, F., Mosse, J., Wright, W., and Hogan, F. (2018). Genetic structure and diversity of the koala population in South Gippsland, Victoria: a remnant population of high conservation significance. *Conservation Genetics* **19**.

Williams, K., Huyvaert, K., Vercauteren, K., Davis, A., and Piaggio, A. J. (2018). Detection and persistence of environmental DNA from an invasive, terrestrial mammal. *Ecology and Evolution* **8**, 688-695.

Yang, M., Sun, Y., Zhang, W., Yuan, H., and Zhang, M. (2019). Variation in winter daily range area of red deer (*Cervus elaphus xanthopygus*) based on DNA extracted from fecal samples. *Journal of Forestry Research* **30**, 1951-1958.

Zalewski, A., Piertney, S., Zalewska, H., and Lambin, X. (2009). Landscape barriers reduce gene flow in an invasive carnivore: geographical and local genetic structure of American mink in Scotland. *Molecular Ecology* **18**, 1601-1615.