Managing eastern grey kangaroos (*Macropus giganteus*) in peri-urban areas: Developing a novel contraceptive delivery system and assessing welfare outcomes from lethal and non-lethal management

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A thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

September 2025

Statement of originality

This thesis is the result of my work and has not been submitted for any other degree or qualification. All information obtained from the published or unpublished work of others has been acknowledged in the text, and a list of references is provided at the end of the thesis. During the preparation of this thesis, Grammarly was used for text enhancement purposes (such as spelling and sentence structure). No content produced by generative AI tools has been used in the preparation of this thesis.

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Date: 30/09/2025

Acknowledgements

This thesis represents a collective effort, made possible by the incredible support, wisdom and motivation provided by several people.

This research was supported by an Australian Government Research Training Program (RTP) Scholarship. It was funded by The University of Sydney, Nelson Bay Golf Course, NRMA Darlington Beach Holiday Park and Coffs Harbour City Council. All fieldwork conducted was licensed under scientific permits from the New South Wales National Parks and Wildlife Service and with animal ethics approval from the University of Sydney's Animal Ethics Committee.

I am profoundly grateful to the many individuals who contributed to this research, particularly the field volunteers whose tireless work and commitment were essential to data collection at all field sites. I would like to acknowledge the contribution of my primary supervisor, Professor Catherine Herbert (USYD), and thank her for the invaluable insights and direction throughout my PhD. The support and guidance provided by my second supervisor, Professor Peter Banks (USYD), was also very much appreciated.

A special thanks to Dr Andrew Bengsen for his patience towards the end of my thesis, his guidance and wisdom, and for giving me the time to work on my thesis during the most stressful times.

I would like to say a big thank you to my fellow PhD students and now friends, Dr Maquel Brandimarti, Dr Holly Cope, and Dr Georgia Thomas, for the many field trips we did together and for all the support, advice and kindness during these years. Thank you, Claire Wimpenny and Dr Melissa Snape (ACT Environment, Planning and Sustainable Development Directorate, Department of Primary Industries and Regional Development), for arranging access to the ACT

sites, assisting in sample collection and providing invaluable support. I would like to sincerely thank Dr Derek Spielman, Professor Alex Chaves and Dr Rebecca Hobbs for their generous contributions to this project.

I am extremely grateful to Dr Tamara Keeley (University of Queensland), who taught me how to run an infinite number of assays in the lab. Without her support, I would never have been able to complete all my laboratory work. I would also like to thank Dr Evelyn Hall for her statistical expertise, guidance and patience, and Dr Sebastien Comte for helping me create and interpret all the Bayesian models.

Finally, to my long-suffering friends and family: thank you for tolerating my hermit-like existence over the past five years, and especially during the final, intense year of thesis writing. Your love and support never diminished, even when I virtually disappeared from social life. My dad Luiz Carlos, mum Terezinha, sisters Fernanda and Flavia have been amazing in keeping me inspired and supporting my university journey. Their love and patience will forever be appreciated. To my husband Paulo Mendes, you are my cheerleader and biggest supporter, I cannot fully express how profoundly grateful I am for your presence in my life and for all the patience you demonstrated throughout these years, and your genuine enthusiasm for understanding every aspect of my research. You were not just supportive, but a constant source of motivation that helped me maintain my sanity during this challenging journey. This PhD would simply not exist without your unconditional love and support. I would never have finished this PhD if it wasn't for all of you in my life.

I dedicate this thesis to my father, Luiz Carlos, whose unwavering love and support continue to inspire me. His presence is greatly missed, but his influence lives on in every step I take.

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Thesis abstract

The eastern grey kangaroo (*Macropus giganteus*) is one of Australia's most iconic marsupial species. Population increases and local overabundance have become common in some areas, mainly due to anthropogenic changes such as improved pasture quality, provision of artificial water sources, and the reduction or removal of natural predators. In locations where kangaroo densities are high, evidence-based management strategies are needed to manage population growth and reduce human-wildlife conflicts. Wildlife managers often regard culling as an effective population control technique. However, its implementation can be influenced by ethical frameworks that consider issues including animal welfare, public perception, and logistical feasibility. Fertility control has been suggested as an alternative management strategy, but its effective use may be limited by the need to physically capture animals for treatment, although remote delivery techniques and other non-capture methods are also used in some management programmes for particular species.

In this thesis, a remote delivery system was developed for treating female eastern grey kangaroos with a long-acting contraceptive implant (Suprelorin®). The contraceptive efficacy and duration were assessed in three kangaroo populations in New South Wales (NSW), with Suprelorin® administered via remote (dart) delivery (4.7 mg) or hand injection at one of two doses (4.7 mg or 9.4 mg) after capture. The duration of contraception was similar between females in the remote treatment group and the hand-injected group. Comparable contraceptive efficacy and duration were also achieved regardless of dose (4.7 mg or 9.4 mg).

The impacts of both delivery methods on animal welfare were evaluated in a fourth peri-urban population in NSW. A before-after-control-impact (BACI) experimental design was used to

assess changes in behaviour, physiology and movement patterns in individual animals after treatment by remote delivery or capture and hand-injection. Neither method induced a stress response in kangaroos, with faecal glucocorticoid metabolite (FGM) concentrations remaining stable after both methods of treatment. Nor was there any change in behaviour or movement patterns that would indicate pain post-treatment. Based on these observations, the remote delivery system was found to be an effective and efficient strategy for administering Suprelorin® to peri-urban kangaroo populations. It negates the need to capture and handle kangaroos, which reduces the cost of treatment, with no indication of adverse impacts on animal welfare.

Although non-lethal management strategies, such as fertility control, are perceived by the community to raise fewer welfare concerns when managing overabundant populations, logistical challenges remain for managing large open populations. Therefore, culling continues to be the most frequently employed method for managing large kangaroo populations, despite concerns regarding negative welfare outcomes. One specific concern relates to the potential for negative welfare outcomes for survivor conspecifics, stemming from the psychological stress and disruption of social structures caused by culling. These potential welfare implications were examined by measuring changes in FGM concentrations in seven kangaroo populations subjected to one of three treatments: culling, disturbance (no culling) or a non-disturbance control. Culling did not increase stress levels in surviving kangaroos at culled sites. Instead, a decrease in FGM concentrations was observed post-culling, challenging existing beliefs regarding the stress caused by culling. However, due to the complexity of the stress response in wild animals, additional research should be conducted to validate these findings using larger sample sizes, and/or assessing multiple physiological or behavioural stress markers.

This research has implications that reach beyond kangaroo management, providing insights that may influence wider wildlife management policies and practices for other wildlife species. Future research should address logistical challenges associated with remote delivery methods, such as the capacity to identify treated individuals. This methodology should also be adapted to use with longer-lasting contraceptives, as the relatively short duration of action of Suprelorin® (~ 1 year) will remain a fundamental barrier to its broad-scale use. There is also a need to develop more comprehensive protocols to assess stress in free-ranging animals, so that animal welfare assessments can be more frequently incorporated into the evaluation of wildlife management techniques. This thesis provides evidence-based recommendations for implementing management strategies that benefit kangaroos, humans, and the environment while considering the welfare implications of these methods.

A note on the style and layout of this thesis

This thesis is presented as a series of chapters and manuscripts submitted for publication. Chapters 2, 3 and 4 are papers that have been submitted for publication and are under review. Manuscripts are listed in the following section. A single reference list is provided at the end of this thesis.

Manuscripts included in this thesis

Each data chapter has been formatted in accordance with the author guidelines of each journal. Chapters 2 and 4 are formatted using British English, while Chapter 3 is in American English.

Silva, FRO, Cope, H, Keeley T, Brandimarti, ME, Spielman, D, Thomas, G & Herbert, CA 2025, 'Hitting the mark: comparing the efficacy and duration of remote dart and hand-delivered deslorelin contraceptive implants in eastern grey kangaroos (*Macropus giganteus*)'. Manuscript submitted for publication. **(Chapter 2).**

Silva, FRO, Comte, S, Keeley T, Brandimarti, ME & Herbert, CA 2025, 'Assessing physiological, behavioral and movement responses to Suprelorin® implant delivery methods in free-ranging eastern grey kangaroos'. Manuscript submitted for publication. **(Chapter 3).**

Silva, FRO, Wimpenny, C, Snape, M, Comte, S & Herbert CA 2025, 'A tale of two stressors: Stress response of eastern grey kangaroos (*Macropus giganteus*) to culling and human disturbance in peri-urban landscapes'. Manuscript submitted for publication. **(Chapter 4).**

Author contributions

Confirmation of co-authorship of published work.

Author contributions to Chapter 2

Manuscript submitted for publication

Silva, FRO, Cope, H, Keeley T, Brandimarti, ME, Spielman, D, Thomas, G & Herbert, CA 2025, 'Hitting the mark: comparing the efficacy and duration of remote dart and hand-delivered deslorelin contraceptive implants in eastern grey kangaroos (*Macropus giganteus*)'. Manuscript submitted for publication.

Author contributions

F.R.O. Silva and C.A. Herbert conceived and designed the study. F.R.O. Silva, H. Cope, M. E. Brandimarti, D. Spielman, G. Thomas and C.A. Herbert collected the data. F.R.O. Silva performed all statistical analyses. F. R. O. Silva conducted laboratory analyses for Heritage Park and Darlington Park samples, and H. Cope conducted laboratory analyses for Nelson Bay samples, supported by T. Keeley. F.R.O. Silva wrote the manuscript. All co-authors reviewed drafts of the manuscript and provided feedback and revisions.

I, as co-author, endorse that the level of contribution by myself and the candidate indicated above is appropriate.

(All authors have signed a copy of the above and this is available upon request)

Author contributions to Chapter 3

Manuscript submitted for publication

Silva, FRO, Comte, S, Keeley T, Brandimarti, ME & Herbert, CA 2025, 'Assessing physiological, behavioral and movement responses to Suprelorin® implant delivery methods in free-ranging eastern grey kangaroos'. Manuscript submitted for publication.

Author contributions

F.R.O. Silva and C.A. Herbert conceived and designed the study. F. R. O. Silva, H. Cope, M. E. Brandimarti, and C. A. Herbert collected the data. S. Comte conducted GPS analyses and statistical analyses for GPS data. F.R.O. Silva performed all other statistical analyses with guidance from S. Comte. F.R.O. Silva, supported by T. Keeley conducted laboratory analyses. F.R.O. Silva wrote the manuscript. All co-authors reviewed drafts of the manuscript and provided feedback and revisions.

I, as co-author, endorse that the level of contribution by myself and the candidate indicated above is appropriate.

(All authors have signed a copy of the above and this is available upon request)

Author contributions to Chapter 4

Manuscript submitted for publication

Silva, FRO, Wimpenny, C, Snape, M, Comte, S & Herbert CA 2025, 'A tale of two stressors: Stress response of eastern grey kangaroos (*Macropus giganteus*) to culling and human disturbance in peri-urban landscapes'. Manuscript submitted for publication.

Author contributions

F.R.O. Silva and C.A. Herbert conceived and designed the study. F.R.O. Silva, C. Wimpenny, M. Snape, collected the data. F.R.O. Silva performed all statistical analyses with guidance from S. Comte. F.R.O. Silva conducted all laboratory analyses. F.R.O. Silva wrote the manuscript. All co-authors reviewed drafts of the manuscript and provided feedback and revisions.

I, as co-author, endorse that the level of contribution by myself and the candidate indicated above is appropriate.

(All authors have signed a copy of the above and this is available upon request)

Attesting author contribution statement

As supervisor for the candidature upon which this thesis is based, I can confirm that the authorship attribution statements above are correct.

Signed:

Catherine Hebert

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Date: 27th of February 2025

Abbreviations

ACT	Australian Capital Territory
ACTH	Adrenocorticotropic hormone
ADG	Average daily gain
AICs	Corrected Akaike Information Criterion
BACI	Before-After Control-Impact
BD	Becton Dickinson
BOM	Bureau of Meteorology
BRB	Biased random bridges
CI	Confidence Interval
cm	Centimetre
CrI	Credible interval
CV	Coefficient of variation
DP	Darlington Park
DPIE	Department of Planning, Industry & Environment
EGK	Eastern grey kangaroo
EIA	Enzyme immunoassay
EMM	Estimated marginal mean
ESS	Effective sample size
F	Female
FGM	Faecal glucocorticoid metabolite
g	Gram
GAMG	Goat anti-mouse immunoglobulin G
GAMM	Generalised additive mixed model

GC	Glucocorticoid
GLM	Generalised linear model
GLMM	Generalised linear mixed models
GnRH	Gonadotrophin-releasing hormone
GPS	Global positioning system
Ha	Hectares
HMC	Hamiltonian Monte Carlo
HP	Heritage Park
HPA	Hypothalamic-pituitary-adrenal
IASP	International Association for the Study of Pain
ID	Identification
kg	Kilograms
KMU	Kangaroo Management Unit
LAMN	Look at Me Now Headland
LH	Luteinizing hormone
LM	Linear model
LPY	Large pouch young
M	Male
MCMC	Markov Chain Monte Carlo
mg	Milligram
mL	Millilitre
MPY	Medium pouch young
n	Sample size
NB	Nelson Bay Golf Club

ng	Nanogram
NPY	No pouch young
NSW	New South Wales
PA	Pennsylvania
PY	Pouch young
RSPCA	Royal Society for the Prevention of Cruelty to Animals
s.e.	Standard error of the mean
SCHD	Subcutaneously high dose
SD	Supplementary data
SE	Standard error of the mean
SL	Scientific license
SST	Serum Separator Tube
TM	Trademark
TMB	3,3′,5,5′-Tetramethylbenzidine
USA	United States of America
USYD	The University of Sydney
VT	Vermont
YAF	Young at foot
µg	Microgram

Overview of the thesis chapters

This thesis investigates three aspects of eastern grey kangaroo (kangaroos hereafter) management practices: the efficacy and longevity of Suprelorin® contraceptive implants for fertility control; the physiological and behavioural responses of kangaroos to different contraceptive delivery methods; and the physiological effects of culling on conspecific kangaroos. It develops a remote delivery system as a novel alternative for the application of Suprelorin® contraceptive implants to peri-urban populations, exploring the efficacy and effectiveness of different doses, as well as the impacts of the contraceptive delivery methods on the physiology and behaviour of kangaroos. In addition, it assesses the welfare implications of culling by measuring changes in stress hormone concentrations at the population level before and after culling. Management strategies, both lethal and non-lethal, are widely used to control overabundant kangaroo populations in Australia. This thesis provides evidence-based recommendations for implementing management strategies that benefit peri-urban kangaroos, humans, and the environment while considering the welfare implications of these methods.

Chapter 1 reviews the effects of urbanisation on kangaroos in Australia, including current population control strategies for managing peri-urban kangaroo populations and their welfare implications. This chapter summarises the current scientific knowledge of kangaroo management practices, highlighting the limitations of current methods and the potential animal welfare implications of fertility control and culling strategies. It then presents the broad aims of the thesis.

Chapter 2 evaluates the efficacy of Suprelorin® contraceptive implants, as a non-lethal alternative to culling. The study develops a novel remote delivery method for the application of

Suprelorin®, assesses the efficacy of two different dosages (4.7 mg and 9.4 mg) and discusses the efficiency of remote delivery via darting compared with traditional hand-injection methods in three populations on the eastern coast of New South Wales. The high dose (9.4 mg) of Suprelorin® has been the dose of choice for kangaroos to date, but the efficacy of a single dose (4.7 mg) has not yet been assessed in comparison. Treatment with a single low-dose implant is desirable when developing a remote, intramuscular delivery technique. Therefore, Chapter 2 compares the efficacy and duration of the low dose using both delivery methods, and compares this to hand injection of the standard, high dose.

Chapter 3 uses the remote delivery method, and the single-dose implant assessed in Chapter 2, to investigate the stress and pain responses of female kangaroos to the two methods of contraceptive delivery using a Before-After Control-Impact (BACI) framework design. This study represents the first comprehensive examination of pain and stress associated with different delivery methods of Suprelorin® in a free-ranging population of kangaroos. Behavioural scoring of video footage and analysis of GPS tracking data were used to assess changes in behaviour and movement which may indicate pain associated with the delivery method. Changes in FGM concentrations were also assessed as a measure of the stress response to different treatment regimes. The findings will inform the development of best-practice protocols for remote delivery of contraceptives to free-ranging kangaroo populations.

Fertility control achieves population control through the suppression of breeding, thereby reducing the recruitment of new animals into the population. As such, there is not an immediate reduction in population size. Instead, the population will initially stabilise, with a decline only observed as adult animals naturally die within the population. This results in a delay between the implementation of control measures and a decrease in population abundance. This issue is further

intensified in kangaroo populations due to their unique reproductive biology. Such a lag can be problematic when more immediate management action is necessary. Therefore, a more effective approach may be to combine fertility control with culling strategies, initially reducing the population by removing kangaroos via culling and then maintaining the population by fertility suppression. Chapter 4 uses a BACI design to assess the impact of culling on the stress levels of conspecific kangaroos. FGM assessment was used as a proxy measure for stress in kangaroo populations across seven sites subjected to one of three treatments: 1. culling, 2. disturbance control (simulating the disturbance associated with culling, but without culling animals), and 3. control (no culling and no disturbance). This research will help to inform debates about the impact of culling on physiological stress responses in kangaroos and their welfare implications.

Chapter 5 provides a general discussion and conclusions for the thesis, emphasising the practical applications of the research and its implications for kangaroo management.

1. General introduction

1.1 The eastern grey kangaroo

The eastern grey kangaroo (*Macropus giganteus*, hereafter kangaroo) is one of Australia's most iconic marsupials and is an emblem of Australia's unique wildlife. These kangaroos inhabit a wide range of environments in the eastern and southern regions of Australia, including open forests, grasslands, woodlands, forests, and shrublands, thriving in areas with annual rainfall greater than 250 mm (Poole 1982; Russell 1974). Their distribution along the densely populated eastern coast often leads to their presence in peri-urban areas like golf courses (Figure 1.1), parks, and reserves (Herbert et al. 2006). While kangaroos are celebrated as an iconic symbol of Australia, attracting both domestic and international tourism (Higginbottom et al. 2004), their populations can become overabundant in certain areas, resulting in increased grazing pressure on native vegetation, declines in local biodiversity, and adverse impacts on primary industry productivity due to pasture competition (Coulson 2007). Consequently, they are subjected to management strategies such as culling or fertility control (Adderton 2004).

Figure 1.1. Eastern grey kangaroos resting in a golf course in Nelson Bay, NSW, Australia. Photography courtesy of Professor Catherine Herbert.

1.1.1 Kangaroo biology

Kangaroos are gregarious herbivores (Taylor 1984), feeding predominantly on grasses, and are most active during the late afternoon and early morning hours when they emerge from cover to forage in groups (Clarke et al. 1995; Russell 1974). The species is recognised for its distinct sexual dimorphism, with males weighing ≤85 kg and females ≤42 kg (Coulson 2008a; Poole 1982). They have a polygynous social structure, where dominant males often monopolise reproductive success (Montana et al. 2020). Males reach maturity from 43 months of age, and females from 14 to 18 months, with the onset of maturity in females indicated by eversion of the teats (Poole & Catling 1974). Kangaroos are monovular and polyoestrous, typically giving birth to a single offspring throughout the year, with a peak in births during summer (Poole & Catling 1974). Gestation lasts approximately 37 to 38 days, after which highly altricial young are born (Poole 1975). The newborn instinctively makes its way to the mother's pouch, where it continues its development during an extended lactation period (Poole 1975). The first emergence of young from the pouch occurs around 283 days after birth, and the final vacation occurs at approximately 319 days (Poole 1975). After the young permanently leaves the pouch, the female typically gives birth to a second young (Poole et al. 1982). During mid to late lactation, females can enter oestrus and mate, with successful mating leading to embryonic diapause when conditions are favourable (Clark & Poole 1967).

1.1.2 Embryonic diapause

Embryonic diapause is a reproductive adaptation in which the development of an embryo is temporarily suspended, delaying immediate implantation in the uterus (Clark & Poole 1967). In eastern grey kangaroos, embryonic diapause is closely linked to lactation and pouch occupancy but differs from that of most other macropods due to the absence of post-partum oestrus (Clark &

Poole 1967). Typically, females do not return to oestrus until after the young vacates the pouch. However, a minority of females may exhibit oestrus and mate while the young in the pouch is between 160 to 209 days old (Poole & Catling 1974). Successful mating will lead to the development of a new embryo. The resulting embryo enters diapause at the blastocyst stage, and resumes development after several months so that birth coincides with the first young permanently vacating the pouch (Clark & Poole 1967; Poole & Catling 1974) (Figure 1.2).

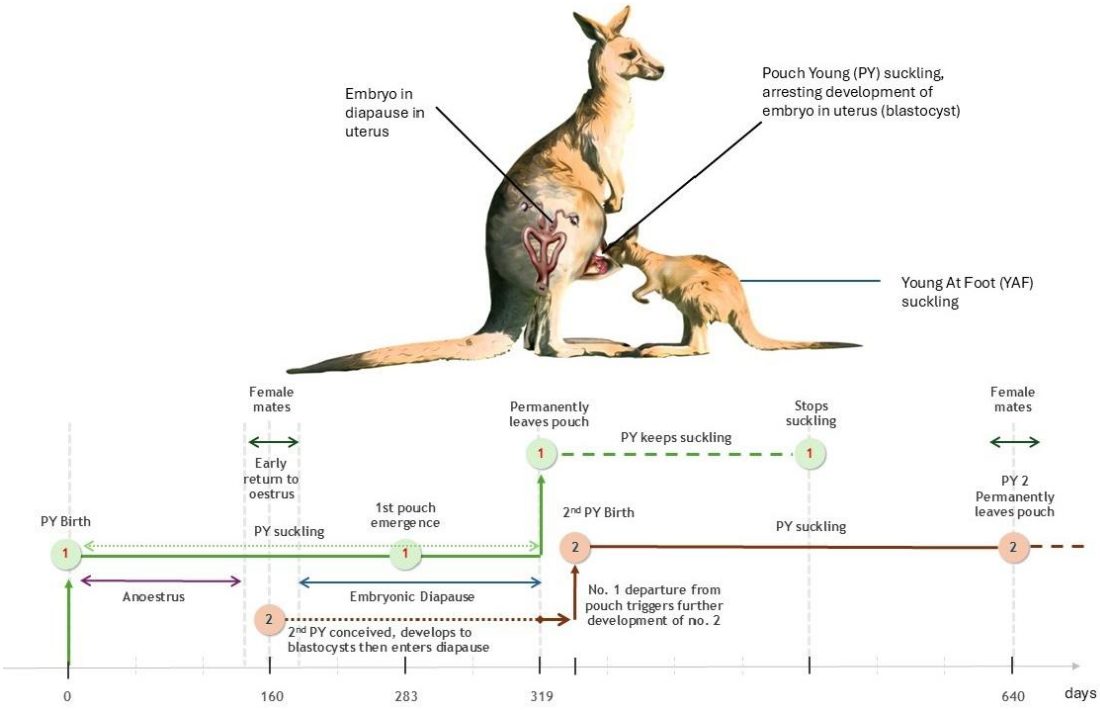

Figure 1.2. Embryonic diapause in the female eastern grey kangaroo. When the first pouch young (PY) is between 160 and 209 days old, the female may return to oestrus with successful mating resulting in an embryo. The embryo enters diapause at the blastocyst stage, resuming development so that birth coincides with the first PY permanently leaving the pouch.

Besides kangaroos, embryonic diapause is also observed in several other mammalian species, including eutherians such as the mouse (*Mus musculus*) and mink (*Neovison vison*), as well as marsupials like the tammar wallaby (*Notamacropus eugenii*), feathertail glider (*Acrobates*

pygmaeus) and honey possum (*Tarsipes rostratus*) (Renfree & Fenelon 2017). This reproductive strategy allows mammals to time the birth of their young to when environmental conditions are optimal for survival (Renfree & Shaw 2000), but it makes fertility control efficacy more challenging, as this strategy needs to be considered.

1.2 Kangaroo management in urban and peri-urban areas of Australia

Since European settlement over 230 years ago, Australia has experienced extensive environmental transformations that have greatly impacted its landscape and wildlife dynamics (Gordon et al. 2021). The introduction of livestock grazing, along with new water sources and the creation of pastures, have shifted habitat conditions considerably (Gordon et al. 2021). Land clearing for agriculture, primarily for cattle pastures, has been the main driver of forest loss in eastern Australia, accounting for 75% of this decline (WWF 2021b). Additionally, the effects of climate change, manifesting as more severe bushfires, prolonged droughts and extreme heat events, further threaten the stability of ecosystems and the survival of native species (Australian Government 2021).

Australia has one of the highest urbanisation rates globally, with over 76% of its population residing in major cities (Australian Government 2021). This increasing urban density, along with sprawling development, imposes additional pressures on the natural environment, leading to extensive land clearing, pollution and a consequential loss of biodiversity (WWF 2021a). Urban expansion contributes to habitat fragmentation, intensifies human-wildlife conflicts, and increases the occurrence of vehicle collisions (Australian Government 2021; Herbert et al. 2021). Two types of habitats arise due to increased urbanisation: ‘grey spaces’, where more than 80% of the area consists of buildings and hard surfaces, and ‘green spaces’, which encompass both

managed vegetation areas, such as gardens and golf courses, and also patches of unmanaged or remnant natural vegetation (Coulson et al. 2014).

These habitats modifications, combined with the near-eradication of the dingo (*Canis lupus dingo*) on the mainland and the suppression of indigenous hunting on kangaroo species (Johnson 2015), have led to increases in the distribution and density of several large macropod species across Australia, including the eastern grey kangaroo, red kangaroo (*Osphranter rufus*), western grey kangaroo (*M. fuliginosus*), and several wallaby species (Gordon et al. 2021). However, their ranges are geographically distinct, with the eastern grey kangaroo widespread across eastern and southern eastern Australia, the western grey kangaroo found mostly in southern and western regions and the red kangaroo inhabiting arid and semi-arid inland areas across central and western Australia (Croft & Witte 2021). In parts of their ranges, the two grey species can reach high densities in some peri-urban areas (Coulson 2008a; Coulson 2008b), causing conflict with people and presenting a unique set of management challenges (Herbert et al. 2021).

The eastern grey kangaroo is considered the most urban of these large macropods and it is known to use both grey and green spaces within urban and peri-urban areas (Coulson et al. 2014). In certain regions, populations occur within and on the fringe of cities, increasing the risks of vehicle collisions and occasional kangaroo attacks on people (Coulson et al. 2014). Managing kangaroo populations sparks controversy, as they are viewed as a treasured national wildlife symbol (important for tourism and the national identity), and as a 'problem' species in need of management (Pople & Grigg 1999).

Managing kangaroos in rural areas is primarily achieved through fencing or shooting. Shooting may be conducted for different purposes, including culling by individual landholders to mitigate damage at the property level, commercial harvesting for meat, leather and pelts (Hercock 2004),

and government-regulated culling. Commercial harvesting is regulated by each jurisdiction's government under the approval of the Australian Government Wildlife Trade Office (Australian Government 2025). Within each commercial harvest zone, a quota is typically set between 10% and 15% of the population based on environmental conditions and population densities determined through aerial surveys (NSW Government 2022). Commercial harvesters must comply with the *National Code of Practice for the Humane Shooting of Kangaroos and Wallabies for Commercial Purposes* (AgriFutures Australia 2020). Landholders must apply for a non-commercial licence and are issued tags for a finite number of kangaroos by the local government wildlife management office (NSW Government 2024). Government-regulated culling is implemented as part of a structured wildlife management program that aims to reduce kangaroos' impacts on some aspects of the biotic or abiotic environment and is conducted by professional contracted shooters (ACT Government 2017). Contracted shooters must comply with *The National Code of Practice for The Humane Shooting of Kangaroos and Wallabies for Non-Commercial Purposes* and aim for headshots to "ensure that sudden and humane death" is consistently achieved (Australian Government 2008). Despite efforts to regulate culling, animal welfare concerns still persist regarding the treatment of animals during culling operations, particularly the potential for suffering among individuals that are not killed instantly and the orphaning of dependent young (Descovich et al. 2015; Hampton & Forsyth 2016; McLeod 2004).

In urban areas, concerns about animal welfare and public safety often discourage the use of culling to manage overabundant macropod populations (Adderton 2004). Public opinion tends to support non-lethal management strategies such as the construction of exclusion fences (Wiggins et al. 2010), translocation (Higginbottom & Page 2010; Thompson et al. 2022), the use of

deterrents (e.g. predator odours used as repellents) (Cox et al. 2010), or the implementation of fertility control (Coulson et al. 2013; Herbert 2004; Wimpenny & Hinds 2018; Woodward et al. 2006). However, these non-lethal options are less frequently implemented, often due to the risks of redistributing the overpopulation problem, potential disease transmission, concerns for animal welfare, or high cost-benefit ratios (Asa & Porton 2005; Massei et al. 2010). Despite these challenges, fertility control has been proposed as a promising method for managing overabundant kangaroo populations, particularly in urbanised areas (Wimpenny et al. 2021).

1.3 Fertility control

Fertility control aims to reduce population growth by limiting the recruitment of new individuals into the population (Massei 2023). As fewer young are born, the population growth rate declines, which initially can lead to a stabilisation of the population and a subsequent reduction in the population size as older individuals gradually die (Asa & Porton 2005). By targeting the reproductive capacity of individuals within the population, fertility control agents act to disrupt the natural reproductive cycles that drive population increase, leading to a reduction in the number of offspring produced over time (Massei & Cowan 2014).

However, fertility control for wildlife management faces numerous limitations, particularly regarding the lag between the initiation of the treatment and the observable reduction in population sizes (Hobbs & Hinds 2018). Unlike lethal control methods (or other methods that remove animals from the population, e.g. translocation), infertility induced by contraceptives does not immediately result in a decrease in population size because the treated animals may continue to live for several years until their natural death (Massei 2023), especially for long-lived species. In saying that, managing long-lived species with fertility control (e.g. kangaroos) is generally more feasible than managing short-lived species (e.g. rodents) due to the dynamics of

population reproduction and lifespan (Massei 2023). For instance, in long-lived species, a lower percentage of the population needs to be rendered infertile to achieve the desired effect on population growth. Individuals with longer lifespans reproduce less frequently over their lifetime and if a portion of the population is made infertile, it can still impact the overall reproductive output over time (Massei 2023). In addition, many species have compensatory mechanisms that can counteract the effect of reduced births through increased survival and immigration, which can lead to diminished effectiveness of fertility control (Ransom et al. 2014).

Another consideration is assessing whether fertility control is biologically suitable for specific wildlife populations. This will depend on factors including local population dynamics, such as whether the population is open (with ongoing emigration and immigration) or closed (with little or no movement of individuals into or out of the population), sex ratios, age structure, estimated fecundity and mortality rates, and population size (Fagerstone 2002). Depending on these parameters, some populations treated only with contraceptives may remain at a high density for several years after initiating a contraception program, and thus it may be more effective to first reduce the population to a desired density using other management techniques, and then use fertility control to maintain that level (Fagerstone 2002).

The social acceptance of fertility control as a wildlife management tool, although generally higher than lethal control, can also pose challenges (Fagerstone 2002). Wildlife managers are often sceptical about using contraceptives instead of traditional lethal methods, such as shooting or toxicants, and some agencies remain reluctant to embrace fertility control as an effective management method (Fagerstone et al. 2010). Human safety and delivery mechanisms are additional limitations to consider, since most fertility control methods require capturing and sedating animals, which could pose potential hazards to those administering the agents.

(Fagerstone et al. 2010), as well as risks to the animal receiving the contraceptive (Hampton et al. 2015b). The higher economic costs of fertility control are often another barrier to implementation (Massei & Cowan 2014).

Several studies have investigated the use of fertility control in managing kangaroo populations (Coulson et al. 2008; Coulson et al. 2013; Coulson & Wilson 2024; Herbert 2004; Woodward et al. 2006), and a number of comprehensive reviews on this topic have been published in recent years (Hampton et al. 2015b; Kirkpatrick et al. 2011; Massei 2023; Massei & Cowan 2014; Wimpenny et al. 2021). Wimpenny et al. (2021) identified several key characteristics of developing effective fertility control methods in wildlife management. These include high species specificity; high efficacy ideally achieved with a single, fast-acting and long-lasting treatment; minimal negative effects on animal welfare, including social behaviour; safety during gestation and lactation; a manageable frequency of repeat treatments; and cost-effectiveness of both the contraceptive substrate and its delivery system. The authors also noted that fertility control strategies targeting females are generally more effective, with fewer adverse side effects on behaviour and welfare, longer duration, and greater overall cost-effectiveness. This is because sterilising every male in a population is impractical, and untreated males can continue to mate successfully with many females (Wimpenny et al. 2021). Fertility control techniques that have been trialled in eastern grey kangaroos and their mechanisms of action and mode of administration are summarised below.

1.3.1 Surgical sterilisation

Surgical sterilisation techniques, such as tubal ligation and ovariectomy in females, and castration and vasectomy in males, offer permanent solutions for controlling fertility in kangaroos (Wimpenny et al. 2021). Tubal ligation and vasectomy are surgical contraception

methods that block reproductive pathways (sperm and egg transport) without removing the gonads whereas castration and ovariectomy are procedures that remove the gonads entirely (i.e. gonadectomy), preventing reproduction and disrupting hormone production (Hampton et al. 2015b). These techniques have been used in captive and semi-captive kangaroo populations (Colgan & Green 2018; Tribe et al. 2014; Wimpenny et al. 2021) and prevent fertility permanently.

1.3.2 Immunocontraception

Immunocontraceptive vaccines work by stimulating an immune response against reproductive proteins or hormones required for reproduction, thereby disrupting key reproductive processes such as ovulation, sperm production, or fertilisation and are often combined with adjuvants that enhance the body's immune response to the vaccine (Massei 2023). Two contraceptive vaccines have been trialled on kangaroos: zona pellucida (ZP) and gonadotrophin-releasing hormone (GnRH)-based vaccines (GonaCon™).

ZP vaccines target the zona pellucida, a protective layer of proteins that surrounds the egg. They block egg fertilisation by stimulating the immune system to produce antibodies that interfere with sperm recognition and binding (Wimpenny et al. 2021). A study using a recombinant marsupial ZP vaccine in kangaroos resulted in a significant and sustained antibody response, preventing offspring production in all immunised kangaroos throughout the 13-month duration of the trial (Kitchener et al. 2009). This outcome followed an initial immunisation and two booster doses, administered at 12 and 28 weeks after the initial injection (prior to the breeding season). However, the study did not determine the long-term duration of infertility, thus further research is needed. To date, no other studies appear to have been published on the use of recombinant ZP vaccines in kangaroos.

The GonaCon™ immunocontraceptive vaccine induces infertility by stimulating the production of antibodies that target GnRH, inhibiting the release of reproductive tropic hormones, follicle-stimulating hormone (FSH) and luteinising hormone (LH), essential for reproductive function (Pinkham et al. 2022). By immunising against GnRH, the vaccine prevents signalling at the gonads, suppressing ovulation in females and spermatogenesis in males. The GnRH is conjugated to a highly immunogenic foreign carrier protein and combined with an oil-in-water adjuvant containing killed *Mycobacterium avium* (Baker et al. 2023), which stimulates a persistent immune response, resulting in prolonged antibody production against GnRH. This formulation can induce long-term infertility with a single injection, as observed in various species, including white-tailed deer (*Odocoileus virginianus*) and wild boar (*Sus scrofa*) (Evans et al. 2015; Massei et al. 2008; Miller et al. 2008). It has been tested in eastern grey kangaroos, whereby all females treated as subadults with a single injection were infertile for three years, and over 80% of females were infertile for 8 years (Wimpenny et al. 2021). GonaCon™ is currently being trialled as a remote dart delivery method for kangaroos, with preliminary results indicating similar efficacy between hand injection and a single shot remotely delivered to kangaroos, with both methods inducing 90% infertility three years post-treatment (Wimpenny et al. 2021). However, in feral horses (*Equus ferus caballus*), Baker et al. (2023) found that GonaCon™ is most effective and suppresses fertility for longer when the initial vaccination is followed by a booster dose administered every 4 to 6 years.

1.3.3 Synthetic progestins

Slow-release implants containing a synthetic progesterone analogue, such as levonorgestrel, work by inhibiting multiple reproductive processes in females. The primary mechanism of action is the inhibition of follicular development and ovulation by suppressing the pre-ovulatory surge

of LH (Hynes et al. 2007). Additionally, they thicken the cervical mucus, reducing its volume and increasing its viscosity, which impedes sperm progression through the cervical canal (Segal et al. 1991). They also interfere with endometrial development, making it unfavourable for implantation of fertilised eggs (Segal et al. 1991). Several studies have evaluated the efficacy of levonorgestrel implants for fertility control in kangaroos (Coulson et al. 2008; Nave et al. 2002; Poiani et al. 2002; Wilson & Coulson 2016). Results indicate that a higher dose of levonorgestrel is necessary for long-term effective fertility control. When three implants were used (3 × 70 mg), efficacy surpassed 90% infertility during the first five years and remained above 57% eight years after treatment (Wilson & Coulson 2016).

1.3.4 GnRH agonists

The use of GnRH agonists, primarily deslorelin, has received considerable attention as a potential fertility control method for eastern grey kangaroos (Descovich et al. 2015; Herbert et al. 2006; Wilson & Coulson 2016; Wilson et al. 2013; Woodward et al. 2006) and it is also the focus of interest of this thesis. Deslorelin, formulated into a slow-release implant (Suprelorin®, Virbac Australia, Macquarie Park, NSW, Australia), was originally developed for the domestic dog (Trigg et al. 2001). Suprelorin® implants contain 4.7 mg of the GnRH agonist deslorelin (D-Trp^{6}-Pro^{9}-des-gly^{10}-GnRH ethylamide), measuring 2.3 x 12.5 mm. The continuous release of deslorelin from the implant exerts its contraceptive effect by inhibiting the synthesis and release of LH and FSH by the pituitary gland, thus disrupting follicular development and oestrous cycles in females, prolonging anestrus (Figure 1.3) (Herbert et al. 2005). This contraceptive has been successfully trialed in many marsupials, including brushtail possums (*Trichosurus vulpecula*) (Eymann et al. 2007; Lohr et al. 2009), tammar wallabies (*Notamacropus eugenii eugenii*) (Herbert et al. 2007; Herbert et al. 2013), Tasmanian devils (*Sarcophilus harrisii*) (Cope et al.

2019), captive eastern grey kangaroos (Herbert et al. 2006; Woodward et al. 2006), and free-ranging eastern grey kangaroos (Wilson et al. 2013), with minimal effects on behaviour (Cope et al. 2018a; Herbert & Trigg 2005; Wilson et al. 2013). Herbert et al. (2006) found that a pre-commercial implant formulation of deslorelin successfully inhibited reproduction in captive female kangaroos for up to 24 months, with a minimum contraceptive duration of 19 months in females that received a higher dose (2 x 10 mg) and 7.5 months for those receiving a lower dose (10 mg). In a free-ranging population, Wilson et al. (2013) found that high-dose (9.4 mg) Suprelorin® implants initially suppressed fertility effectively in 100% of treated females in the first year of treatment, but the effects diminished over time, with fertility reversing to normal rates by year three.

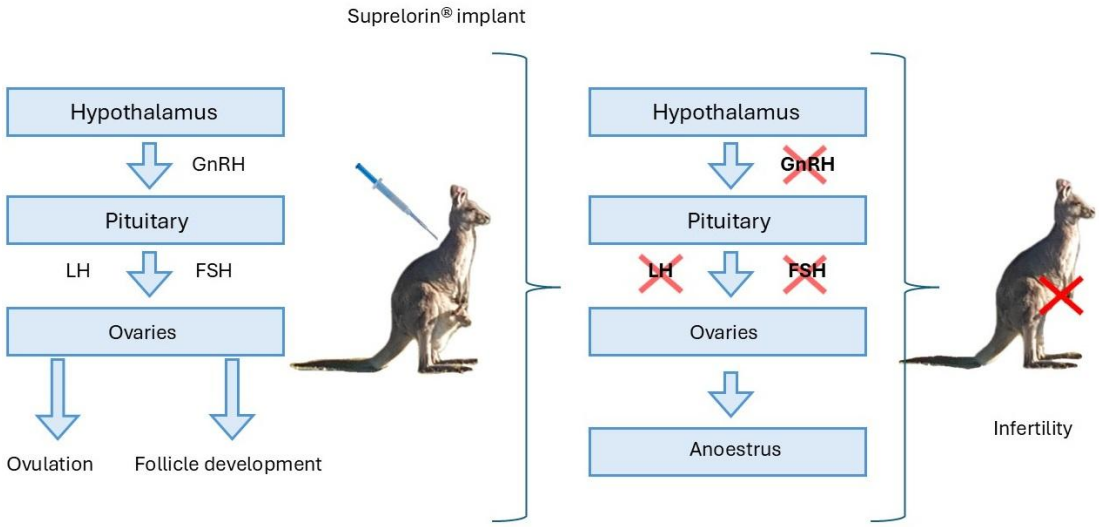

Figure 1.3. Diagram showing the suppression of reproduction by Suprelorin® implants. Suprelorin® contains deslorelin (a gonadotrophin-releasing hormone (GnRH) agonist) that is released slowly from the implant over time. The continuous release of deslorelin leads to the desensitisation of GnRH receptors in the pituitary gland, resulting in a suppressed production of follicle-stimulating hormone (FSH) and luteinising hormone (LH). This suppression inhibits ovulation and follicular development in the ovaries. Consequently, oestrogen and progesterone levels are reduced, and the female enters a state of anoestrus (Herbert et al. 2005).

1.3.5 Current limitations of fertility control

While there have been many advances in the use of fertility control in kangaroos in the last two decades, and multiple options are available, they are not without limitations. For example, all methods currently used in non-experimental settings require animal capture for treatment, which has inherent welfare risks and is resource-intensive. The duration of action, mode of delivery and ease of administration post-capture vary between the different approaches and create a different risk-benefit (or cost-benefit) profile for each agent (Table 1.1). Currently available contraceptive implants and vaccines for macropods do not interfere with active pregnancy, lactation or pouch young development, so they can be safely delivered to females who are pregnant or supporting young (Herbert et al. 2006; Nave et al. 2002; Snape 2012; Wilson et al. 2013).

1.3.5.1 Kangaroo capture

Capturing kangaroos for fertility control usually involves trapping (Nave et al. 2002; Wilson et al. 2013), immobilisation via darting (Tribe et al. 2014; Wimpenny & Hinds 2018) or pole syringe (Wilson & Coulson 2016), or herding and darting (Colgan et al. 2019). The most commonly used anaesthetic for this purpose is a combination of tiletamine hydrochloride and zolazepam hydrochloride, marketed as Zoletil® 100 (Virbac Pty. Ltd, Milperra, Australia) (Brandimarti et al. 2021; Herbert et al. 2020; Mayberry et al. 2014), with recovery times varying from 1 to 4 hours (King et al. 2011). After the contraceptive is applied and other required procedures conducted, the animal is left to recover in a shaded place, typically with the head covered, with monitoring required until full recovery to prevent the animal from getting injured (Brandimarti et al. 2021).

1.3.5.2 Modes of delivery

Suprelorin® implants are administered subcutaneously between the shoulder blades of the animal (Figure 1.4) (Herbert et al. 2006; Wilson et al. 2013), while GonaCon™ is a viscous, oily emulsion (1 mL = 1000 µg) that is administered intramuscularly in the rump region (Wimpenny & Hinds 2018). Remote dart delivery is currently being investigated in kangaroo populations in New South Wales and the ACT, for both Suprelorin® (this thesis) and GonaCon™ (Wimpenny et al. 2021). Levonorgestrel implants are also inserted subcutaneously between the shoulder blades, but their larger size (40 x 2.5 mm) and need for multiple implants require a 3-5 mm incision under local anaesthesia (Coulson & Wilson 2024; Wilson & Coulson 2016), making them unsuitable for remote delivery. Limited data is available regarding the ZP immunocontraceptive vaccine for kangaroos. In Kitchener et al. (2009), the ZP vaccine was administered as four injections: subcutaneously to two sites in the groin and intramuscularly to two sites in the rump (0.25 mL per site = 100 µg), with a booster applied 12 weeks later to maintain the immune response. Multiple doses were needed for a lasting effect, which limits their suitability for use in free-ranging populations. However, ZP vaccines have been used via remote dart delivery in feral horse populations, where administration involves a single intramuscular injection followed by a booster (Rutberg et al. 2017; Schulman et al. 2024), highlighting the need for further research to adapt ZP vaccines for remote delivery in kangaroos. Surgical sterilisation procedures require more invasive techniques involving surgery conducted by veterinarians and requiring the animal to be under general anaesthesia (Colgan & Green 2018; Tribe et al. 2014).

Table 1.1. Fertility control methods that have been trialled in eastern grey kangaroos, duration and cost-effectiveness.

Method	Type	Sex targeted	Duration	Cost-effectiveness	Reference
Surgical sterilisation (gonadectomy, vasectomy or tubal ligation)	Surgery	Males and/or females	Permanent	Low as it incurs additional expenses with veterinarians and surgical equipment	1, 2
Zona pellucida	Vaccine	Females	<2 years; requires additional boosters	Low; boosters required 1- 4 weeks after initial treatment	3
GonaCon™	Vaccine	Females	>3 years	Medium if hand delivered, high if remote delivery is effective	4
Levonorgestrel	Hormonal implant	Females	3 – 7 years, dose-dependent	Medium, multiple implants needed and extra costs for incision equipment	5, 6
Deslorelin	Hormonal implant	Females	1 – 2 years	Medium if hand delivered, high if remote delivery is effective. Reapplication is required for a longer duration.	5, 7, 8, 9, 10

References: 1 Colgan and Green 2018; 2 Tribe et al., 2014; 3 Kitchener et al. 2009; 4 Wimpenny and Hinds, 2018; 5 Wilson and Coulson, 2016; 6 Coulson and Wilson, 2024; 7 Coulson et al., 2013; 8 Wilson et al., 2013; 9 Woodward et al., 2006; 10 Herbert et al., 2006.

1.3.5.3 Accessibility of the method

Kangaroo fertility control methods differ in the "accessibility" of the drug formulations and the degree of training required for operators to administer them. Surgical sterilisation requires access to skilled veterinarians and surgical resources, but the techniques are well-established (Colgan & Green 2018). Contraceptive implants are much easier to administer, particularly in the case of Suprelorin® and Gonacon™, which require a simple subcutaneous or intramuscular injection that

animal managers (non-veterinarians) can be trained to administer. Levonorgestrel requires more skill because the implantation procedure is more invasive due to their larger size and the need for multiple implants (Coulson & Wilson 2024; Wilson & Coulson 2016).

The contraceptive agents also vary in their relative availability in Australia. Suprelorin® is registered for use in dogs and is widely available by veterinary prescription in Australia (Herbert et al. 2006; Wilson et al. 2013). It can be used "off-label" in kangaroos under permits issued by the Australian Pesticides and Veterinary Medicines Authority (APVMA) (Australian Pesticides and Veterinary Medicines Authority 2025). GonaCon™, however, is not commercially available or registered in Australia yet, and requires special import permits (Wimpenny & Hinds 2018), which limits availability to different operators. Levonorgestrel implants are produced locally in relatively large numbers for government-operated koala fertility control programs in the southern states and are therefore, relatively easy to access for kangaroo management in Australia (Coulson & Wilson 2024; Wilson & Coulson 2016).

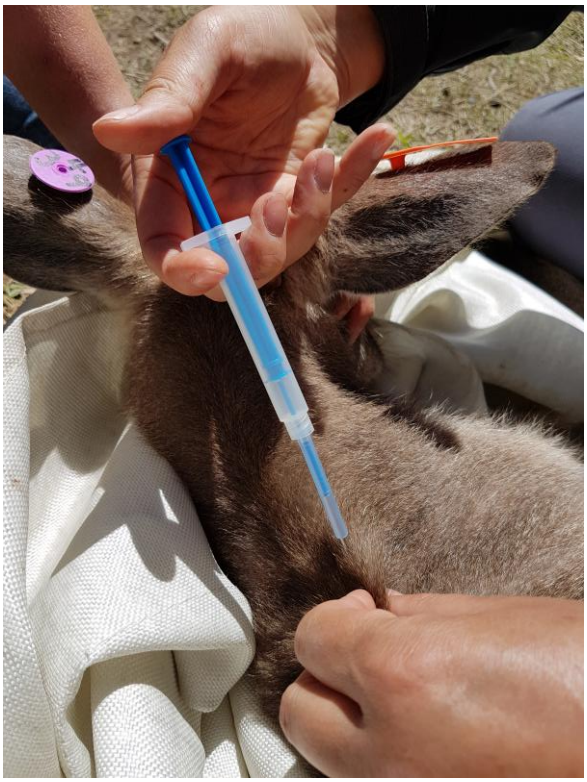

Figure 1.4. A Suprelorin® implant being subcutaneously inserted between the shoulder blades of a tagged female eastern grey kangaroo.

1.3.6 Welfare and Fertility Control

From a welfare perspective, fertility control could potentially be seen as more harmful than lethal strategies, due to stress imposed by manually handling, darting or anaesthetising animals (Hampton et al. 2015b; Hampton et al. 2019) and/or depriving animals of positive welfare states and natural behaviours such as mating, rearing offspring and dispersal (Hampton et al. 2019; Mellor & Beausoleil 2015). Although empirical data exist on the behavioural responses of kangaroos to fertility control treatment (Poiani et al. 2002; Wilson et al. 2013; Woodward et al. 2006), limited data are available on the welfare impacts associated with the fertility control

treatment process. This gap makes it difficult to critically evaluate the overall welfare impacts of this management approach for kangaroos.

Moreover, in urban and peri-urban areas, lethal management of overabundant populations may be impractical due to the proximity to residential areas, public safety concerns and logistical constraints (Wimpenny et al. 2021). In such contexts, fertility control may represent a non-lethal alternative that, despite its welfare considerations, avoids some of the immediate risks and public opposition associated with culling.

1.4 Culling

Culling by shooting is widely used for managing kangaroo populations to minimise their impacts on agriculture and native vegetation, and to minimise human-wildlife conflicts in rural areas (Gordon et al. 2021). However, public attitudes toward culling kangaroos in nature reserves and parks near urban areas are mixed and context-dependent. While opposition exists, especially among animal welfare advocates (Ramp 2013), ACT government surveys have also found that a significant proportion of the local population supports culling under certain circumstances, particularly for conservation purposes (McIlroy 2019). Shooting must conform to the *National Code of Practice for The Humane Shooting of Kangaroos and Wallabies for Non-Commercial Purposes* (Australian Government 2008), which stipulates that adult kangaroos must be shot in the head for an instant and painless death. The code specifies that only centrefire rifles are to be used for shooting larger kangaroo species, while rimfire rifles may be used for smaller species or in particular situations. Using firearms in urban areas requires careful consideration due to significant safety concerns for people, which often renders shooting a challenging management method in these locations (Austiger Conservation 2023).

In addition to safety concerns for humans, ethical concerns arise regarding the treatment of animals during culling operations. Critics argue that culling raises moral questions about how kangaroos are treated and their "right" to live undisturbed in their natural habitats, reflecting broader societal debates about animal welfare and intrinsic value (McKinnon et al. 2018; RSPCA Australia 2022). Even when culling is conducted with precision and conforms with guidelines provided to minimise suffering, public concerns persist regarding the pain and suffering inflicted on individual animals during the process and their surviving conspecifics thereafter (Descovich et al. 2015). Culling can also induce psychological stress in surviving individuals, disrupting social structures (Shannon et al. 2013) and potentially exacerbating conflict with humans (O'Neill et al. 2017). These discussions promote critical inquiries into the physiological and ecological effects of culling on targeted populations.

1.4.1 Culling in the Australian Capital Territory (ACT)

In the Australian Capital Territory (ACT), kangaroo management is carried out via a structured conservation culling program designed to maintain ecological balance and protect native flora and fauna (ACT Government 2018). This management strategy is guided by the Nature Conservation (Eastern Grey Kangaroo) Culling Calculator, which calculates the appropriate kangaroo densities necessary to preserve grassland conservation values (ACT Government 2018). Culling is conducted to mitigate the impacts of overgrazing, which can cause a reduction in the structural complexity of ground layer vegetation, thereby threatening species present in this habitat, such as the critically endangered yellow box-red gum grassy woodland and natural temperate grassland, the superb parrot (*Polytelis swainsonii*) and the striped legless lizard (*Delma impar*) (ACT Government 2018). The ACT's diverse ecosystems, characterised by a mix of grasslands, woodlands, and wetlands, support a variety of native species, making it vital to

manage kangaroo populations effectively. By closely monitoring kangaroo populations and conducting culling in consultation with ecological experts, the ACT aims to maintain a sustainable environment that balances the needs of wildlife with the preservation of its distinctive biodiversity (ACT Government 2018). However, the appropriateness of culling as a long-term population management strategy is subject to ongoing debate, prompting calls for more evidence-based research on its impact on animal welfare and consideration of alternative methods (Miguel et al. 2020).

1.4.2 Welfare and Culling

In Australia, kangaroos are mainly killed using firearms, which occurs through licensed culling or commercial harvesting (Descovich et al. 2015). Concerns exist about the welfare of animals during culling operations, especially regarding the suffering of animals that are not killed immediately (Descovich et al. 2015; Hampton & Forsyth 2016; McLeod 2004). Despite the adoption of codes of practice aimed at minimising suffering, there is still some public anxiety about the pain experienced by individual animals and the extent to which these codes are consistently applied and enforced (Ben-Ami et al. 2014). Additionally, the impact on surviving conspecifics is an important consideration (McKinnon et al. 2018), as it may lead to psychological stress and disruption of social structures (Shannon et al. 2013) These changes can alter the population's sex ratio and age distribution over time, impacting breeding success and survival (Beasley et al. 2013). While some potential welfare issues can be mitigated by ensuring compliance with the codes of practice, questions regarding the welfare impacts on survivor conspecifics and the effects of disrupted social structures remain unquantified.

1.5 Assessing animal welfare

Assessing welfare in wild animals can be challenging, as it encompasses a range of physical and environmental elements, and their influence on the animal's emotional state. While it is difficult to measure the emotional state of an animal, physiological markers can be used to make inferences about the welfare status of a wild animal (Hemsworth et al. 2015).

Physiological markers provide an objective measure of an animal's response to experienced sensations (Beaulieu 2024). There is a range of physiological markers that can be employed in the assessment of animal welfare, including the absence of illness, immune markers, heart rate, respiratory rate, and stress hormone concentrations, predominantly cortisol (Polgár et al. 2019), but their utility in free-ranging animals varies. For free-living wildlife, it is important to employ measures that are easily validated in a new species, can be collected/measured non-invasively and with minimal disturbance, and have a known link to the affective state of an animal (Beaulieu 2024). For these reasons, stress hormone concentrations, measured using non-invasive sample collection methods, are used as the primary method to assess the welfare outcomes of kangaroos in this thesis (described in more detail in section 1.6). Behavioural assessments are also incorporated in Chapter 3 as an additional measure of pain and welfare.

It should be noted that there is some debate about the use of the term "humaneness" in conservation biology, predominantly because it is often reduced to a binary measure (i.e. a method is either "human" or "inhumane") (Hampton et al. 2020). However, given that the term is still embedded in the wildlife management literature, for example: humaneness is referenced as a key desirable characteristic for wildlife control (Wimpenny et al. 2021), this term is used in this thesis to describe the relative impacts of different management techniques on animal welfare.

1.6 Stress

Stress is defined as physiological and psychological responses to perceived demands or challenges that exceed an individual's coping abilities (Dobson & Smith 2000). These responses disturb the body's internal balance, called homeostasis (Novak et al. 2013). It involves a complex interaction between environmental factors and an individual's perception of these factors, which can lead to a range of responses across various biological systems, including hormonal, behavioural, autonomic nervous system, and immune responses (Palme et al. 2005).

Previous studies have indicated that stressors from seasonal variations, food availability, population density, and human impacts can affect the behaviour and movement of wildlife (Favreau et al. 2014). Capture, darting, and handling may also induce stress in these animals (Brivio et al. 2015; Herbert et al. 2020), as well as culling, which may cause psychological stress for the remaining conspecifics, potentially altering social dynamics (Shannon et al. 2013) and heightening conflicts with humans (O'Neill et al. 2017). Additionally, acute stress can result in myopathy, a condition in which large muscle masses may be damaged, leading to the release of significant quantities of myoglobin, which can cause death either immediately following capture or several days later (Breed et al. 2019).

Indicators of stress, both behavioural and physiological, have been utilised to evaluate the welfare of kangaroos (Brunton et al. 2020; Herbert et al. 2020; Sherwen et al. 2015). For instance, Herbert et al. (2020) assessed the stress responses of kangaroos to lightweight tracking collars using behavioural indicators and the measurement of faecal glucocorticoid metabolite (FGM) concentrations. In another study, Brunton et al. (2020) found that in urban areas with dense human populations and high levels of development, the stress levels in kangaroos correlate with increased FGM concentrations, while kangaroos in less disturbed, non-urban areas showed lower stress levels.

The duration and intensity of stressors determine whether the response is acute (short-term) or chronic (prolonged) (Rao et al. 2024). Acute and chronic stressors can cause variations in the responsiveness of the hypothalamic-pituitary-adrenal (HPA) axis and the secretion of glucocorticoids (GC) (Touma & Palme 2005) (Figure 1.5). GC are one of the most common physiological measures of stress, and their metabolites can offer valuable insights into the physiological state of wildlife populations (Sheriff et al. 2011).

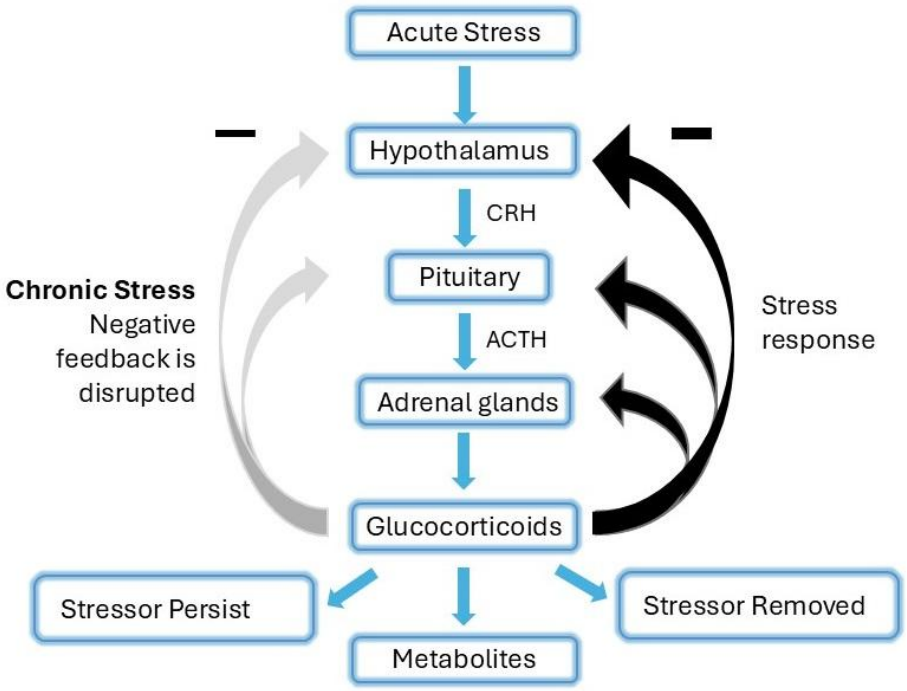

Figure 1.5. Activation of the hypothalamus-pituitary adrenal (HPA) in response to stress and its effects on biological functions and feedback loops. A stressor triggers the hypothalamus to release corticotrophin-releasing hormone (CRH), which stimulates the pituitary to secrete adrenocorticotropic hormone (ACTH). ACTH stimulates the adrenal glands to produce glucocorticoids (GC) such as cortisol or corticosterone. GC metabolites are excreted in faeces (Touma & Palme 2005). In acute stress, the negative feedback loop (black arrows) efficiently regulates the HPA axis. Under chronic stress, prolonged exposure to high GC levels can desensitise GC receptors in the hypothalamus and the pituitary, weakening the negative feedback loop (grey arrows) and leading to dysregulation of the HPA and persistent GC production.

1.6.1 Glucocorticoids and the HPA axis

Glucocorticoids are steroid hormones that are released in response to a stressful stimulus (stressor) via activation of the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis plays a central role in the production and secretion of GC (Touma & Palme 2005). When an animal perceives a stressor, the hypothalamus detects the stress signals and releases corticotrophin-releasing hormone (CRH), a tropic hormone that stimulates the pituitary gland to secrete adrenocorticotropic hormone (ACTH) into the bloodstream (Smith & Vale 2006). ACTH travels through the blood to the adrenal glands and prompts them to release GC hormones (cortisol and corticosterone), referred to as stress hormones (Herman & Cullinan 1997; Sapolsky et al. 2000). Once the stressor is removed or addressed, the elevated levels of GC exert negative feedback on both the hypothalamus and pituitary glands to reduce the production of CRH and ACTH, thereby lowering cortisol levels back to baseline (Touma & Palme 2005). This feedback mechanism maintains homeostasis. However, various factors can influence this process, including genetic predispositions, environmental conditions, social structure, age and the individual's reproductive status (Dantzer et al. 2014).

Under normal circumstances, GC play a crucial role in energy regulation, glucose metabolism, inflammation control, and reproduction (Fanson et al. 2017). During episodes of acute stress, GC facilitate energy mobilisation and promote adaptive behaviours such as flight responses (Möstl & Palme 2002). However, prolonged increases in glucocorticoid levels resulting from chronic stress can adversely affect an individual's health, compromising the immune system, causing muscle deterioration, and impacting reproductive health (Fanson et al. 2017). Given the important role of GC secretion in the physiological stress response, GC are frequently measured to determine stress responses on animals using a range of biological media (Sheriff et al. 2011).

Most wildlife studies focus on determining concentrations of GC metabolites in faeces, as these samples can usually be collected without inducing an acute stress response to sampling (Palme et al. 2005).

1.6.2 Faecal glucocorticoid metabolites (FGM)

Faecal glucocorticoid metabolites (FGM) are the waste products of glucocorticoid hormones. After entering the bloodstream and performing their functions in the body, GC are metabolised by the liver and excreted as conjugates into the gut via the bile, generating different metabolites that are expelled via faeces (Cyr & Romero 2008; Touma & Palme 2005). When assessing GC levels in faecal samples, metabolites are measured rather than the intact steroid (Möstl et al. 2005). FGM analysis is used as a non-invasive method to monitor stress levels in wildlife, which can offer valuable insights into the health and well-being of animal populations without the necessity of capturing or handling individuals (Fanson et al. 2017). Elevated FGM concentrations, measured as metabolites of GC hormones excreted in faeces, are often correlated with increased stress levels, which can arise from natural environmental challenges or anthropogenic disturbances (Cyr & Romero 2008). Studies have shown that disturbances caused by human activities can lead to significant alterations in FGM levels. For example, koalas (*Phascolarctos cinereus*) affected by environmental disruptions caused by humans (e.g. land clearing, vehicle collision, and burns from bushfires) had higher mean FGM levels compared to healthy koalas without signs of stress (Narayan 2019). Handling and captivity also led to an increase in FGM levels in female honey possums (*Tarsipes rostratus*), suggesting that these animals are vulnerable to chronic stress in a captive environment (Oates et al. 2007). Kangaroos subjected to capture for collar deployment had an acute stress response demonstrated by

increased FGM levels 24 hours post-deployment, compared to uncaptured control animals (Herbert et al. 2020).

The time it takes for FGMs to reflect a stressor is closely linked to the gastrointestinal transit time of the species being studied (Keay et al. 2006), as this determines how quickly the metabolites are produced, metabolised, and excreted (Touma & Palme 2005). Generally, GC released in response to a stressor can be detected within a few hours, but the specific timing varies depending on the species and individual physiological factors, such as gut passage rates (Fanson et al. 2017). For many vertebrates, gut transit times can range from a few hours to several days, consequently impacting when FGM become detectable in faecal samples. Some species of marsupials have a relatively long gut passage and a FGM peak can occur up to 4 days after exposure to a stressor (Fanson et al. 2017). For kangaroos, the lag between exposure to a stressor and peak FGM concentrations is approximately 19 hours (range = 10-34 hours) (Fanson et al. 2017).

1.7 Pain

Procedures causing pain to animals are among the most emotionally contentious public concerns regarding animal welfare (Grandin 2014). The methods used to manage animals, whether lethal or non-lethal, that lead to pain, discomfort or diminish their capacity to express normal behaviour are key areas of public concern (Weary et al. 2006).

Pain is defined by the International Association for the Study of Pain (IASP) as “an unpleasant sensory and emotional experience associated with, or resembling that associated with actual or potential tissue damage” (IASP 1994). It encompasses sensory, cognitive, and affective dimensions that different individuals experience uniquely (Walker et al. 2009). Techniques for assessing animal pain typically involve evaluating general body functions, including changes in

feed consumption, physiological responses such as alterations in cortisol concentrations, and behavioural alterations (Weary et al. 2006). Pain behaviours may include changes in posture (e.g., spending more time lying down or standing), specific movement changes (e.g. trembling, limping, or ear flicking), decreased locomotion, reduced feeding activity, and increased lethargy (Walker et al. 2009). For example, horses experiencing acute pain may exhibit changes in posture (e.g., standing rigidly in a "parked" position), tremors, reluctance to move, reduced feeding, and increased lethargy as indicators of pain (National Research Council 2009). Pain-related behaviours vary between species and can persist for days or weeks following a painful procedure due to tissue damage, inflammation and repair processes (Walker et al. 2009). While there is inherent uncertainty in evaluating experiences like pain in free-ranging animals, certain indicators can offer valuable insights. For instance, all mammals pain guard after an injury (Grandin & Deesing 2002). Thus, if an animal exhibits guarding behaviour toward an injured area, such as limping from a leg injury, we can assume the animal is in pain.

Nonetheless, some prey species exhibit stoicism, a survival mechanism where they do not show signs of pain for fear of attracting predators, instead displaying escape-avoidance reactions (Walker et al. 2009; Weary et al. 2006). For instance, a chicken that exhibits pain guarding by holding its leg up will cease this behaviour when introduced to a frightening new environment (Gentle & Corr 1995). Macropods are also considered stoic and may not show obvious signs of pain, therefore observing subtle alterations in their behaviour, such as hunched posture, limping, and lethargy, can indicate that they are in pain (NSW Government 2021)

Physiological assessment, such as determining FGM concentrations, can also be used as an indicator of physiological stress, which may be associated with pain in free-ranging populations. However, distinguishing between the effects of pain and other stressors on FGM concentrations

can be challenging (Santamaria et al. 2021). For example, when tame cattle are restrained, their lack of fear means that the pain associated with hot iron branding is likely the primary factor contributing to increased cortisol levels (Lay Jr et al. 1992). In contrast, cattle that are not used to handling will experience a significant rise in cortisol levels due to the stress of restraint, nearly reaching levels comparable to those seen with hot iron branding (Lay Jr et al. 1992). In marsupials such as koalas, GC levels have been shown to fluctuate seasonally, with elevated cortisol concentrations observed during the breeding season compared to the non-breeding season, highlighting the importance of considering natural reproductive cycles when interpreting stress hormone data (Santamaria et al. 2021).

Given the challenges associated with stoicism and pain detection. I used a combination of methods to determine if kangaroos experienced pain in response to different methods of contraceptive delivery. Behavioural observations were used to determine if kangaroos displayed guarding behaviour or if there were more subtle changes in movement patterns or frequency of specific behaviours. These observations were paired with FGM analysis, which should remove any confounding effects of stoicism in pain assessment.

1.8 Conclusions

The eastern grey kangaroo is a large, iconic macropod of Australia, commonly found in peri-urban areas where land clearing and development occur frequently. Managing kangaroo populations is a contentious issue as they are considered a cherished symbol of the country's wildlife, significant for tourism and national identity, yet also regarded as a problem species that requires management (Pople & Grigg 1999). In peri-urban areas where kangaroos can reach high densities, concerns regarding animal welfare and public safety frequently hinder the use of culling for overabundant populations (Adderton 2004). GnRH agonists, such as Suprelorin®,

have shown promise as fertility control alternatives for kangaroos, but limitations include potential welfare concerns associated with the need for capture and restraint of the animal. Development of a remote delivery system for Suprelorin® implants could potentially increase the efficacy of Suprelorin® treatment and minimise the risks associated with capture and handling.

Both lethal and non-lethal population control methods can cause stress in free-ranging populations, resulting in behavioural and physiological alterations (Favreau et al. 2014). Remote dart delivery of contraceptives offers a less invasive option that could reduce stress and the risks associated with capture and restraint, however further research to understand the impacts of contraceptive delivery methods on the welfare of kangaroos is warranted. The effective use of contraception depends on its capacity to prevent reproduction while avoiding negative effects on the animal's physical condition (e.g., weight loss or injury), physiological function (e.g., hormonal or metabolic disruption), or behaviour (e.g., grooming or movement changes). Ultimately, effective wildlife management should balance population control and conflict reduction with considerations of animal welfare, implementing approaches supported by scientific data and evidence (Descovich et al. 2016; Hampton et al. 2015b).

1.9 Thesis aims

This thesis aims to investigate three critical aspects of eastern grey kangaroo management strategies: the efficacy of Suprelorin® contraceptive implants; physiological and behavioural responses to Suprelorin® contraceptive delivery methods; and the physiological effects of culling on surviving conspecifics. The goal is to provide evidence-based recommendations for implementing management strategies that benefit kangaroos, humans, and the environment while considering the welfare implications of these methods.

Chapter 2 aims to assess and compare the contraceptive effectiveness of Suprelorin® administered via remote delivery and hand injection at two doses in female kangaroos. The overall objective was to develop an effective and efficient method to extend interbirth intervals to manage free-ranging kangaroo populations, without the need for animal capture.

Chapter 3 investigates the stress responses of female kangaroos to two methods of contraceptive delivery. It focuses on the physiological and behavioural responses of females receiving Suprelorin® implants administered remotely via darts as an alternative to the conventional capture-treatment method involving subcutaneous injection. By assessing changes in behaviour and measuring faecal glucocorticoid metabolite concentrations, this chapter evaluates the pain and stress response associated with each delivery method. Furthermore, alterations in movement patterns using GPS collars were assessed. This study represents the first comprehensive examination of the pain and stress associated with various delivery methods of Suprelorin® contraceptive implants in a free-ranging population. The findings will inform the development of protocols for implementing remote fertility control techniques in free-ranging kangaroo populations, which can be adapted for other long-term fertility control agents, as well as other wildlife species.

Chapter 4 examines the physiological stress responses of kangaroo populations to culling and disturbances in the ACT, Australia. It assesses FGM levels as an indicator of stress among kangaroo populations at seven locations, each exposed to one of three scenarios: 1. culling, 2. disturbance (without culling), and 3. control (no culling or disturbance). The disturbance (without culling) control was implemented to mimic the disturbance caused by culling without actually removing (killing) any individuals. This is intended to evaluate if culling results in higher physiological stress levels in kangaroo populations compared to disturbance alone. This

research aims to enhance our understanding of stress physiology in kangaroos, enabling researchers to more effectively assess management interventions designed to reduce negative welfare outcomes at both individual and population levels. Additionally, the findings will contribute to discussions on how culling affects physiological stress responses in kangaroos and the potential acute stress reactions in their conspecifics, potentially enhancing public perceptions of culling as a humane and essential management practice.

Finally, Chapter 5 discusses the main research findings of this thesis, including management implications of fertility control methods and culling, real-world application and welfare concerns, making a general conclusion regarding the use and selection of management practices. This chapter also discusses the challenges encountered and suggests improvements in experimental design.

2. Hitting the mark: comparing the efficacy and duration of remote dart and hand-delivered deslorelin contraceptive implants in eastern grey kangaroos (*Macropus giganteus*)

2.1 Abstract

Context. Managing kangaroo populations in urban and peri-urban areas presents considerable challenges that require effective strategies to control population growth and reduce human-wildlife conflicts. This study evaluated the efficacy of remotely delivered Suprelorin®, a long-acting deslorelin contraceptive implant, as a non-lethal alternative for inhibiting fertility in overabundant kangaroo populations.

Aims. This study aimed to assess and compare contraceptive effectiveness of Suprelorin® treatment administered via remote-delivery and hand-injection at two doses in female eastern grey kangaroos. The overall objective was to develop an effective method to extend interbirth intervals to manage free-ranging kangaroo populations.

Methods. Sexually mature female kangaroos were randomly assigned to four treatment groups: Dart (4.7 mg implant remote intramuscular delivery, n = 44); SC (4.7 mg implant hand-injected subcutaneously, n = 42); SCHD (two 4.7 mg implants hand-injected subcutaneously, n = 31); and Control (capture minus implant, n = 39). The study was conducted at three coastal sites in NSW, Australia. Pouch status was monitored regularly to assess efficacy, and a subset of females was challenged with a gonadotrophin-releasing hormone to determine if their pituitary was suppressed, indicating effective contraception.

Key results. Females in all treated groups exhibited significantly longer interbirth intervals post-treatment compared to the control group. There was no significant difference in contraceptive

duration between females receiving remotely delivered or hand-injected implants, or between the 4.7 mg and 9.4 mg doses. While contraceptive treatment increased the interbirth interval, the somewhat high prevalence of blastocysts in diapause (i.e. conceived pre-treatment; Dart = 12%, SC = 29%, and SCHD = 31%) reduced the effective contraceptive longevity.

Conclusions. Remote delivery of contraceptive implants showed promise as an effective and efficient approach for contracepting kangaroos in peri-urban environments without the need for animal capture. This study highlights the potential for remote non-lethal approaches to fertility control.

Implications. This study demonstrated the effectiveness of remotely delivering contraceptive implants to kangaroos and will likely enhance the efficacy of using fertility control approaches for controlling overabundant kangaroo populations in peri-urban regions. Remote delivery improves the scalability of contraceptive distribution, addresses logistical challenges, and reduces the risks associated with lethal control methods. The remote delivery system described here can be adapted for longer-term contraceptive agents, which could facilitate population management strategies and minimise animal welfare concerns associated with culling or capture-and-treat methods.

2.2 Introduction

The eastern grey kangaroo (*Macropus giganteus*) is an iconic marsupial species and represents a symbol of Australia's unique wildlife, populating vast areas of eastern Australia (Poole et al. 1982). The species is deeply ingrained in the country's cultural identity. Therefore, their management poses significant challenges, particularly where populations reach high densities, resulting in human-wildlife conflict, such as kangaroo-vehicle collisions and aggressive encounters (Herbert et al. 2021). Often, population management strategies for eastern grey

kangaroos (EGK) are influenced by social and political factors, with lethal techniques such as shooting considered by some as ethically problematic (Herbert et al. 2021). Additionally, the proximity of residential areas often precludes culling due to safety concerns (Wimpenny et al. 2021).

In response, non-lethal strategies have emerged, focusing on controlling the fertility of these populations. Fertility control methods aim to reduce population growth by limiting recruitment, thereby reducing or stabilising population size (Asa & Porton 2005). By targeting reproductive capacity, these methods disrupt reproduction, leading to fewer offspring over time (Asa & Porton 2005; Massei & Cowan 2014; Wimpenny et al. 2021).

The EGK is recognised for its distinct sexual dimorphism, with males weighing 40 to 90 kilograms and females from 20 to 40 kilograms (Poole et al. 1982). Females are monovular and polyoestrous, typically giving birth to a single offspring throughout the year, with a peak in summer (Poole & Catling 1974). Gestation lasts 37 to 38 days, when highly altricial young are born (Poole 1975). The newborn instinctively makes its way to the mother's pouch, where development continues. The young first emerge from the pouch around 283 days after birth and final vacation occurs at approximately 319 days (Poole 1975).

EGK are capable of embryonic diapause, a reproductive adaptation whereby the embryo’s development remains suspended, refraining from immediate implantation in the uterus (Clark & Poole 1967). Embryonic diapause is intricately linked to lactation and pouch occupancy (Clark & Poole 1967), but differs from that of most other macropods due to the absence of post-partum oestrus. In some females, oestrus may return when the young occupying the pouch is 160 to 209 days old (Poole & Catling 1974). If mating is successful, the blastocyst that forms remains in

diapause until it resumes development so that birth occurs when the first young permanently leaves the pouch (Clark & Poole 1967; Poole & Catling 1974).

Suprelorin® (Virbac Australia, Macquarie Park, NSW), a slow-release implant containing the gonadotrophin-releasing hormone (GnRH) agonist deslorelin, has shown promise as a contraceptive for macropods (Wimpenny et al. 2021). These implants inhibit luteinising hormone (LH) production by the pituitary gland, thus disrupting follicular development and oestrus cycles in females, which prolongs anoestrus (Herbert & Trigg 2005). This contraceptive was successfully trialed in brushtail possums (*Trichosurus vulpecula*) (Eymann et al. 2007; Lohr et al. 2009), tammar wallabies (*Macropus engenii*) (Herbert et al. 2007; Herbert et al. 2013), Tasmanian devils (*Sarcophilus harrisii*) (Cope et al. 2019), captive EGK (Herbert et al. 2006; Woodward et al. 2006) and free ranging EGK (Wilson et al. 2013). The treatment showed no or minimal effects on behaviour (Cope et al. 2018a; Herbert & Trigg 2005; Wilson et al. 2013). These implants are typically hand-injected subcutaneously, but their small size makes them conducive to remote delivery, addressing the efficiency concerns that have hindered fertility control operations (Massei & Cowan 2014; Rutberg 2013; Wimpenny et al. 2021).

To ensure the feasibility of remote delivery of Suprelorin® implants, it is important to compare the release rate of deslorelin administered intramuscularly (remotely) versus subcutaneously via hand injection. If the implant breaks or cracks upon impact it could cause an increase or burst in the rate of drug release (Stewart et al. 2020). Therefore, the contraceptive efficacy and duration between animals receiving remote vs. hand-injected implants must be compared. Remote delivery also favours smaller implants to minimise impact on the animal, making a lower dose (4.7 mg) the most practical option. Consequently, it is vital to assess the contraceptive efficacy of

this lower dose compared to the more commonly used doses (9.4 or 10 mg) in this species (Herbert et al. 2006; Wilson et al. 2013; Woodward et al. 2006).

This study aimed to evaluate the contraceptive effectiveness of Suprelorin® administered at two doses (4.7 and 9.4mg) and via two routes (remote-delivery and hand-injection) in female EGK at three sites in the mid-north coast region of New South Wales (NSW), Australia. The specific aims were to compare the: (1) contraceptive efficacy and (2) contraceptive duration and interbirth interval between different delivery methods and doses relative to untreated control animals. Contraceptive efficacy is assessed by calculating the percentage of females giving birth in the first year following treatment and monitoring reproductive success over time (Wilson & Coulson 2016; Wilson et al. 2013). Contraceptive duration was evaluated by changes to the interbirth interval, defined as the time between the birth of pouch young present at the time of treatment and the first pouch young conceived post-treatment (Herbert et al. 2006; Woodward et al. 2006). This metric is more relevant than the traditional approach of measuring time to first birth post-treatment, as it accounts for the complexities of reproduction (i.e. embryonic diapause) in macropods (Herbert et al. 2006) and the resultant net change in reproductive output that is most relevant to management objectives. By comparing the efficacy and duration of contraceptive effects between treatment groups, this study aimed to identify optimal doses and administration routes for inhibiting reproduction in overabundant EGK populations.

2.3 Material and Methods:

2.3.1 Animals

The study was carried out on free-ranging sexually mature female EGK at three sites located in the mid-north coast of NSW. Sexual maturity was determined by eversion of their teats (Poole and Catling 1974), or the presence of young in the pouch or at foot. This work was approved by the University of Sydney's Animal Ethics Committee under the permit number: # N00/7-2012/3/5791, 2015/917, 2016/1062, and the NSW National Parks and Wildlife Service under scientific licence SL100961.

2.3.2 Study Sites

Three sites with abundant kangaroo populations were used: (1) Nelson Bay Golf Club (NB), (2) Darlington Park (DP) and (3) Heritage Park (HP). NB is in Port Stephens, 160 km northeast of Sydney (32°72′8″S, 152°15′0″E). At the time of the study, the kangaroo density at NB was 3.4 individuals ha^{-1} (Herbert et al. 2021). DP and HP are in the Coffs Harbour Northern Beaches region of NSW. DP is approximately 35 km northeast of Coffs Harbour (30°04′8″S, 153°19′1″E) in Arrawarra, with a kangaroo density of 1.4 individuals ha^{-1} (Brandimarti et al. 2020). HP is in Moonee Beach, approximately 16 km northeast of Coffs Harbour (30°18′3″S, 153°14′9″E) with a kangaroo density of 1.2–1.5 individuals ha^{-1} (Henderson et al. 2018).

2.3.3 Experimental design

Sexually mature females were chosen randomly and allocated to one of four treatment groups: high dose (9.4 mg implant hand-injected subcutaneously, SCHD); low dose (4.7 mg implant hand-injected subcutaneously, SC); remotely delivered (4.7 mg intramuscular implant, Dart); and

control group (normal capture minus implant, Control). All animals used in this study were captured and ear tagged for individual identification prior to the study. Table 2.1 describes the timing of treatment and sample sizes at each site.

The reproductive status of the animals was monitored post-treatment through monthly observations of their pouch in the field. A subset of females was re-captured to inspect their pouch to confirm reproductive status and to determine the female's hormonal competence using a gonadotrophin releasing hormone (GnRH) challenge technique (Herbert et al. 2007). The reproductive status of females at the start of treatment was recorded either from pouch inspection at capture (hand-injected and control animals) or by pouch observations at the time of dart-delivery and consulting any recent capture records.

2.3.4 Suprelorin® implant

Suprelorin® (Virbac Australia, Macquarie Park, NSW) is a slow release long-acting implant, containing 4.7 mg of the GnRH agonist deslorelin (D-Trp^{6}-Pro^{9}-des-gly^{10}-GnRH ethylamide), measuring 2.3 x 12.5 mm. Females in the low dose group (SC) received one subcutaneous implant whilst the high dose group (SCHD) received two implants each, injected subcutaneously between the shoulder blades following capture. Females in the dart group received one implant (4.7 mg) delivered intramuscularly into their rump using a prototype dart (Figure 2.1). Females in the control group received the same capture and processing minus the implant.

2.3.5 Dart delivery

Females in the dart group received one implant (4.7 mg) delivered intramuscularly using a prototype dart (Pneudart, Williamsport, PA, USA) and a tranquiliser firearm (Pneu-dart X-caliber; Williamsport, PA, USA). These prototype darts were similar to the standard 1cc Type P

darts but had a larger calibre needle to accommodate the implant. The body of the dart was pre-loaded with 1mL of water for injection (NB site) or 1mL of Meloxicam (Metacam; Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany) (DP and HP sites) prior to firing. Meloxicam was added to the dart following a previous NB treatment where a subset of animals was observed limping within the first 48 hours post treatment (Herbert, unpublished data). The implants were pre-loaded into a gelatin sheath (made by Pneu-dart using proprietary technology, Figure 2.1) and were loaded into the cannula of the dart immediately before firing. This was necessary to ensure that the gelatin did not soften in the cannula because of prolonged contact with moisture, which can result in the implant getting lodged in the cannula. Darts were fired into the rump of the pre-tagged (and therefore identifiable) kangaroos from a distance of 10 m.

2.3.6 Animal Capture

Kangaroos were immobilised using Zoletil® 100, a drug combining tiletamine hydrochloride and zolazepam hydrochloride at a 1:1 ratio (Virbac Pty. Ltd, Milperra, Australia). Each female received a dose of approximately 125 mg (~5 mg/kg for a 25kg female), administered as a 1 mL intramuscular injection using a pole syringe, particularly at NB and DP, where the kangaroos were accustomed to human presence. Alternatively, Zoletil® was delivered using a dart gun (Type P, 1cc, ¾" gel collars darts fired from an X-Caliber, both from Pneu-dart, Williamsport, PA, USA) at distances up to 30 m when the animals were too skittish for a close approach (Brandimarti et al. 2020). This was commonly employed for animals at HP, and occasionally at NB and DP. Immobilised kangaroos were transferred into a capture bag and immediately transported to a nearby processing site. After processing, the animals were released in a tranquil area near the processing site, with their heads covered, and were monitored until they had recovered and were fully mobile.

2.3.7 Data Collection

During processing, animals were weighed (Digital hanging scale Model WS603, Wedderburn, Ingleburn, NSW, Australia), and had lower leg length measured according to Poole et al. (1982). Up to 5 mL of blood was collected from the lateral tail vein for health assessments (as part of an additional study, see Brandimarti et al. (2020) and/or luteinizing hormone (LH) analysis (see GnRH challenge below). The pouch was inspected and reproductive status (pouch and teat condition) was recorded. Teat condition was categorised as (1) no signs of recent activity, (2) elongated teat with milk (indicating suckling of young at foot or very recent loss of young), or (3) regressing teat (indicating recent loss or weaning of young). Any young had pes (foot) and head length measured using vernier calipers, for aging using established growth curves (Poole et al. 1982). The date of birth of pouch young (PY) was calculated by subtracting the age of PY from the date of capture. Each female was micro-chipped (FDX-B Injectable Transponder Implant, Allflex, Capalaba, Qld, Australia) and ear tagged with a unique colour, shape and number (left and right ear) combination (Allflex sheep button or mini tags, Capalaba, Qld, Australia).

Following treatment, field observations were conducted monthly for 18-36 months, where pouch status was monitored. This involved searching the entire site and identifying tagged animals using binoculars (10 x 42; Nikon Monarch, Sydney, NSW, Australia) or a spotting scope (20-60 X; Nikon Fieldscope, Sydney, NSW, Australia). The presence and size of any young (medium or large) was recorded, based on the extent of a visible pouch bulge. A subset of females was re-captured for a GnRH challenge whereby they had all measurements re-taken and reproductive status recorded. Any PY at the time of recapture was also measured and aged.

When the female was sighted but not captured, the observer recorded pouch status, as determined by the size of a pouch bulge. Females were categorised as either having no PY (NPY, no discernible bulge), or a medium (MPY) or large (LPY) PY, or young at foot (YAF). Using an unpublished guide (Herbert 2018) the age of PY was determined to be: MPY = 146 ± 4 d, LPY = 253 ± 4 d, YAF = 342 ± 7 d. The date of birth was calculated by subtracting the age of PY from the date of sighting.

2.3.8 Interbirth interval

The inter-birth interval was calculated as the time between birth of the PY at treatment and the first PY born after treatment (interbirth interval 1). Subsequent inter-birth intervals were also determined for successive young (interbirth interval 2 is the interval between the first and second young after treatment and so on).

2.3.9 GnRH challenge

A subset of females from each treatment group from all sites was re-captured for GnRH challenge, 8 to12 months post-treatment (Table 2.2). Blood samples (4-5 mL) were collected from the lateral tail vein using a 23-gauge winged infusion set and 5mL syringe (Time -15). A second sample was collected 15 minutes later (Time 0). Immediately after collecting the Time 0 sample, the needle was left in place and the blood collection syringe was replaced with a second syringe containing 2 μg kg^{-1} of synthetic GnRH (Fertagyl; Intervet, Sydney, NSW, Australia) made up to a 1 mL injection with 0.9% sterile saline. The time of GnRH injection was recorded, and two subsequent blood samples were collected 15 (Time 15) and 30 (Time 30) minutes after the GnRH injection. Samples were transferred to 5 mL BD Vacutainer SST II Advance tubes and

stored on ice until centrifuged. Whole blood was centrifuged for 15 minutes at 3000 rpm, and serum was stored at -20 ºC until LH analysis.

2.3.10 LH assay

A double antibody enzyme immunoassay (EIA) (Graham et al. 2002), previously modified for EGK (Wilson et al. 2013) was used to measure serum LH concentrations. The plates were read at 450 nm using a microplate reader (800^{TM} TS, Biotek Instruments Winooski, VT, USA). All samples and standards were assayed in duplicate following the methods described in Wilson et al. (2013).

LH assays for the NB site were conducted in 2013. The sensitivity of the assay was 0.168 ng mL^{-1} (lowest readable standard). The inter-assay coefficient of variation (CV) measured from pooled samples with high (2.502 ng mL^{-1}) and low binding (0.418 ng mL^{-1}) LH concentrations were 2.7% and 19.9%, respectively from 5 plates. The intra-assay CV was 7.0% and 3.6% respectively from 8 replicates run on the same plate.

LH assays for DP and HP were conducted in 2020. The sensitivity of the assay was 0.156 ng mL^{-1}. The inter-assay CV measured from pooled samples containing high (2.651 ng mL^{-1}) and low binding (0.636 ng mL^{-1}) LH concentrations were 7.9% and 12.9%, respectively (n=5 plates). The intra-assay CV was 3.1 % and 5.0 % from nine replicates run on the same plate, respectively.

2.3.11 Determining female reproductive status at the time of treatment

To accurately compare contraceptive efficacy between different delivery techniques, we needed to determine whether the first birth after contraceptive treatment represented a new conception (i.e. a contraceptive failure or expiration of contraceptive effect) or whether the female was pregnant at the time of treatment (i.e. with a blastocyst in diapause, or active pregnancy). This

was determined by accounting for the reproductive status of the female (and age of current PY) at the time of treatment and developing a reproductive timeline for each female using data from subsequent re-sightings, recaptures, and GnRH challenge results (Figure 2.2). A PY was classified as likely resulting from a reactivation of a blastocyst in diapause if they met the following criteria :

- The female was non-responsive to the GnRH challenge. A surge of LH is essential for ovulation (and subsequent conception), therefore, the inability to respond to an injection of synthetic GnRH with a significant elevation of LH 15-30 min later, strongly suggests that the female was incapable of having conceived post-treatment (Herbert et al. 2013).
- PY at treatment was > 160 days old (Poole 1975; Poole & Catling 1974).
- Estimated interbirth interval 300-338 days (Poole 1975).

A female was considered to have an active pregnancy at the time of treatment if the first young following contraception, was born within 40 days of treatment.

2.3.12 Contraceptive duration

Contraceptive duration was defined as the period from treatment to the first parturition of a young conceived post-treatment, thereby excluding pregnant females (either an active pregnancy or a blastocyst in diapause) when treated. In such instances, the contraception duration was defined as the period until the second PY born post-treatment, as deslorelin does not inhibit the development of existing embryos (Herbert et al. 2006; Wilson & Coulson 2016). Females that did not have a PY after treatment were deemed to still be contracepted and duration was considered to be the time until last pouch resighting without a PY.

2.3.13 Data analyses

All data were analysed in the statistical program R version 4.2.1 (R Core Team 2023). The assumption of normal distribution of error was tested using descriptive graphs (histograms, quantile-quantile plots and standardised residual plots). Where the assumption of normality was not met, natural log transformations were performed. Results were reported as mean ± standard error of the mean (SE). Plots were created using the ggplot2 package (Wickham 2016). Statistical significance was set at $p < 0.05$ for all analyses.

To assess differences in female weight and leg length between groups at the time of treatment, we employed Linear models (LMs) and conducted post-hoc Estimated Marginal Means (EMMs) using the emmeans package (Lenth 2024) on significant results, to predict means and SE of means. Changes in female weight were compared by computing the average daily gain (ADG) between the time of treatment and weights taken at recaptures, corrected for the number of days between captures. We also used LM and EMM to estimate ADG.

Logistic regression models were used to compare the percentage of females carrying PY at the time of treatment, one-, two- and three-years post treatment. The glmer function from the lme4 package (Bates et al. 2015) was used to analyse the effects of treatment, time and their interactions on the binary outcome of females carrying PY. The models included a random intercept for site to account for potential variability and clustering at the site level. To assess the significance of the main effects and interactions, an analysis of deviance was performed using the Anova function from the car package (Fox & Weisberg 2019), which provides Type III Wald chi-square tests. EMMs and pairwise comparisons between treatments and sites at each time point were obtained using the emmeans package. The p-value for the pairwise comparisons were adjusted for multiple comparisons using Tukey's method.

To analyse the effects of treatment and site on PY's birth season, a multinomial logistic regression model was fitted using the multinom function from the nnet package (Ripley & Venables 2002). The model included treatment and site as independent variables and the PY's birth season as the dependent variable with four levels (Autumn, Spring, Summer and Winter). EMMS and pairwise contrasts were tested using the Tukey method to adjust for multiple comparisons and control the family-wise error rate.

Individual females were considered to respond significantly to the GnRH challenge if their plasma LH levels at Time 15 and/or Time 30 were at least twofold greater than the standard deviation of the baseline (Herbert et al. 2013). The baseline was determined by calculating the mean LH levels at Time -15 and Time 0. LMs were employed to estimate the effects of treatment, sampling time (Time 15 and Time 30), and their interaction on the percentage change in plasma LH concentrations. Additionally, a generalised linear model (GLM) with a binomial distribution was carried out to evaluate differences in the likelihood of individuals displaying a positive LH response to the GnRH challenge between treatment groups. In this analysis, the binary LH response was the outcome variable with Treatment as the predictor variable. Another GLM with a binomial distribution was used to assess whether the timing of the GnRH challenge, relative to the time since contraception, influenced the likelihood of a positive LH response. In this model, the binary LH response served as the outcome variable, while the time post-treatment was designated as the predictor variable.

By combining data from the GnRH challenge, contraceptive failure, rate of blastocysts and pregnancy, we calculated the duration of treatment and employed LMs and EMM to assess the relationship between the response variable (duration) and the independent variables: treatment and site.

GLMs with a binomial distribution were employed to assess the effects of treatment and site on three binary outcomes: presence of blastocysts in diapause, pregnancy, and contraceptive failure or expirations. We initially used the dataset with only females that underwent GnRH challenge in the analysis, where females were considered to have a blastocyst in diapause if they had a PY but were non-responsive to GnRH challenge; to be pregnant if they had a PY born up to 40 days post treatment; and failure if they were responsive but had no PY or data were inconsistent. We assessed four models: Model 1 included both treatment and site as variables; Models 2 and 3 evaluated the effects of treatment and site independently; and Null Model without the predictor variables (site and treatment). Each model's fit was quantitatively assessed by calculating the corrected Akaike Information Criterion (AICc) for small datasets, and confidence intervals for the estimated probabilities were derived by transforming the logistic regression coefficients using the inverse logit function (plogis). Due to the small dataset, the Null Model was determined to be the best fit for the data; however, it did not provide meaningful information regarding the effects of sites or treatments in the data. New GLMs were then employed using the dataset with all females, where the presence of blastocysts in diapause, pregnancy and contraceptive failure/expiration were determined using the criteria previously explained above in the section: '*Determining female reproductive status at the time of treatment*'.

To compare the interbirth intervals across treatment groups, we used LMs and EMM to estimate the relationship between the response variable (e.g. interval birth) and the independent variables: treatment and site.

2.4 Results

2.4.1 Weight and leg length

There was a significant difference in weight of females across sites (DP 28.4 ± 0.9 kg, HP 27.3 ± 0.4 kg, NB 26.4 ± 0.4 kg; $F_{2,149} = 3.969$, $p = 0.021$). Females at DP were 7.6% heavier, on average, than the females at NB ($t = 2.615$, $p = 0.026$). However, when comparing treatment groups (regardless of site), the weight of females at the time of treatment was similar (Control 26.9 ± 0.6 kg; Dart 27.2 ± 0.5 kg; SC 26.9 ± 0.6 kg; SCHD 27.3 ± 0.7 kg; $F_{3,149} = 0.127$, $p = 0.944$).

There was no significant difference in ADG between treatment groups (Control 2.9 ± 0.9 g; Dart 3.0 ± 0.6 g; SC 0.3 ± 1.1 g; SCHD 1.1 ± 1.7 g; $F_{3, 104} = 2.080$, $p = 0.107$) or between sites (DP 3.6 ± 1.4 g, HP 1.3 ± 1.5 g, NB 1.4 ± 0.43 g; $F_{2,104} = 1.914$, $p = 0.153$). There was no significant interaction in ADG between treatment groups and sites ($F_{5,99} = 1.692$, $p = 0.091$). Additionally, the leg length of females was not statistically different between treatment groups (Control 50.9 ± 0.4 cm; Dart 50.7 ± 0.5 cm; SC 50.8 ± 0.3 cm; SCHD 51.2 ± 0.7 kg; $F_{3,149} = 0.253$, $p = 0.859$), or across study sites (DP 50.3 ± 0.8 cm, HP 51.4 ± 0.3 cm, NB 50.8 ± 0.2 cm; $F_{2,149} = 1.699$, $p = 0.186$).

2.4.2 Reproductive data

At the time of treatment, there were no significant differences in the proportion of females carrying PY between treatment groups ($\chi^2 = 0.997$, df = 3, $p = 0.802$), or between sites ($\chi^2 = 1.413$, df = 2, $p = 0.493$). Additionally, there was no significant interactions between treatment groups or time (measured as one, two or three years post treatment) ($\chi^2 = 15.415$, df = 9, $p = 0.080$), nor between sites and time ($\chi^2 = 3.211$, df = 6, $p = 0.782$).

However, a significant change was noted in females carrying PY over time ($\chi^2 = 14.393$, df = 3, $p = 0.002$). One-year post-treatment, there was a significant decline in the proportion of females with PY compared to the time of treatment. Specifically, females in the Dart, SC and SCHD groups showed significant declines of 37.1% (estimate = 3.099, $p = 0.019$), 41.2% (estimate = 3.261, $p = 0.013$), and 44.6% (estimate = 3.285, $p = 0.014$) respectively, when compared to the Control group (Figure 2.3). No statistically significant differences were observed two- and three-years post-treatment in females with PY. The overall number of females with PY declined across time due to missing data, as some animals died or disappeared from the study over the three years.

There was a significant difference between treatment groups in the age of PY at the time of treatment ($F_{3,133} = 6.298$, $p = 0.001$). Particularly, PY in the Dart group were, on average, 64 ± 16 days older than those in the control group ($t = -3.831$, $p = 0.001$) and 47 ± 16 days older than females in the SC group ($t = 3.029$, $p = 0.015$) at the time of treatment (Figure 2.4a). Furthermore, a significant difference in PY age was observed across different sites ($F_{2,133} = 8.597$, $p = 0.000$. PY were, on average, 52 ± 14 days older in NB compared to HP ($t = -3.686$, $p = 0.001$; Figure 2.4b).

Most PY present at the time of treatment were born in summer. However, the distribution of PY birth season differed significantly among treatment groups ($\chi^2 = 18.422$, df = 9, $p = 0.031$) and across study sites ($\chi^2 = 12.964$, df = 6, $p = 0.044$) (Table 2.3).

No mortalities occurred during capture or treatment. Post-treatment, five females died from causes unrelated to the treatment: three from the HP site and two from the DP site succumbed to unknown natural causes or vehicular collisions.

2.4.3 Contraceptive efficacy

There were significant differences in the post-treatment interbirth intervals between treated groups. Dart, SC and SCHD exhibited interbirth intervals that were 31%, 40% and 28% longer, respectively, than the Control group. This effect persisted after excluding pregnant females and those with blastocysts at the time of treatment, with the treated groups showing increases of 42%, 41% and 55% longer interbirth intervals (respectively) than the Control group. There were no significant differences in the post-treatment interbirth interval across sites.

Initially, there were no significant differences between sites or treatment groups in the time to the first PY birth post-treatment. However, excluding females that had already conceived pre-treatment (i.e. those pregnant or with blastocysts in diapause), showed a significant treatment effect, with animals in the treated groups (Dart, SC and SCHD) taking 32%, 47% and 57% longer, respectively, until birth of the first PY post-treatment than those in the Control group.

No significant differences were found in the second interbirth interval or time to second birth post-treatment across either treatment groups or sites, regardless of whether pregnant females and those with blastocysts in diapause were included or excluded from the analysis (see Table 2.4 for statistical details).

2.4.4 GnRH challenge

There was a significant change in LH concentration from the baseline between treatments ($F_{3,150}$ = 15.426, p <0.001). The control group exhibited a pronounced increase in LH peak in response to GnRH injection compared to the SCHD (t = 6.207, p = <0.001), Dart (t = 5.069, p <0.001) and SC (t = 4.265, p <0.001) groups (Figure 2.5). No significant differences were observed between

Time 15 and Time 30 in percentage change of LH concentration ($F_{1,146}$= 0.0005, p = 0.982), nor were there interactions between sampling time and treatment groups ($F_{3,146}$ = 0.150, p = 0.930).

Additionally, there was a significant difference among the treatment groups in the proportion of individuals displaying a positive LH response to the GnRH challenge (χ^2 = 66.345, df = 3, p <0.001). Specifically, 95% of control, 24% of Dart, 18% of SC and 8% of SCHD females demonstrated a significant LH response (Figure 2.6). Furthermore, the timing when the GnRH challenge was applied post contraception did not significantly affect the likelihood of females responding positively with an LH surge between treatment groups (χ2 = 2.965, p = 0.086).

2.4.5 Blastocysts and Pregnancy

Based on a combination of responsiveness to the GnRH challenge and pouch assessment at the time of the challenge, a young born following contraceptive treatment was classified as resulting from the reactivation of a blastocyst in diapause or an active pregnancy conceived before treatment. Specifically, 12%, 29%, and 31% of females in the Dart, SC, and SCHD groups, respectively, were found to have given birth to a young that was present as a blastocyst in diapause at the time of treatment. Additionally, 20% of females in the Dart group, 12% in the SC group, and none in the SCHD group, were categorised as having an active pregnancy at the time of treatment. Among the sites, 9% of contracepted females in DP, 18% in NB, and none in HP were categorised as having an active pregnancy. However, due to the small size of the dataset of females subjected to GnRH challenge, the null models without predictor variables (Site and Treatment) were determined to be the best fit, providing no meaningful information about the effects of Treatment or Site.

When considering the entire dataset (not limited to GnRH challenge data) and applying all criteria outlined in the methods section, a logistic regression analysis was conducted to assess the proportion of females with blastocysts in diapause or pregnant among the treated groups and across sites. This revealed no statistically significant differences in the proportion of females with blastocysts in diapause between the treatment groups (Dart 15%, SC 18%, SCHD 19%; χ^2 = 0.298, df = 2, p = 0.742) or across sites (DP 30%, HP 12%, NB 17%; χ^2 = 3.772, df = 2, p = 0.152). Similarly, there were no statistically significant differences in the proportion of females being pregnant at the time of treatment between the treated groups (Dart 14%, SC 7%, SCHD 13%; χ^2 = 0.602, df = 2, p = 0.740) or between sites (DP 4%, HP 6%, NB 14%; χ^2 = 5.186, df = 2, p = 0.075).

2.4.6 Duration

Using the data that excluded females with pre-conceived young at treatment (blastocysts in diapause or active pregnancy), the analysis revealed that the presence of a PY at the time of treatment did not significantly affect the duration of contraception ($F_{1,62}$ = 0.6436, p = 0.426). Similarly, the age of the PY at treatment did not affect contraceptive duration ($F_{1,63}$ = 0.7402, p = 0.393). There was no significant interaction between treatment groups and study sites ($F_{4,55}$ = 1.319, p = 0.274). Nor were there significant differences in contraceptive duration among the treated groups (Dart 392 ± 36 days, SC 452 ± 39 days. SCHD 488 ± 46; $F_{2, 59}$ = 1.600, p = 0.211). However, a significant difference was noted across sites, with durations of 388 ± 44 days at DP, 370 ± 30 days at HP, 493 ± 36 days at NB ($F_{2, 61}$ = 3.394, p = 0.040). Post-hoc comparisons indicated a non-significant trend suggesting the duration of contraception at NB was longer than at HP (t = -2.382; p = 0.052).

We could not determine the duration of contraception for certain animals due to missing data (these animals either perished or were not resighted post-treatment) (Table 2.5).

2.5 Discussion

Our results indicate that long-acting deslorelin implants (Suprelorin®) may offer a non-lethal alternative for managing populations of overabundant eastern grey kangaroos by suppressing female reproduction. Females across our three treated groups had significantly longer interbirth intervals post-treatment than the control group. There was no significant difference in the duration of contraception between females in the remotely treated group and those that received hand-injected implants. Hence, remote delivery of contraceptive implants appears to be an effective and efficient approach for managing peri-urban populations, addressing past challenges in contraceptive administration (Wimpenny et al. 2021). We also demonstrated that effective contraception can be achieved in kangaroos using lower deslorelin doses without compromising contraception duration. These findings challenge the assumption that larger doses are necessary for large species (Agnew et al. 2021; Bertschinger et al. 2008; Cope et al. 2018b; D'Occhio et al. 2000).

Traditionally, measures of fertility control efficacy focus on contraceptive duration (i.e. the time between treatment and either conception or birth of the next young) as a metric of success. However, within the context of on-ground management, changes to the interbirth interval are a more relevant metric, particularly for species with complex reproductive strategies, such as kangaroos, as this accounts for the reproductive status at the time of treatment. In our study, females in the treated groups exhibited significantly extended interbirth intervals, with averages of 536, 533, and 586 days for the Dart, SC, and SCHD groups, respectively. In contrast, the control group had an average interbirth interval of 378 days, similar to the interbirth intervals of

approximately 386 days reported in non-treated females at other sites (King et al. 2017), suggesting a treatment induced increase in interbirth interval of between 155 and 208 days for different groups. This is longer than the delay that would be estimated by comparing the time to first birth post-treatment, which varied from 260 days in the control group to between 343 to 408 days in treatment groups (a difference of between 83 and 148 days).

The consistently high natural fertility rates initially observed across all kangaroo populations in our study emphasise the reproductive robustness typical of this species (Coulson et al., 2008; Wilson and Coulson, 2016; Wilson et al., 2013). At treatment, about 90% of all females had PY. However, one year post-treatment, we observed that 58% of treated females were carrying PY, sharply contrasting with the 97% in the control group. By the second year post-treatment, the fertility among treated females rose to 69%, approaching the control group's reduced rate of 86%. This is consistent with the diminishing contraceptive effects reported by Wilson and Coulson (2016) and Wilson et al. (2013). By the third year, the contraceptive effects appeared to have waned almost entirely, with fertility among treated females returning to 72%, compared to rates of 55% in control animals at the same time. As anticipated in long-term field studies that passively follow known individuals over time, there was attrition in the number of females available for monitoring, with only 41% of treated and 49% of control females observable by the third year, presenting a significant challenge in assessing long-term trends. The reduction in the proportion of females re-sighted three years post-treatment likely resulted from a combination of mortality, dispersal, sampling intensity (especially associated with COVID-19-induced travel restrictions in year 3), and detectability issues. Some females died during the study period, reducing the pool of individuals available for re-sighting. At one site (HP), site disturbances such as residential expansion and installation of new fences may have prompted emigration from the

site, and almost certainly impacted our capacity to observe animals (as previously unoccupied public land became private residences). The reduced fertility in control animals in year 3 probably reflects a combination of chance effects (associated with smaller sample size), possibly compounded by climatic factors, as the period from April to July 2019 in Coffs Harbour was unusually warm with below average rainfall, consistent with the wider drought across NSW that year (Bureau of Meteorology (BOM) 2019). For example, at HP, only three out of 11 control females were re-sighted three years post-treatment, with just one observed with a PY. Future studies could incorporate more intensive monitoring approaches to counter this attrition over time to provide a more robust evaluation of fertility trends and extended contraception.

The method of contraceptive delivery did not significantly influence the outcomes of Suprelorin® implants, suggesting remote delivery can be as effective as subcutaneous delivery in managing kangaroo fertility. Females in the Dart group exhibited similar suppression of pituitary LH responsiveness to those in the SC group, which shared the same dose (4.7 mg), indicating both methods achieve comparable effectiveness and release rates. Additionally, our study highlights the importance of addressing the risks associated with projectile delivery methods, particularly the potential for implant breakage, which could lead to an increased release rate of the active compound upon treatment (Stewart et al. 2020) shortening the longevity of contraception. Prior to this study, carcass trials were conducted to assess penetration depth and potential breakage, leading to the adoption of a gelatin sleeve for the Suprelorin® implants (Herbert unpublished data). The comparable contraceptive duration between the Dart and SC delivery methods suggests that little or no breakage occurred during darting.

Our findings suggest a potential nonlinear association between the dose of implants and their contraceptive effects. Specifically, increasing the dose did not necessarily increase the magnitude

of suppression of the reproductive axis proportionally. Low-dose implants (SC and Dart) suppressed the reproductive axis less than the high-dose treatment (SCHD) at the time of the GnRH challenge. However, doubling the dose did not increase the contraceptive duration proportionally. Studies in other species have found a relationship between deslorelin dose and the maximum duration of reproductive axis suppression, rather than the degree of suppression itself (Cope et al. 2019; Herbert et al. 2006; Junaidi et al. 2009; Trigg et al. 2001). However, this dose-response pattern was not discernible in our study, as the maximum duration of contraception did not significantly differ between the low and high dose treatments.

The contraceptive effect of Suprelorin® can be masked by births of young that were in utero at the time of treatment (i.e. either a blastocyst in diapause or an active pregnancy) (Herbert et al. 2006; Wilson & Coulson 2016). We found varying rates of embryonic diapause across our study sites. At DP, at least 30% of females exhibited embryonic diapause, compared to 12% at HP and 17% at NB. These percentages are slightly lower than those documented by Poole (1973) at Mt. Hope (36.1%) and Clark & Poole (1967) in Toobeah (28%), Queensland, Australia. The higher prevalence of diapause at DP may be attributed to the superior habitat quality and favourable environmental factors. This is consistent with Wilson et al. (2013), who investigated three sites in Victoria, Australia, and noted a greater prevalence of embryonic diapause at Plenty Gorge, which boasted the highest pasture biomass, lowest kangaroo density, and heaviest females. We consider DP to be a high-quality habitat, as it represents a mixed environment comprising coastal bushland, a caravan park, a residential area, private farmland and a golf course, all with improved pastures. This habitat offers abundant food resources from coastal bushland, visitor rubbish, gardens, golf course and pastures, along with ample water from natural and artificial bodies of water. Notably, DP also exhibited the heaviest females and the lowest population

density. Additionally, DP had the lowest decline in fertility rates following treatment, which could correlate with the highest prevalence of embryonic diapause observed at this site.

Consistent with previous research, our study found that EGK exhibit a peak in births in summer, from December to March (Poole 1983). Therefore, as suggested by Wilson et al. (2013) the optimal window for implanting females should be between April and May, when most females will have given birth, minimising the probability of being pregnant at the time of treatment. Additionally, the pouch young carried by females at this time will mostly be under 100 days old, reducing the likelihood that females have a blastocyst in diapause (Poole 1983). However, the timing of contraceptive treatment in our study did not align with this optimal window. Females at the HP and DP sites were treated from April to June, while those at the NB site were treated from June to October. Interestingly, the mean age of pouch young at the time of treatment was 156 days at DP, 134 days at HP, and 186 days at NB, suggesting that the ideal treatment timing for our study would have been from February to March, rather than April to June. This mismatch could have affected the effectiveness of the implants and highlights the need to account for local population characteristics when planning fertility control operations. Aligning the treatment period with the peak birthing season could improve the contraceptive outcomes.

2.5.1 Management Implications

We demonstrated that deslorelin implants offer an effective non-lethal approach for inhibiting fertility in overabundant kangaroo populations. Remote delivery via darts was found to have comparable efficacy to hand-injection after capture, providing an efficient contraceptive treatment for free-ranging populations. To our knowledge, this is the first published account of successful remote delivery treatment with Suprelorin® contraceptive implants.

While our findings of prolonged interbirth intervals and delayed time to first pouch young born post-treatment highlight the efficacy of our prototype contraceptive delivery method (achieved in collaboration with Pneu-dart Pty Ltd.), challenges remain. Contraception effects diminish over time, with fertility rates returning to normal by the third-year post-treatment. The lack of significantly different interbirth intervals between the control group and the treated animals thereafter indicated that contraceptive effect disappeared before the second PY was born. This contraceptive duration will likely not reduce the population density in long-lived species. Repeated treatments or alternative approaches with a longer contraceptive duration (e.g. GonaCon™) (Wimpenny & Hinds 2018), may be necessary to sustain the desired level of fertility suppression in target populations, unless a long-lasting Suprelorin® implant can be developed without increasing implant size. The remote delivery approach described here may be more suited to semi-captive populations, or populations with unknown long-term trajectories.

To maximise contraceptive efficacy, administration should be carefully timed, particularly in high-quality habitats where embryonic diapause is more likely (Wilson et al. 2013). The optimal window for implanting should be when most females are carrying pouch young <100 days old (Poole 1983), and as demonstrated here this could vary between populations. This will reduce the likelihood of pregnancies or females with blastocysts in diapause, which could mask the initial contraceptive effects.

One of the critical challenges with remotely delivered contraception is the reliable identification of individuals when treated and for a period thereafter. This is essential for identifying contracepted and non-contracepted animals and estimating the proportion of treated females within a population. In this study, all treated females were initially captured and identified with unique ear tags, allowing for long-term monitoring of contraceptive efficacy. However, this is

not feasible for remote contraception in large free-ranging populations. One potential solution is to incorporate a temporary dye marker into the contraceptive dart or projectile. Double-chambered darts are available, which can simultaneously deliver drugs and splash dye on the coat of an animal upon impact, leaving a temporary visible mark on treated animals. A study on polar bears (*Ursus maritimus*) assessed the use of modified darts that collect a tissue sample while simultaneously applying a temporary dye upon impact to mark the animal and prevent resampling (Pagano et al. 2014). Pattanarangsan et al. (2022) investigated a double-dart technique for wildlife remote drug delivery, optimising pressure and aim to deliver two darts in a single shot, which could be adapted for drug and dye marking. Blanket treatment of a large proportion of females within a short period of time would then theoretically be possible, without increased risk of repeat treatment of individuals. Studies have explored using darts that leave a paint mark on the coats of treated white-tailed deer (*Odocoileus virginianus*), however the dyes tested were not completely satisfactory (Naugle et al. 2002) and so additional research is needed in this area. Cadaver trials of drug delivery and marking darts in kangaroos (Herbert unpublished data) suggest that the increased weight of darts increases penetration and impact damage. Another approach used a two-shooter method, where contraceptive darts and marker darts were fired simultaneously, but coordination between the two shooters was difficult (Wimpenny & Hinds 2018). Ideally, the contraceptive implant and the marker would be combined in one small, lightweight dart. This or another integrated marking system is required before remotely delivered contraceptives, such as Suprelorin®, could be deployed at a large scale.

The advantages of remote delivery in terms of accessibility, safety, and scalability for managing dispersed populations cannot be overlooked. However, the reproductive biology of most macropod species means that longer-term contraceptive agents are needed for long-term

suppression. The remote delivery system described here could be modified to deliver a longer lasting contraceptive such as GonaCon™ (tested in kangaroos in the ACT) (Wimpenny & Hinds 2018), potentially enabling more effective population management strategies while minimising animal welfare and ethical concerns associated with culling or capture-and-treat methods.

Further refinements and studies may optimise the remote delivery system and minimise failures. Long-acting contraceptive implants administered remotely could be a valuable tool for wildlife managers. Remote delivery will enable more efficient and widespread implementation of non-lethal population control strategies for eastern grey kangaroos in urban and peri-urban environments, where traditional management methods may be impractical or undesirable. Future studies should also evaluate the safety profile of remote contraceptive delivery, including investigating potential behavioural and stress impacts, and assessing the overall welfare of animals, to ensure it is superior to current capture-and-treat protocols.

In conclusion, our findings identified the promising potential of remotely delivered deslorelin implants as a practical and effective approach for inhibiting fertility in overabundant eastern grey kangaroo populations, particularly in peri-urban areas. Remote delivery had comparable efficacy to low dose hand-injected subcutaneous implants, exhibiting similar durations of contraception and suppression of reproductive hormones. Remote delivery addresses a significant challenge in administering contraceptives to free-ranging populations, where traditional hand-injection methods may be logistically difficult or pose risks to animals and personnel (Wimpenny et al. 2021).

2.6 Tables and Figures

Table 2.1. Study site, location, population density and treatment information from three discrete populations of eastern grey kangaroos in the mid-north coast region of NSW: NB (Nelson Bay Golf Club), DP (Darlington Park), and HP (Heritage Park). Treatment groups: Dart (low dose, 4.7 mg remote delivered intramuscularly), SC (low dose, 4.7 mg remote delivered subcutaneously); SCHD (high dose, 2 x 4.7 mg delivered subcutaneously); Control (normal capture minus implant).

Site	Geographic Coordinates	Population density (Year)	Size (ha)	Date of Trials	Total animals captured	Sample size	Treatment group
NB	32°72'8"S, 152°15'0"E	3.4/ha^{-1} (2012)[1]	64	June – October 2013	78	20	Dart
						20	SC
						17	SCHD
						21	Control
DP	30°04'8"S, 153°19'1"E	1.4/ha^{-1} (2016)[2,3]	44.5	May – June 2017	28	10	Dart
						9	SC
						2	SCHD
						7	Control
HP	30°18'3"S, 153°14'9"E	1.2 to 1.5/ha^{-1} (2016) [1,2]	415	April – June 2017	50	14	Dart
						13	SC
						12	SCHD
						11	Control

[1] Herbert et al. 2021; [2] Brandimarti et al. 2020; [3] Henderson et al. 2018.

Table 2.2. Subset of female eastern grey kangaroos recaptured for GnRH challenge from different treatment groups at each site: Darlington Park (DP), Heritage Park (HP) and Nelson Bay Golf Club (NB).

Site	Total challenged (n)	Dart (n)	SCHD (n)	SC (n)	Control (n)
HP	16	5	4	2	5
DP	16	6	–	5	5
NB	48	15	10	11	12

n, sample number.

Table 2.3. Percentage of PY born per season pre-treatment across treatment groups (Control, Dart, SC and SCHD) and study sites (Darlington Park (DP), Heritage Park (HP) and Nelson Bay Golf Club (NB)).

	Treatment groups				Study Sites		
	Control (%; n=35)	Dart (%; n =49)	SC (%, n=36)	SCHD (%; 26)	DP (%; n=26)	HP (%; n=44)	NB (%; n=71)
Autumn	26	12	19	27	12	25	21
Spring	23	35	22	15	42	18	25
Summer	46	35	56	50	31	55	41
Winter	6	18	3	8	15	2	13

Table 2.4. Mean (± standard error (s.e.)) interbirth intervals and contraceptive duration (in days) for female kangaroos across treatment and control groups. Mean data are presented for all females in each group or site (all animals), excluding females that had already conceived young before treatment (i.e. a blastocyst in diapause or an active pregnancy). Interbirth Interval 1 = interval from PY present in the pouch at the treatment to the first PY born post-treatment), Interbirth Interval 2 = interval between first and second PY born post treatment.

	Treatment groups				
	Control Mean ± s.e. (days)	Dart Mean ± s.e. (days)	SC Mean ± s.e. (days)	SCHD Mean ± s.e.. (days)	*p* value
Interbirth Interval 1 (all animals)	357 ± 12^{a} (31)	466 ± 27^{b} (35)	456 ± 33^{ab} (26)	500 ± 50^{b} (18)	0.006*
Interbirth Interval 1 (excluding PY conceived pre-treatment)	378 ± 14^{a} (22)	536 ± 33^{b} (23)	533 ± 36^{b} (17)	586 ± 61^{b} (12)	0.000*
1st Birth post treatment	214 ± 17 (34)	253 ± 30 (35)	287 ± 35 (30)	300 ± 49 (21)	0.245
1st Birth post treatment (excluding PY conceived pre-treatment)	260 ± 16^{a} (24)	343 ± 32^{b} (23)	382 ± 35^{b} (20)	408 ± 52^{b} (14)	0.010*
Interbirth Interval 2	366 ± 27 (27)	384 ± 25 (28)	396 ± 30 (18)	361 ± 33 (15)	0.708
Interbirth Interval 2 (excluding PY conceived pre-treatment)	378 ± 39 (17)	358 ± 24 (16)	370 ± 38 (12)	344 ± 31 (10)	0.958
2nd Birth since treatment	555 ± 35 (27)	592 ± 41 (27)	677 ± 35 (18)	678 ± 61 (15)	0.083
2nd Birth since treatment (excluding PY conceived pre-treatment)	616 ± 43 (17)	666 ± 56 (15)	745 ± 33 (12)	774 ± 62 (10)	0.081

Values with different letters (a or b) are significantly different at $p < 0.05$. *significant p value.
(*n*) in parentheses = samples for that Mean ± s.e. calculation

Table 2.5. Percentage of kangaroos exhibiting data gaps preventing treatment duration calculations. These animals disappeared or died after treatment. The data are categorised by site: Nelson Bay Golf Club (NB), Darlington Park (DP), and Heritage Park (HP). The percentages are broken down into treatment groups: remote delivery (Dart), high dose (SCHD), low dose (SC) and control, representing the percentage of females affected by data absence.

	Dart (%)	SCHD (%)	SC (%)	Control (%)	Total (%)
NB	10 (2)	24 (4)	5 (1)	19 (4)	14 (11)
HP	29 (4)	33 (4)	54 (7)	25 (3)	35 (18)
DP	0	0	33 (3)	10 (1)	13 (4)
Total	14 (6)	26 (8)	26 (11)	19 (8)	21 (33)

(*n*) in parentheses = number of kangaroos that disappeared or died after treatment

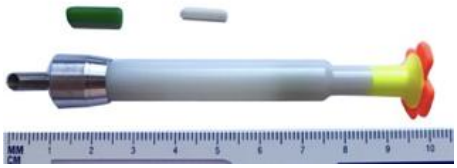

Figure 2.1. Prototype dart (Pneudart, Williamsport, PA, USA), gelatin sheath (green) and contraceptive implant (white).

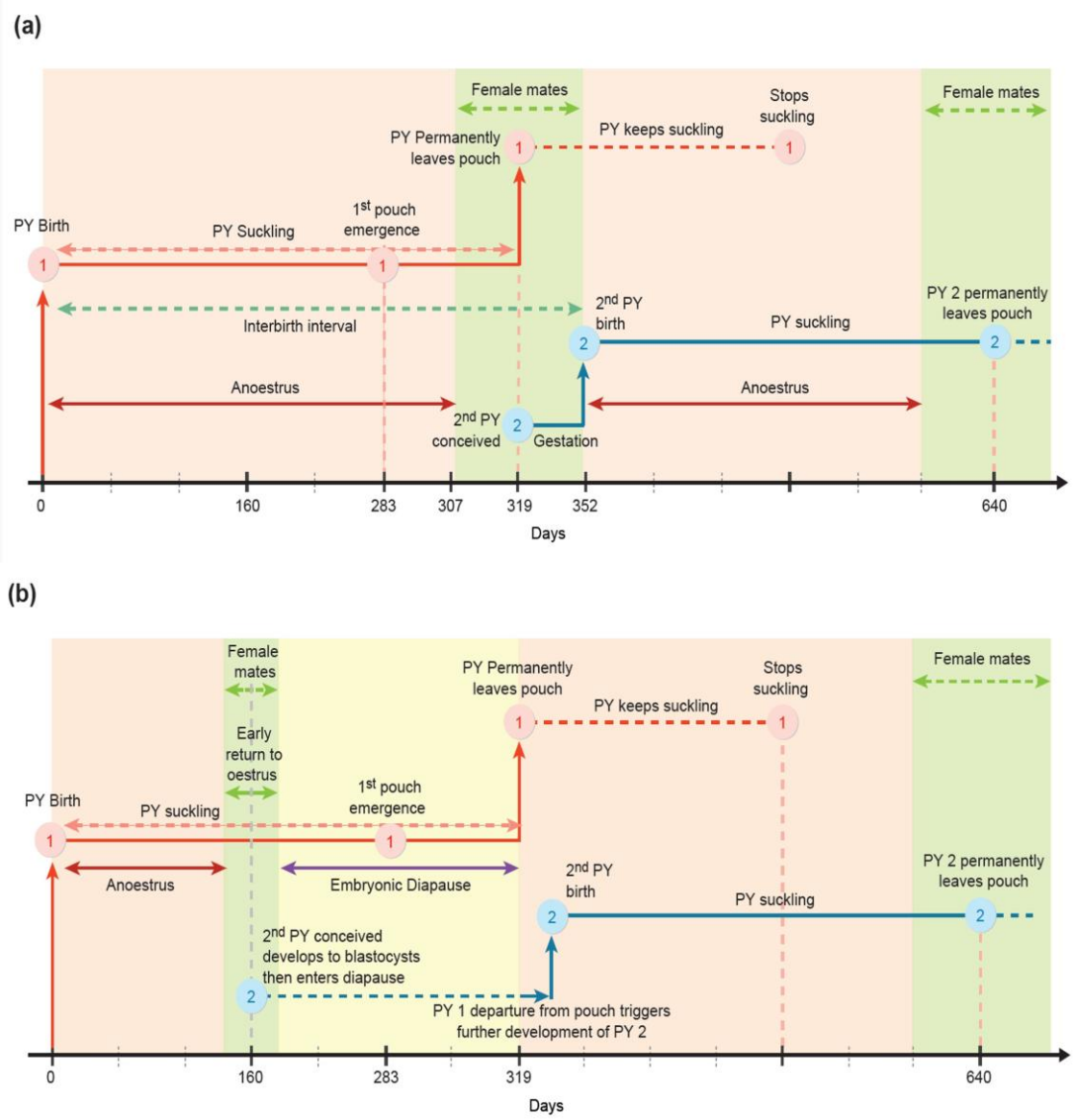

Figure 2.2. Female eastern grey kangaroo reproductive patterns depicting a) the "typical" timeline when a female returns to oestrus after PY loss or just before the PY permanently vacates the pouch; and b) the timeline when a female mates when her previous young in the pouch, resulting in a blastocyst entering embryonic diapause. Not to scale.

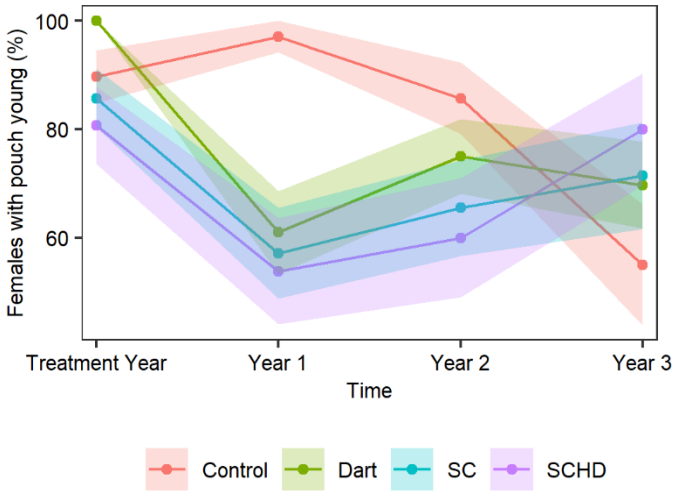

Figure 2.3. Percentage of females carrying PY at the time of treatment (Treatment Year) and one year (Year 1), two years (Year 2) and three years (Year 3) post treatment, for different treatment groups: Control (no implant), Dart (4.7 mg implant dart-delivered), S SC (4.7 mg implant hand injected), and SCHD (2 x 4.7 mg implant hand injected) across all three sites: HP (Heritage Park); DP (Darlington Park); NB (Nelson Bay Golf Club). Shaded polygons represent standard error. Sample sizes indicate females carrying PY out of total females observed (n with PY / total n observed) at each time point, by treatment group: Treatment Year – Control (n = 35/39), Dart (n = 44/44), SC (n = 36/42), SCHD (n = 25/31); Year 1 – Control (n = 33/34), Dart (n = 25/41), SC (n = 20/35), SCHD (n = 14/26); Year 2 – Control (n = 24/28), Dart (n = 30/40), SC (n = 19/29), SCHD (n = 12/20); Year 3 – Control (n = 11/20), Dart (n = 23/33), SC (n = 15/21), SCHD (n = 12/15).

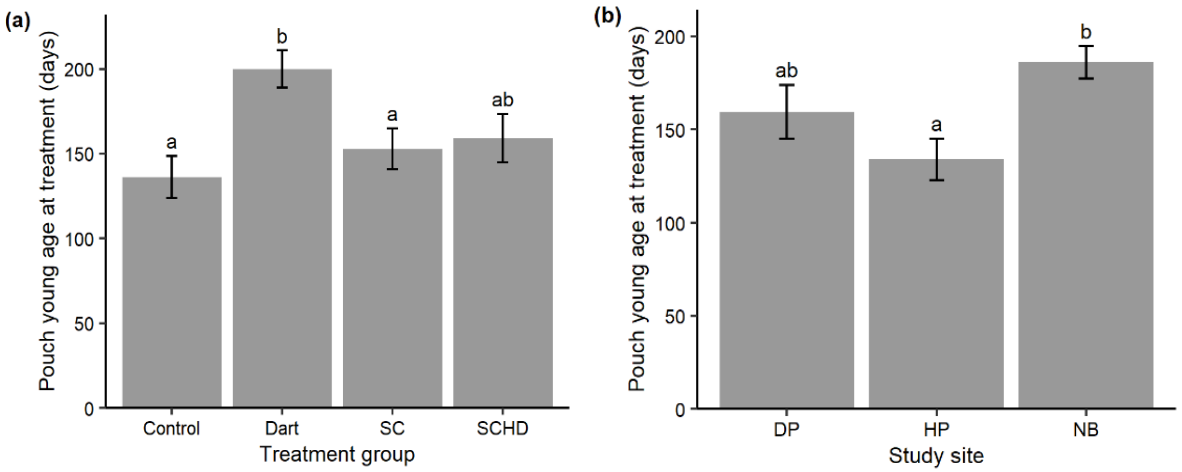

Figure 2.4. Mean age of pouch young (PY) at the time of treatment (± SE, days) (a) across treatment groups: Control; Dart (remote delivered 4.7 mg implant); SC (hand-injected 4.7 mg implant); and SCHD (hand-injected 2 × 4.7 mg implant), and (b) across study sites: HP (Heritage Park); DP (Darlington Park); NB (Nelson Bay Golf Club). Bars with different letters (a or b) are significantly different at $p < 0.05$. Number of females with PY observed at the time of treatment: Control (n = 35), Dart (n = 44), SC (n = 36), SCHD (n = 25); DP (n = 27), HP (n = 44), NB (n = 69).

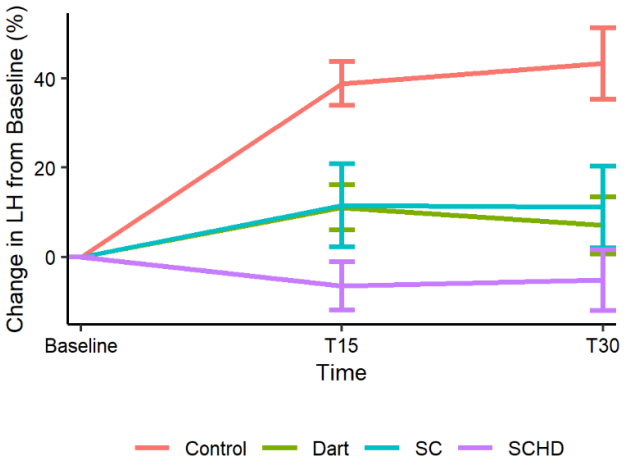

Figure 2.5. Percentage changes in LH concentrations at 15 and 30 minutes post GnRH injection (at Time 0) from baseline levels (average of Time -15 and 0), across treatment groups: Control (capture no implant, n = 22), Dart (remote delivered 4.7 mg implant, n = 25), SC (hand-injected 4.7 mg implant, n = 17), and SCHD (hand-injected 2 x 4.7 mg implant, n = 13); mean ± s.e.. GnRH challenges conducted 8 to 12 months post-treatment.

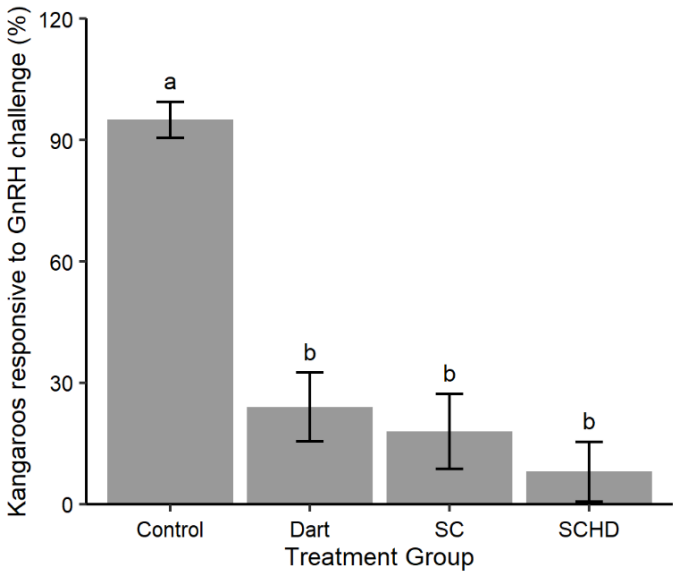

Figure 2.6. Percentages of female kangaroos displaying a significant response to GnRH challenge, across the four treatment groups: Control (capture no implant, n = 22), Dart (remote delivered 4.7 mg implant, n = 25), SC (hand-injected 4.7 mg implant, n = 17), and SCHD (hand-injected 2 x 4.7 mg implant, n = 13) ± s.e.. A subset of females from each treatment group (total n = 77) from three study sites: DP (Darlington Park), HP (Heritage Park) and NB (Nelson Bay Golf Club) were re-captured for GnRH challenge approximately 8 to 12 months post-treatment.

3. Assessing physiological, behavioral and movement responses to Suprelorin® implant delivery methods in free-ranging eastern grey kangaroos (*Macropus giganteus*)

(This chapter uses US spelling, as required by the international journal to which it was submitted).

3.1 Abstract

Overabundant populations of eastern grey kangaroos in peri-urban areas pose ecological and social challenges. Current management strategies are often hindered by animal welfare and logistical concerns. This study aimed to assess the stress responses of eastern grey kangaroos to two contraceptive delivery methods. We specifically assessed physiological and behavioral responses of females treated with Suprelorin® implants delivered remotely (via dart) as an alternative to the traditional capture-treatment method (subcutaneously injected). Sexually mature females were assigned to one of three experimental groups: SC (4.7 mg implant via subcutaneous injection after capture), Dart (4.7 mg implant administered remotely via dart), and Control (no treatment or capture). We used a before-after control-impact framework to quantify any stress-induced response to the two delivery methods through an assessment of changes in behavior (which may also indicate pain) and in fecal glucocorticoid metabolite (FGM) concentrations. Additionally, we assessed changes in movement patterns using GPS collars. No significant changes in FGM concentrations, behavior or movement were detected across treatments or time periods. During the treatment window (5–9 April 2018), the Dart group displayed a temporary reduction in step length while the SC group shifted from nocturnal to crepuscular activity, with both groups returning to pre-treatment patterns after the treatment

window. The SC group also showed weak evidence of an increase in vigilance behavior and decrease in grooming duration through the treatment window, though individual variability was high. Remote dart delivery reduces the risks associated with sedation and capture, offering a safer alternative for kangaroos and humans when applying contraceptives in a peri-urban area. Future research should enhance the utility of remote delivery by addressing logistical challenges (e.g., identifying treated animals) and evaluating its efficacy in other species. The inclusion of remote darting methods in wildlife management programs may improve animal welfare and the effective management of overabundant populations.

3.2 Introduction

The eastern grey kangaroo (*Macropus giganteus*) inhabits extensive regions across eastern Australia. In urban environments where resources are plentiful, kangaroos can reach high densities, leading to overabundance (Wimpenny et al. 2021). This may lead to increased competition for resources, human-wildlife conflicts (e.g. kangaroo-vehicle collisions and aggressive encounters) and negative environmental impacts, such as overgrazing and habitat degradation, which adversely affect other species and the overall environmental health (Herbert et al. 2021).

Management strategies for eastern grey kangaroos (EGK) are often influenced by social and political considerations, where lethal techniques, such as culling, are controversial (Herbert et al. 2021). Public opposition to culling is growing, primarily due to concerns over animal welfare, human safety in urban areas, the environmental impact of certain lethal methods and the perceived ineffectiveness of culling in permanently reducing the size of the target wildlife population (Massei 2023). Additionally, the proximity of kangaroo populations to residential areas limits the feasibility of culling due to safety concerns (Wimpenny et al. 2021). Fertility

control presents a non-lethal alternative for managing overabundant kangaroo populations, particularly in peri-urban areas (Herbert et al. 2021; Silva et al. 2025b)

Suprelorin® (Virbac Australia, Macquarie Park, NSW) is a contraceptive implant containing the gonadotrophin-releasing hormone (GnRH) agonist deslorelin. It inhibits luteinizing hormone (LH) production by the pituitary gland, disrupting follicular development and estrous cycles in females (Herbert & Trigg 2005), presenting a potential solution for achieving controlled and reversible fertility suppression in EGK (Wimpenny et al. 2021). Suprelorin® is typically administered via subcutaneous injection between the shoulder blades of the kangaroo after capture (Herbert et al. 2006; Woodward et al. 2006). In free-living populations, capture requires immobilization by darting, a procedure that carries inherent risks to the operator and may compromise animal welfare (Wimpenny et al. 2021). Recently, remote dart delivery of Suprelorin® was successfully trialled in three wild EGK populations, demonstrating comparable efficacy to hand injection methods while offering a less invasive alternative (Silva et al. 2025b)

Contraceptive treatment with GnRH agonists has not been associated with undesirable behavioral effects (Baker et al. 2002; Bertschinger et al. 2002; Cope et al. 2018a; Herbert & Trigg 2005; Wilson et al. 2013; Woodward et al. 2006). Woodward et al. (2006) found no significant changes to overall crepuscular activity or feeding behavior in females EGK treated with deslorelin implants. A review on the side effects of contraceptive treatment in wildlife, indicated that activity patterns were unchanged after contraceptive treatments in most studies, although in some species, contraception resulted in decreased foraging time, or improved body condition (Gray & Cameron 2010). While the contraceptive has minimal impact, the method of delivery could lead to pain, behavioral changes and increased stress levels (Brivio et al. 2015; Cattet et al. 2008).

Stress is the biological response of an organism to harmful environmental stimuli or stressors that disrupt the body's homeostatic regulatory systems (Novak et al. 2013). The nature and intensity of stressors in an environment can lead to either acute (short-term) or chronic (long-term) stress responses (Rao et al. 2024). Glucocorticoids (GC) are steroid hormones produced by the adrenal glands in response to a stressor (Sheriff et al. 2011). At baseline levels, GC help regulate energy, manage inflammation, influence glucose production and support reproductive health (Fanson et al. 2017). During acute stress, GC facilitate energy mobilisation and may enhance behaviors such as alertness or escape responses (Möstl & Palme 2002). However, prolonged elevation of GC due to chronic stress can lead to negative health effects, including immune system suppression, muscle wasting, and reproductive issues (Fanson et al. 2017).

Glucocorticoid metabolites are processed and excreted over time and these metabolites can be detected in faeces (so called fecal glucocorticoid metabolites, FGMs) for several days following a stress event (Corvalan 2020). FGMs can be measured non-invasively to assess an animal's physiological stress levels without the need for capture or invasive procedures such as blood sampling (Fanson et al. 2017; Sheriff et al. 2011). The detection of GC in faeces can vary based on several factors, including the species, the type of stressor, and the specific assay used (Fanson et al. 2017). FGMs have been previously used as indicators of physiological stress levels in marsupials (Cope et al. 2022; Corvalan 2020; Dowle et al. 2012; Fanson et al. 2017; McKenzie & Deane 2005), allowing researchers to quantify changes in stress response related to different treatments.

EGK exhibit primarily crepuscular and nocturnal behavior patterns, including grazing, variations in posture, and vigilance, while midday periods are typically spent resting (Clarke et al. 1995; Coulson 1997; Favreau et al. 2014; Kaufmann 1975). These patterns can fluctuate in response to

various stressors, including environmental and social factors such as food availability, population density, habitat disruption, and human presence (Favreau et al. 2014). Acute stress responses to darting, capture and handling can disrupt normal wildlife behaviors and alter movement patterns and landscape use (Trondrud et al. 2022). Previous research has reported that capture and manipulation procedures cause acute stress, as indicated by the increased FGMs concentrations in EGK (Herbert et al. 2020) and alpine ibex (*Capra ibex*) (Brivio et al. 2015). Alterations in movement rates following acute stress have also been documented in various species, including mule deer (*Odocoileus hemionus*) (Northrup et al. 2014), grizzly bears (*Ursus arctos*) and American black bear (*U. americanus*) (Cattet et al. 2008), free-ranging bison (*Bison bison*) (Jung et al. 2019), and western grey kangaroos (*Macropus fuliginosus*) (Cowan et al. 2020). Additionally, acute stress can lead to myopathy, a condition where large muscle masses can be damaged resulting in the release of substantial quantities of myoglobin, which can lead to death immediately following capture or up to several days later (Gamble 2004).

Pain is defined as "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage" (IASP 1994). It involves sensory, cognitive, and affective dimensions that individuals experience differently (Walker et al. 2009). Techniques for assessing animal pain typically involve evaluating general body functions, including changes in feed consumption, physiological responses such as alterations in cortisol concentrations, and behavioral changes (Weary et al. 2006). Pain related behaviors vary by species and can persist for days or weeks following a painful procedure due to tissue damage, inflammation and repair processes (Walker et al. 2009).

Assessing pain responses to fertility control agents and their methods of delivery is important to ensure that no negative side effects arise, especially since the justification for using fertility

control often hinges on improving animal welfare (Woodward et al. 2006). The practical application of contraception relies on its ability to effectively inhibit reproduction without causing adverse physical, physiological or behavioral side effects.

This study aimed to investigate whether EGK experience greater pain and stress responses following either intramuscular remote delivery or capture (via immobilization) and subcutaneous hand-injection of Suprelorin® implant. We used video observations and Global Positioning System (GPS) tracking collars to assess behavioral and movement changes (which may be indicative of pain) before and after treatment, and FGM analysis to measure changes in stress hormone concentrations among treated kangaroos. This study is the first systematic investigation into the pain and stress effects of Suprelorin® contraceptive implants delivery methods in a free-range EGK population. The findings will inform the development of best-practice protocols for implementing remote delivery fertility control methods in free-ranging kangaroo populations.

3.3 Materials and Methods

3.3.1 Study Site

The study was conducted at Look at Me Now Headland (LAMN), situated 15 kms north of Coffs Harbour, New South Wales, Australia (30.177°S, 153.189°E). LAMN is part of the Moone Beach Nature Reserve, comprising approximately 24 hectares and including a threatened ecological community of *Themeda*-grasslands and threatened flora species such as *Zieria prostrata* (Hunter & Hunter 2019). The area is also home to free-ranging macropods, including EGK and red-necked wallabies (*Notamacropus rufogriseus*). Direct counts of kangaroos in February 2018 and February 2019 reported densities of 5.4 (CV = 11.3%) kangaroos per hectare

(Brandimarti et al. 2021). LAMN has experienced increasing kangaroo densities, which can have significant impacts on local systems, particularly on endangered species (Herbert et al. 2021).

3.3.2 Experimental design

We used a Before-After Control-Impact (BACI) experimental design (Green 1979) to measure the effect of the two contraceptive delivery methods on kangaroo movements, behavior and stress levels (Figure 3.1). Before the intensive contraceptive treatment and sampling period, a total of 31 sexually mature female kangaroos were immobilized using Zoletil® and processed for ear tagging and collection of physical and reproductive metrics. During the initial capture, a subset of female kangaroos (n = 9) was fitted with solar-powered GPS collars to track their movement pre- and post-contraceptive treatment. During the treatment period, tagged females were randomly allocated to one of three experimental groups: SC (4.7 mg Suprelorin® implant via subcutaneous injection after capture, n = 11), Dart (4.7 mg Suprelorin® implant administered remotely intramuscularly via dart, n = 9), and Control (no treatment or capture, n = 11). Behavioral responses to these treatments were recorded through video observations pre- and post-contraceptive treatment. A total of 243 videos were collected from 27 females (Control n = 9, Dart n = 7, and SC n = 11). Fecal samples were collected to assess stress responses to the treatment via glucocorticoid metabolite concentrations (total fecal samples n = 138; Control n = 38, Dart n = 46, SC n = 54). The study also included long-term monitoring of the reproductive status and an analysis of contraceptive duration. Detailed descriptions of captures, treatments and monitoring procedures are outlined below.

3.3.3 Animal capture and processing

Over three days in early February 2018, 31 female kangaroos were immobilized using a fixed dose of 125 mg of Zoletil® 100 (approximately 5 mg/kg for a 25 kg female), administered as a 1 mL injection intramuscularly in the rump muscles using a dart gun (Type P, 1cc, ¾" gel collars darts fired from an X-Caliber, both from Pneu-dart, Williamsport, PA, USA) at distances of up to 20 m. Following immobilization, the kangaroos were immediately transported to a nearby processing area within 500 m of the capture location. We recorded the weight, leg length and reproductive status of each animal, following Silva et al. (2025b). Each kangaroo was individually injected with a microchip (FDX-B Injectable Transponder Implant, Allflex, Capalaba, Qld, Australia) and identified with uniquely colored and shaped ear tags (Allflex sheep button or mini tags, Capalaba, Qld, Australia). The reproductive status was determined through pouch inspection and teat condition assessment, categorized as: (1) no signs of recent activity, (2) elongated teat with milk (indicating suckling of young at foot or very recent loss of young), or (3) regressing teat (indicating recent loss or weaning of young). For females with pouch young, the offspring's pes (foot) and head length were measured to estimate age (Poole et al. 1982). The birth date of pouch young (PY) was calculated by subtracting the estimated age from the capture date. After processing, animals were released in a tranquil area near the processing site, with their heads covered, and visually monitored remotely for up to 2.5 hours from time of immobilization.

3.3.4 GPS collar deployment and retrieval

We deployed nine solar-powered GPS collars (Radio tag-14, Milsar, Gdansk, Poland) on a random subset of female kangaroos at the time of initial capture. At the time of treatment, we ensured that these animals were split between the two contracepted groups, i.e. Dart (n = 4) and

SC (n = 5), as the relatively small number of collars meant that splitting them between all three groups would render the sample sizes for each too small. Each collar weighed approximately 131 g, which is about 0.5% of an average adult female's body weight. Each collar recorded GPS location fixes every 15 minutes after activation. The success rate, defined as the percentage of scheduled GPS fixes successfully obtained, was used to assess data completeness and collar performance during the tracking period. Under ideal conditions, the GPS accuracy was within 3-5 m. However, accounting for less optimal conditions, 95% of the fixes were expected to be within a 15-20 m radius. To measure the effect of the treatment on kangaroo movements, we considered a 30-day period before the contraceptive application window (5–9 April 2018 = during treatment) and a 30-day period after, resulting in a total of 65 days of tracking data for each female. These time windows provided sufficient data for robust estimation of movements while minimizing seasonal variations in behavior. We discarded all GPS locations associated with a horizontal dilution of precision > 3 and removed unrealistic locations in the sea or those resulting from movement spikes consisting of two consecutive steps with a speed exceeding 10 ms^{-1} and a turning angle < 30° (Gupte et al. 2022). To retrieve the collars, the kangaroos were recaptured (as previously described), approximately 14 weeks after deployment. One collar was retrieved seven months after deployment due to delays in recapturing the kangaroo.

3.3.5 Contraceptive Delivery

Suprelorin® (Virbac Australia, Macquarie Park, NSW) contraceptive implants contain 4.7 mg of the GnRH agonist deslorelin (D-Trp^6-Pro^9-des-gly^{10}-GnRH ethylamide) incorporated into a matrix and formulated into 2.3 x 12.5 mm implants. Suprelorin® implants were delivered by one of two delivery methods: females in the SC group were captured (as above) and had one implant injected subcutaneously between the shoulder blades; females in the Dart group received one

implant delivered intramuscularly using a specialized prototype dart (Pneudart, Williamsport, PA, USA) shot from a tranquilizer firearm (Pneu-dart X-caliber; Williamsport, PA, USA). These custom darts were modified versions of standard 1cc Type P darts, featuring a larger caliber needle to accommodate the implant. The dart body was pre-loaded with 1cc of Meloxicam (Metacam; Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany), a non-steroidal anti-inflammatory drug with analgesic properties, as in Silva et al. (2025b). A previous pilot study conducted on the mid-north coast of New South Wales (Australia), found that a subset of female EGK exhibited limping within the first 48 hours after receiving Suprelorin® implants remotely delivered via dart (Herbert, unpublished data). To address these findings, we incorporated Meloxicam into the remote delivery dart in our study to prevent pain. The implants were encased in a gelatine sheath (made by Pneu-dart using proprietary technology) and loaded into the dart's cannula immediately before firing to prevent premature softening and potential lodging. The darts were fired from approximately 10 m away, targeting the kangaroo's rump area.

3.3.6 Behavioral observations

To assess the behavioral impact of the two contraceptive delivery methods, we filmed kangaroos in ten-minute intervals three times daily (morning, afternoon, and evening), three days before and three days after the treatment (hereafter referred as pre-72, pre-48, pre-24 and post-24, post-48, post-72). These behavioral observations were intended to assess the acute, short-term response to contraceptive delivery (i.e. treatment) methods. This timeframe was selected because previous studies have shown that stress and behavioral changes resulting from capture and darting usually appear within the first few days after the procedure (Herbert et al. 2020; Stiegler et al. 2024) and we were primarily interested in the stress and pain responses to initial treatment. We used a variety of mobile phone cameras (depending on the observer’s personal device) to

capture footage from distances ranging between 5 and 10 m. Each video was reviewed by the same operator, who recorded the frequency and duration of an array of behaviors potentially influenced by the contraceptive delivery, using Solomon Coder, a specialized video coding software for animal behavior (Péter 2017). The kangaroos at LAMN are accustomed to the presence of humans due to the area's popularity as a tourist destination, which minimized any influence from the observers' presence during the recording.

The behavioral responses were categorized into two groups: frequency and duration (Table 1). The frequency group included five short, discrete behaviors (hopping, grooming head, grooming body, pentapedal walk and shaking ears), recorded as the number of observations during the length of each video. The duration group included behaviors recorded as the proportion of time allocated to each behavior throughout the duration of the video: they consisted of four mutually exclusive behaviors (grazing, lying down, locomotion, and standing) and two non-exclusive behaviors (grooming and vigilance). Due to a limited number of observations, we combined all pre- and post-treatment observations into "Before" and "After" treatment categories, respectively. Behaviors such as scratching or touching the left or right rump (subsequently coded as the darted or undarted side), interactions with other kangaroos, and limping on either side, were recorded but excluded from the analyses because of their low frequency of observation. The reviewer recording the behaviors had no knowledge of which side the kangaroo was darted while reviewing the footage.

3.3.7 Fecal sample collection

To measure the effect of the two delivery methods on stress hormones, we collected fecal samples (3-5 pellets/sample) from females pre- and post-treatment, during capture and video recording sessions, when available. A total of 138 samples were collected from 31 individuals

(Pre-treatment: Control n = 22, Dart n = 20, SC n = 30; Post-treatment: Control n = 16, Dart n = 26, SC n = 24). Each sample was placed in a ziplock bag, identified with the animal's ID, kept in an ice box and was subsequently frozen at -20 °C for later FGM analysis. Usually, the metabolites of GC released in response to a stressor can be detected in feces within a few hours, although the exact timing depends on the species and individual physiological factors like gut passage rates (Fanson et al. 2017). In many vertebrates, gut transit times vary from a few hours to several days, affecting when FGM become detectable in faecal samples. In EGK, the time from stress exposure to the highest FGM levels is approximately 19 hours, ranging from 10 to 34 hours (Fanson et al. 2017). Therefore, the selected sampling window effectively captures the immediate stress responses to treatment delivery.

3.3.8 Assessment of fecal glucocorticoid metabolites levels

In the laboratory, the fecal samples were thawed and dried for approximately 18 hours in a Qualtex Thermostat laboratory oven set to a temperature of 60 °C. Once dried, the samples were crushed with a mallet and passed through a fine mesh sieve. The resulting fine fecal powder was stored in vials at -20 °C until ready for steroid metabolites extraction. Fecal powder, ranging from 0.19 to 0.22 g, was weighed and placed into test tubes with 2.5 mL of 80% methanol (Sherwen et al. 2015). The tubes were vortexed for 15 seconds and subsequently shaken on a Thermo Fisher Scientific orbital shaker at 210 rpm for one hour at room temperature and then centrifuged using a Sorvall general-purpose RC-3 automated refrigerated centrifuge at 2500 g for 15 minutes. The clear supernatant containing the extracted FGMs was then transferred into labelled glass vials and stored at -20 °C. The 37e enzyme immunoassay, originally developed for wild mice (*Mus musculus)* (Touma et al. 2003) and later validated for EGK (Fanson et al. 2017), was employed for this study. This assay was specifically designed to target FGM and is suited

for broad-spectrum FGM analysis (Möstl et al. 2005), measuring GC metabolites with a 5α-3β, 11β-diol structure. All necessary standards, antibodies, and labels were obtained from R. Palme from the Institute of Biochemistry at the University of Veterinary Medicine, Austria.

The assay used microtiter plates (Costar 96 well, high binding assay plate, Corning Inc.) pre-coated with goat anti-mouse immunoglobulin G (GAMG) coating antibody. Plates were thawed and washed three times prior to use. A volume of 0.05 ml of steroid standard diluted fecal extract (1:20 in Trizma buffer), or control, was added to each well, followed by 0.1 ml of biotinylated steroid and 0.1 ml of primary antibody. Equilibrium binding occurred during an overnight incubation at 4°C. After washing the plates three times, 0.25 ml of streptavidin-POD-conjugate was added to each well and incubated for 45 minutes at 4°C. The plates were then washed six times before 0.25 ml of TMB substrate (3,3′,5,5′-Tetramethylbenzidine) was added. The reaction was stopped with 0.05 ml of sulfuric acid. The optical density was measured at 450 nm using an ELx808 BioTek microplate reader with Gen5 Software, employing a reference wavelength of 630 nm. Results were uploaded to MyAssays.com and calculated using four-parameter logistic curve analysis. The assay's reliability was demonstrated by intra- and inter-assay coefficients of variation of <10% and <15% respectively, for all assays. To ensure consistency, all samples from an individual were run on the same plate.

3.3.9 Long-term monitoring

All female kangaroos were monitored monthly for approximately 13 months following contraceptive delivery. Observations primarily involved visual monitoring of pouch status using binoculars or a spotting scope where the presence and approximate size of any young were recorded based on observable pouch bulges. PYs were categorized as medium (MPY), large (LPY), or young at foot (YAF), with ages estimated as 146 ± 4 days, 253 ± 4 days, and 342 ± 7

days, respectively, based on visible pouch bulges (Herbert unpublished data; Silva et al. 2025b). At the end of the monitoring period (April 2019), the females were recaptured, their pouches inspected, and all measurements retaken.

3.3.10 Contraceptive duration

We determined contraceptive longevity, for treated kangaroos, based on the time of first young born following contraceptive treatment and whether the PY resulted from a new conception (indicating contraceptive failure or expiration of its effect) or if the female was already pregnant at the time of treatment (either with a blastocyst in diapause or an active pregnancy), as Silva et al. (2025b).

3.3.11 Statistical Analysis

All data analyses were performed in the statistical program R version 4.2.1 (R Core Team 2023).

3.3.11.1 Reproductive and contraception data

To compare female weight, leg length and PY age between treatment groups at the time of treatment, we used gaussian Bayesian generalized linear models (GLMs) with the 'brms' package (Bürkner 2017). We created models where dependent variables (weight, leg length or PY age) were influenced by the variable 'treatment' (Control, SC or Dart). We used diffuse normal priors N (0, 10) for all model parameters. We ran four Markov Chain Monte Carlo (MCMC) chains of 10,000 iterations each, with 5,000 warm-up (burn-in) iterations, ensuring an effective sample size (ESS) > 1000 for each parameter estimate. We assessed the convergence of the MCMC chains visually and using the Gelman-Rubin statistic (Brooks & Gelman 1998).

3.3.11.2 GPS data

Acute stress responses to darting or captures can influence animal behavior, including their movement patterns and use of the landscape (Herbert et al. 2020; Trondrud et al. 2022). We used the movement-based kernel density estimation (Benhamou & Cornélis 2010) to describe the kangaroo's occurrence range area based on the 99% and the 50% utilization distribution calculated by biased random bridges (BRB; (Benhamou 2011). For each level, we calculated the overlap (0 = no overlap, 1 = complete overlap) between the 30-day post-treatment range and the 30-day pre-treatment range. We also estimated the relative change in ranging area as the post-treatment area divided by the pre-treatment area.

Intense disturbances can also impact the circadian activity of wildlife resulting in altered diel activity cycles (Tablado & Jenni 2017). As treatment in our study happened during daylight hours, we expected to observe a shift of movement activity towards nighttime. We used the linear distance moved by the kangaroos between two consecutive locations (step length, m) taken 15 minutes apart. We fitted a gaussian generalized additive mixed model (GAMM) with treatment (Dart, SC) and period (before, during and after treatment) specific splines ($N = 6$) to the step length (m) data. We used cubic cyclic structure for the splines to account for the circular nature of the hour of the day and included animal ID as a random effect (means and smooth terms). The time was standardized to sun time (sunrise = 6 am, sunset = 6 pm). The GAMMs were fitted using the 'mgcv' package (v1.8.42) (Wood 2011) in R and visually checked for an absence of pattern in the model residuals and a k-index close to 1.

3.3.11.3 Behavioral data

We assessed the impacts of contraceptive treatments (Control, Dart and SC) on kangaroo behaviors using Bayesian generalized linear mixed models (GLMMs) with the following BACI design (Comte et al. 2023; Pardini et al. 2018):

$$Y_i = \alpha + \beta_1 BA + \beta_2 BA + \beta_3 BA{:}CI + \theta_j,$$

where Y_i represents the response variable (behavior duration or frequency), α is the model intercept reflecting the baseline behavior (Control group) before treatment; β_1 is the expected change in behavior from before to after (BA) in absence of treatment, β_2 is the difference in behavior between groups before the treatment (CI), β_3 is the expected true effect of treatment (interaction between the treatment types and the observation periods) on kangaroo behavior (BA:CI), and θ_j is a random effect accounting for individual variability among kangaroos, considering repeated measures within subjects.

We used a negative binomial distribution to address overdispersion in the count data, with a log link function due to the nature of the response variable. To account for variations in observation time among individual kangaroos (duration of video), we included an offset in our models, representing the logarithm of the total observation time for each subject. This adjustment allowed us to model rates of behaviors rather than raw counts. We used diffused normal priors N (0, 10) for all model parameters. We fitted all models with four MCMC chains, each running for 40,000 iterations after 20,000 warm-up steps with a thinning of 10. Model fit and convergence were checked as previously described.

Percentage changes were calculated as the difference between posterior estimates after and before treatment, divided by the before value and multiplied by 100.

3.3.11.4 Fecal glucocorticoid metabolites data

We used a similar BACI design to investigate the effects of treatment on female kangaroo stress response. As FGM concentrations are strictly positive values, we used a gamma Bayesian GLMM with a log link function. Preliminary screening of the data showed no apparent evidence of differences between sampling periods (Figure 3.5. Supporting information), so we decided to group all the samples before and after to maximize the convergence of the model. We still included the period (pre-72, pre-48, pre-24 and post-24, post-48, post-72), the time of day (morning, afternoon and evening), and the individual ID as nested random effects (i.e. time of day within day within animal ID) to account for sampling design. We used the same priors, model structure and model check as for the behavior models.

3.4 Results

3.4.1 Initial capture data

The mean weight for females in the Control group (n = 11) was 27.9 kgs (95% Credible Intervals (CrI): 25.6–30.1 kg), and leg length was 51.6 cm (95% CrI: 50.2–53.0 cm). Females in the Dart group (n = 9) had similar weight and leg length (CrI including zero) to females in the Control group. Females in the SC group (n = 11) were 15% (95% CrI: 4–26%) lighter and 5% (95% CrI: 1-9%) smaller (indicated by leg length) than the females in the Control group (Table 3.5. Supporting information).

At the time of treatment, all females in the Control and Dart groups had PY compared to six females (55%) in the SC group. There was no evidence of a difference in PY age between the three groups, with a mean age of 110 days (95% CrI: 85–146 days; see Table 3.6. Supporting information).

3.4.2 Behavioral analysis

A total of 243 videos were collected from 27 kangaroos (Control n = 9, Dart n = 7, and SC n = 11). The mean video length was 9.5 minutes (range: 1.6 to 13.4 min). On average, the kangaroos allocated their time as follows: 49 % (± 2 % SE) grazing, 8 % (± 2 %) lying down, 1 % (± 0.1 %) moving (locomotion), 42% (± 2 %) standing. In addition, kangaroos showed grooming and vigilant behaviours for 6% (± 1 %) and 17 % (± 1 %) of time, respectively (Figure 3.2A). Our model showed no evidence of females changing their time budget allocation (Figure 3.2B) to the four exclusive behaviors (grazing, lying down, locomotion and standing) (Table 3.7. Supporting information) after contraceptive treatment (Dart and SC).

Before treatment, the three experimental groups showed similar levels of grooming and vigilance (β_1, see Table 3.8. Supporting information) and, in absence of treatment (β_2, see Table 3.8. Supporting information), these behaviors remained stable. The remote darting delivery (Dart) did not affect females' grooming or vigilance (β_3, Figure 3.3) behaviors. Still, our models suggest that the subcutaneous delivery (SC) may be associated with a -24% (95% CrI: -52–4%) decrease in grooming and a 19% (95% CrI: -64–103%) increase in vigilance (β_3) behaviors, but the variance was greater than the effect size (i.e. the credible intervals still included zero, Figure 3.3).

In terms of frequency of behaviors, female kangaroos groomed their heads an average of 1 time (± 0.1 SE), their bodies 5 times (± 0.3), hopped 4 times (± 0.5), engaged in pentapedal walking 5 times (± 0.3), and shook their ears an average of 5 times (± 0.6) per video (Figure 3.2C). Our models showed no evidence of frequency of hopping, grooming head or pentapedal walk being influenced by survey period (i.e. before or after) or treatment groups (see Table 3.9. Supporting information). Our results showed that the frequency of body grooming before

treatment was similar between the SC group and the Control group, but was 14% (95% CrI: 1–24%) less frequent in the Dart group (β_1, see Table 3.9. Supporting information). There was no evidence of change in body grooming frequency for any group after the treatment (i.e., all credible intervals included zero; see Table 3.9. Supporting information). The frequency of ear shaking was similar between all groups before treatment (β1, see Table 3.9. Supporting information) and decreased by 22% (95% CrI: 8–32%; β_2) after treatment in both the SC and Control groups. There was weak evidence that females in the SC group did not reduce their ear shaking behavior after treatment, but the variance was greater than the effect size (β_3, Figure 3.3).

No kangaroos were observed limping after treatment. During the filming sessions, 11 kangaroos briefly touched or scratched their rumps where they received the dart: 3 (33%) individuals from the Dart group touched their rumps a total of 3 times (1 time each), while 8 (73%) individuals from the SC group touched their rumps a total of 15 times (mean = 1.9, max = 3 times).

3.4.3 Fecal glucocorticoid metabolite concentrations

A total of 138 fecal samples were collected from 31 individual kangaroos, 72 samples before treatment (Control n = 22, Dart n = 20, SC n = 30) and 66 after treatment (Control n = 16, Dart n = 26, SC n = 24) (Table 3.4). The mean FGM concentration was 5.2 ng/g (95% CrI: 4.3–6.1 ng/g) across the dataset. Our model showed no evidence of differences between FGM concentrations across treatment groups, time of the day, pre- and post-treatment periods between groups (see Table 3.10. Supporting information).

3.4.4 Kangaroo movement

From the nine GPS collared females (SC = 5 and Dart = 4), one individual from the SC group failed to collect enough locations before and after the contraceptive application window (5-9 April 2018, referred here as during treatment). Between 4 March and 8 May 2018 (i.e., 65 days), the remaining eight collars collected animal locations every fifteen minutes for a total of 43772 locations with a mean success rate of 87.7% (range: 54.6–97.7%, Table 3.3). Before treatment, the mean monthly range (BRB99%) for female kangaroos was 43.8 ha (range: 38.2–49.7 ha) and the core area (BRB50%) was 6.4 ha (range: 5.2–7.8 ha) or 15% of the BRB99% area. There was no evidence that females changed their ranging areas after the treatment for both the BRB99% (mean = -0.004; 95% CrI: -0.334–0.329) and the BRB50% (mean = 0.011; 95% CrI: -0.320–0.336). All females showed high site fidelity with the mean overlap between the post-treatment and the pre-treatment ranges of 98.7% (95% CrI: 92.5–100%) and 94.5% (95% CrI: 83.8–99.7%) for the BRB99% and the BRB50%, respectively.

Before treatment, both groups showed movement activity across the whole day with longer movements between sunset and sunrise than during daylight. Females subjected to dart delivery showed a 10.4% (95% CrI: 7.4–13.4%) reduction of their average step length during the five days of treatment, with a more even distribution of movement throughout the day. Females that received subcutaneous injections showed no change in mean step length during the five days of treatment, but their movement pattern shifted to a crepuscular pattern with peaks of long movements around sunrise and just after sunset (Figure 3.4). After the treatment application period, both groups returned to their pre-treatment movement patterns (mean step length and diel pattern).

3.4.5 Contraceptive efficacy

Suprelorin® implants suppressed reproduction in both treatment groups. In the Dart group, the contraceptive treatment was effective for at least 367 days (± 33 days) on average, and 383 days (± 10) in the SC group, on average. At the end of the monitoring period (396 ± 9), five out of nine animals from the Dart group had a PY, yet three of those likely resulted from birth of a young that was conceived prior to treatment (two active pregnancies and one reactivation of a blastocyst in diapause at treatment) (Table 3.2). The other two PY were born approximately 186 and 211 days post treatment, suggesting contraceptive failure or early reversal. In the SC group, three females had PY after treatment, all resulted from active pregnancies conceived prior to treatment (Table 3.2).

3.5 Discussion

This study represents the first comparative assessment of behavioral, physiological and movement changes to different delivery methods of long-acting deslorelin (Suprelorin®) contraceptive implants. Our findings indicate that there were no significant changes in behavior or FGM concentrations that would indicate compromised welfare after either capture and hand-injections, or intramuscular remote delivery of Suprelorin® implants to female eastern grey kangaroos. There were no significant changes in time allocation to key behaviors such as grazing, vigilance and resting, nor any detectable increase in fecal glucocorticoid metabolite concentrations, or changes in movement patterns that may be symptomatic of pain, following treatment with either delivery method. Remote dart delivery was equally effective as the traditional subcutaneous injection capture-and-treatment method with minimal welfare impacts and comparable contraceptive efficacy. These results provide critical insights into the use of

remotely delivered contraceptive treatment as a humane fertility control tool, with potential utility for managing overabundant populations, particularly in peri-urban environments.

In our study, we utilized a prototype dart for remote delivery that was similar to the standard dart used for immobilization of kangaroos within the SC group, but it had a larger caliber needle to accommodate the implant (Silva et al. 2025b). In previous field trials, the dart was pre-loaded with 1 mL of water for injection (which helps to propel the implant into the animal), but 20-25% of animals were observed limping during the first 48 hours after dart delivery (Herbert, unpublished data). This led us to replace the water for injection with 1 mL of Meloxicam (a non-steroidal anti-inflammatory drug with analgesic properties) to manage any pain associated with remote delivery. We did not observe limping in kangaroos from either group post-darting or post-capture in the current study, and behavioral observations did not detect any other subtle changes in behavior that may be indicative of pain. A few females briefly touched or scratched their rumps where they received the dart, but this behavior was more prevalent among females in the SC group than in the Dart group. This could potentially indicate a more pronounced discomfort after capture since SC animals did not receive analgesia during darting for capture.

A study on a golf course on the Gold Coast, Queensland, Australia, documented pain reactions to darting displayed by EGK during capture sessions for Suprelorin® contraceptive administration (Tribe et al. 2014). They categorized pain reactions to dart into four scores: score 1– minimal reaction with no flight response; 2 – jumping at impact with irritation; 3 – immediate flight response; and 4 – rapid or immediate collapse. Most kangaroos displayed minimal reactions to dart impact, suggesting that darting generally does not inflict significant pain. As macropods are considered stoic (NSW Government 2021), it is possible that reactions documented by Tribe et al. (2014) were primarily flight responses rather than direct responses to pain. Although we did

not directly monitor reactions to darting, we assessed pain through visual observations and FGM to evaluate physiological stress effects caused by contraceptive delivery methods. The lack of observed differences in FGM concentrations between treatment groups and time periods suggests that neither delivery method induced considerable stress post treatment.

Gut transit times can vary widely among species, affecting how quickly circulating glucocorticoids are detected following a stressor (Keay et al. 2006). In marsupials, FGM peaks have been observed between 0.3 to 4 days following a stressor. For EGK, the lag between exposure to a stressor and peak FGM concentrations is 19 hours (range = 10-34 hours) (Fanson et al. 2017). As such, we hypothesise that if our treatments increased stress levels, we would have identified rises in FGMs with our sampling within the first 48 hours post-treatment. Using the same enzyme immunoassay, Herbert et al. (2020) observed a significant increase in FGM levels in EGK 24 hours after a stressor, but concentrations returned to baseline by 48 hours.

In our analysis, we observed considerable variability in FGM concentrations among individuals. For instance, one female (ID 307) had FGM concentrations of 17.16 ng/g before treatment (pre-48 morning), which then decreased to 3.96 ng/g (pre-48 afternoon) and fluctuated thereafter up to 14.83 ng/g (post-48 afternoon). She was notably the only animal displaying concentrations above 10.5 ng/g. To better understand individual variabilities and provide a clearer picture of FGM levels in kangaroos, future studies should consider collecting a larger number of samples from the same individual over an extended period before and after treatment. This approach would help detect potential increases or decreases in FGM over time and contribute to a more comprehensive understanding of stress responses in free-ranging kangaroo populations.

Failure to detect an acute stress response to contraceptive treatment may be related to high baseline FGM concentrations in this population due to chronic stress. In 2018, LAMN recorded

one of the highest population densities of EGK in New South Wales, with 5.4 individuals per hectare (Brandimarti et al. 2021). A health assessment conducted at the start of the current study established that this population was experiencing chronic nutritional stress, nonregenerative anaemia, nutritional deficiencies, and heavy parasitic burdens (Brandimarti et al. 2021), believed to be linked to high population density and environmental pressures associated with urbanization. Although FGM concentrations are generally expected to rise in response to acute stress, their dynamics can be more complex in chronically stressed populations. In some species, chronically stressed individuals may reach a physiological limit above which, additional GC rises are curtailed (e.g. starlings (*Sturnus vulgaris*)) (Cyr & Romero 2008), whereas in other species, acute stress responses are still detectable in chronically stressed individuals (e.g. koalas (*Phascolarctos cinereus*) (Narayan 2019). In our study, however, no significant increases in FGM concentrations were detected post-treatment. This highlights the need for further research comparing chronically stressed and unstressed populations to better understand these dynamics in free-ranging kangaroo populations.

Our models showed no significant changes in the time budget allocation for the four exclusive behaviors (grazing, lying down, locomotion, and standing) following contraceptive treatment, regardless of the delivery method. Similarly, Woodward et al. (2006) found no significant differences in the diel activity budgets (including scanning, feeding, grooming, and interactions with other animals) between deslorelin and placebo treated female EGKs, before and after treatment. That study only utilized the SC method to treat females after manual capture and restraint.

While the Dart method did not significantly affect time spent grooming or vigilant in the current study, the SC method showed potential associations with decreased grooming and increased

vigilance. However, the high variance relative to the effect size means these results should be interpreted cautiously. The observed increase in vigilance following SC treatment may be linked to the more intensive handling procedure nature of this method, which involves animal immobilization and handling, rather than the contraceptive treatment. Increased vigilance allows animals to monitor and assess their surroundings in response to perceived threats (Pecorella et al. 2016). Consequently, the SC method may have driven kangaroos to engage in heightened anti predator behaviors, associated with acute stress.

We did not observe significant differences in the frequency of most behaviors assessed. However, some interesting patterns emerged for body grooming and ear shaking. Females in the Dart group initially groomed their bodies less frequently than those in other groups, but increased this behavior after treatment, while the Control and SC groups decreased grooming frequency. Despite an increase in grooming frequency in the Dart group, the overall time spent grooming did not change. Grooming is a complex behavior and can vary among species and individual animals. The overall amount of grooming must be interpreted carefully in relation to stress, as both high and low stress can lead to increased grooming activity (Smolinsky et al. 2009). As for the increase in ear shaking behavior in kangaroos in the SC group, it could indicate an association with increased vigilance, although not much is mentioned in the literature about this behavior other than representing an irritation with ear tags (Sofo 2010) or serving the purpose of warding off insects (Procter-Gray & Ganslosser 1986). As such, their likely significance in the current study is unclear.

Our results on the movement patterns of kangaroos indicated no significant changes in the ranging areas post-treatment for either group, suggesting that the contraceptive treatments did not disrupt the spatial ecology of the kangaroos. The high site fidelity observed further supports

this conclusion, indicating that these females maintained their established home ranges despite the disturbance associated with treatment. High site fidelity and small home ranges are common characteristics exhibited by females in peri-urban areas (Brunton et al. 2018; Coulson et al. 2014; Herbert et al. 2020; Herbert et al. 2021). Previous research at LAMN indicated that females exhibited high fidelity within their small home ranges (Smith 2020).

Prior to treatment, both groups displayed consistent movement activity throughout the day, with longer movements occurring during nocturnal hours (between sunset and sunrise). However, females in the Dart group experienced a reduction in average step length by 10.4% during the treatment application period (5–9 April 2018). This reduction was accompanied by a more even distribution of movements throughout the day, suggesting that darting may have induced a change in how these kangaroos navigated their environment, leading to shorter steps and less distance covered. This shift suggests that the kangaroos adjusted their behavior to maintain activity levels despite the decrease in step length, engaging in shorter movements more frequently throughout the day rather than longer movements concentrated at certain times. Such a change could reflect an adaptation to potential discomfort or changes in mobility resulting from the darting, allowing them to maintain some level of activity while minimizing exertion during peak movement times. Animals often adapt their behavior or change their mobility in response to discomfort or disruptions, allowing them to remain active while reducing energy expenditure during high-demand times (Cattet et al. 2008). From an animal welfare perspective, it is important to reiterate that there was no evidence of limping or changes in grooming directed to the dart-injection site that were indicative of a clear pain response. In contrast, females receiving the SC treatment did not exhibit changes in step length, but their movement pattern shifted to a crepuscular rhythm, increasing activity around dawn and dusk. This alteration may reflect an

adaptive response to the capture and a heightened perception of threat. Animals can adapt their behavior in response to environmental stimuli (Tyler et al. 2016). By shifting to a crepuscular activity pattern during the treatment application window, these kangaroos may be employing a strategy to avoid predation while maximising foraging opportunities. The dim light of twilight makes it more challenging for both predators and prey to see each other, providing a balance between safety and resource availability (Bleicher et al. 2019). After the treatment application window, both groups returned to their pre-treatment movement patterns regarding average step length and diel activity distribution, indicating that while there were some initial changes potentially associated with the treatment delivery methods, these were temporary and did not result in lasting changes to their movement ecology.

Overall, our findings suggest that while the contraceptive treatments did not alter the kangaroos' primary behavior patterns, there were subtle changes in specific behaviors, particularly associated with the SC method. The increased vigilance and changes in grooming patterns in the SC group might indicate a mild stress response to the more intensive handling procedure. It's important to note that these behavioral changes were relatively minor and did not affect core activities like grazing or resting. This suggests that both delivery methods, have minimal impact on the kangaroos' daily behavioral patterns and overall welfare. Replicating this study in different free-ranging populations of kangaroo would increase the robustness of our findings.

The efficacy of the contraceptive in our study (for both treated groups) was similar to a preliminary study conducted at three other sites on the NSW mid-north coast, which found that the duration of contraception was 392 ± 36 (SE) days for the Dart group and 454 ± 38 days for the SC group (Silva et al. 2025b). These durations suggest that annual treatments would effectively suppress female reproduction for extended periods and lengthen inter-birth intervals

(Silva et al. 2025b). By suppressing reproductive activities, kangaroos can potentially improve their own physical wellbeing, reallocating resources that would typically be devoted to nurturing offspring (Forrester et al. 2024).

Several limitations warrant further investigation. First, the small sample size in our study may have limited our ability to detect subtle differences in stress, behavior, and movement patterns. Second, individual variability in FGM concentrations, as observed in kangaroo ID 307, underscores the need for longitudinal studies with larger sample sizes to better understand stress responses over time. Additionally, environmental factors, such as high population density, overgrazing and urban pressures (e.g. human presence and habitat modification), may have contributed to elevated baseline stress levels, potentially masking treatment effects. Further research should include a greater frequency of post-treatment sampling to capture potential short-term changes in FGM levels and behavioral responses. Investigating the use of longer-lasting contraceptives, such as GonaCon™, and their impact on kangaroo populations would also provide valuable insights (Wimpenny & Hinds 2018). Finally, studies should explore the scalability of remote delivery methods for other species and contexts. In particular, the widespread operational use of a dart delivered contraceptive for wildlife management would require modification of the dart to simultaneously mark treated individuals (Wimpenny & Hinds 2018).

In conclusion, the similarity in contraceptive efficacy, physiological, behavioral and movement outcomes between the darted and hand-injected groups suggests that neither delivery method induces a significant stress response in kangaroos, and the efficacy of Suprelorin® implants is consistent regardless of the delivery method. This consistency is vital for management strategies focused on fertility control, where the primary goal is to achieve effective contraception without

compromising animal health, welfare or behavior. Delivering contraception remotely increases the feasibility of treating larger populations and reduces logistical challenges and animal welfare concerns associated with capturing and handling wild animals. Furthermore, remote darting can be particularly advantageous in urban and peri-areas with high kangaroo population densities where animal capture may be less feasible. This method facilitates a wider application of contraceptives with minimal disruption to the animals and their environment, aligning with conservation goals that emphasise humane and minimally intensive handling practices.

3.6 Tables and Figures

Table 3.1. Type and description of behaviors of eastern grey kangaroos assessed in video recordings. The Duration Group consisted of behaviors recorded as the proportion of time allocated to each behavior throughout the duration of the video: four mutually exclusive behaviors and two non-exclusive behaviors. The Frequency Group consisted of five short, discrete behaviors, recorded as the number of observations during the length of each video.

Behavior	Description	Group	Type
Grooming	Scratching or licking any part of the head or body	Duration	Non-exclusive
Vigilance	Lifting their heads above the horizontal position while either crouched or standing upright, and either gazing intently in one direction or scanning their environment[1]	Duration	Non-exclusive
Grazing	Eating or actively searching for food	Duration	Mutually exclusive
Lying down	Sleeping or resting	Duration	Mutually exclusive
Locomotion	Moving by either hopping or pentapedal walking	Duration	Mutually exclusive
Standing	Either in two or four limbs, stationary	Duration	Mutually exclusive
Hopping	Leaping off the ground with both feet	Frequency	Discrete
Grooming head	Grooming any part of the head, including face, ears and eyes	Frequency	Discrete
Grooming body	Licking or scratching any part of the body, including torso, arms, legs and tail	Frequency	Discrete
Pentapedal walk	When kangaroos use their tail as well as their front and hind limbs to walk	Frequency	Discrete
Shaking ears	Flicking or shaking ears	Frequency	Discrete

[1]Favreau et al. 2014

Table 3.2. Number of eastern grey kangaroo females per treatment group, number of females with pouch young (PY) at the time of treatment, and number of females with PY at the end of the monitoring period (396 ± 9 days). The 'PY at the end of monitoring period' is broken down into active pregnancies and blastocysts in diapause (conceived prior to treatment), failure or reversal of contraception, and total (sum of all PY states at the end of monitoring). Note: active pregnancies and blastocysts were not calculated for the control group, as this group did not receive contraceptive implants.

Treatment group	Total females p/ group	PY at treatment	PY at the end of the monitoring period			
			Active pregnancy	Blastocyst in diapause	Failure or early reversal	Total
Dart	9	9	2	1	2	5
SC	11	6	3	0	0	3
Control	11	11	-	-	-	-

Table 3.3. GPS tracking summary and range estimates (ha) using biased random bridges (BRB) for female kangaroos at Look at Me Now Headland, mid-east coast of Australia. Success rate (%) represents the proportion of scheduled GPS fixes successfully recorded by the GPS collar.

Animal ID	Treatment group	GPS locations	Success rate (%)	BRB99% (ha)		BRB50% (ha)	
				Before	After	Before	After
308	Dart	6094	97.7	43.24	40.53	7.00	6.51
304	Dart	5533	88.7	42.68	48.42	5.18	5.51
322	SC	5894	94.5	49.67	47.87	7.80	8.01
359	SC	6001	96.2	38.20	39.99	5.59	5.53
356	Dart	5086	81.5	46.17	37.97	6.46	5.43
358	Dart	3405	54.6	39.51	50.54	6.44	7.98
306	SC	5819	93.3	41.99	44.05	6.60	6.79
326	SC	5940	95.2	48.62	38.73	5.95	5.81

Table 3.4. Number of kangaroo samples collected for fecal glucocorticoid metabolites (FGM) analysis, separated by Before and After treatment. Collection periods: 3 days before (pre-72, pre-48 and pre-24) and 3 days after (post-72, post -48 and post -24) treatment – morning, afternoon, and evening.

	pre-72			pre-48			pre-24			
	Morning	Afternoon	Evening	Morning	Afternoon	Evening	Morning	Afternoon	Evening	Total
Control (n)	0	1	0	5	3	3	5	3	2	22
Dart (n)	0	2	0	2	5	1	6	3	1	20
SC (n)	1	2	2	3	6	4	6	6	0	30
Total samples (n)	1	5	2	10	14	8	17	12	3	72
	post-24			post-48			post-72			
	Morning	Afternoon	Evening	Morning	Afternoon	Evening	Morning	Afternoon	Evening	Total
Control (n)	1	5	1	3	2	3	1	0	0	16
Dart (n)	5	2	4	5	4	5	0	1	0	26
SC (n)	2	4	2	5	5	4	0	1	1	24
Total samples (n)	8	11	7	13	11	12	1	2	1	66

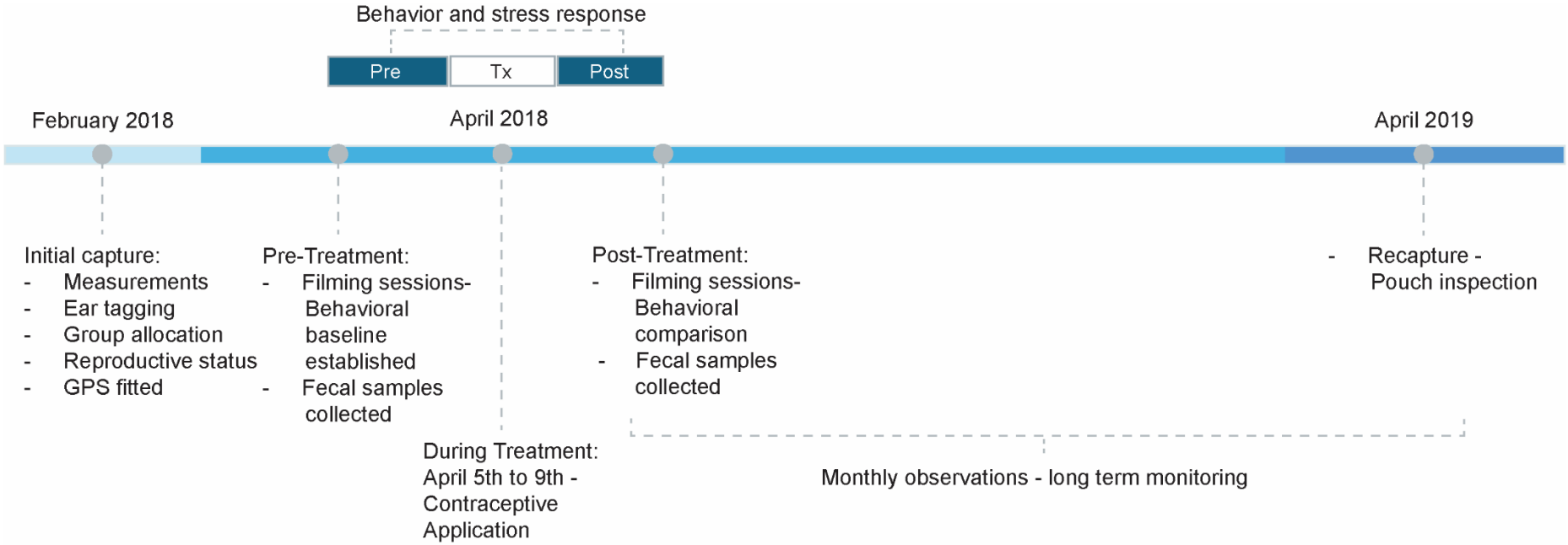

Figure 3.1. Timeline of the study design and data collection process for monitoring the effects of contraceptive treatments in female eastern grey kangaroos. Key events include the initial capture, tagging and GPS fitting in February 2018; contraceptive delivery in April 2018 with pre- and post-treatment filming sessions for behavioral analyses and fecal sample collections for physiological analyses; and long-term monthly observations until recapture in April. Note: timeline not to scale.

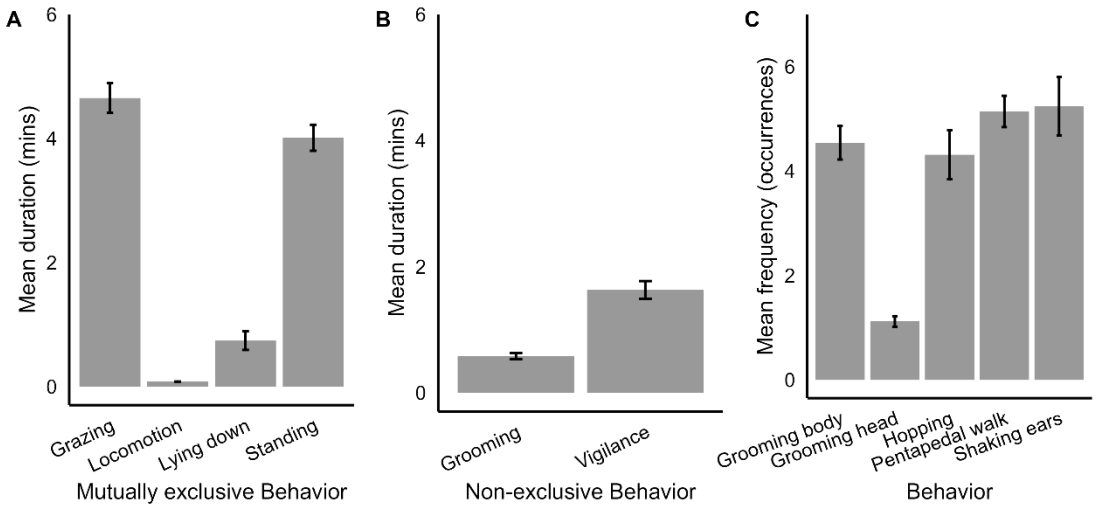

Figure 3.2. Video observations collected for 27 female eastern grey kangaroos before and after treatment (Control n = 9, Dart n = 7, and SC n = 11) from Look at Me Now Headland, Australia. A) Mean duration of the mutually exclusive behaviors – grazing, locomotion, lying down and standing - expressed in minutes; B) Mean duration of the non-exclusive behaviors – grooming and vigilance - expressed in minutes; C) Mean frequency of behaviors – grooming body, grooming head, hopping, pentapedal walk, shaking ears – expressed in number of occurrences.

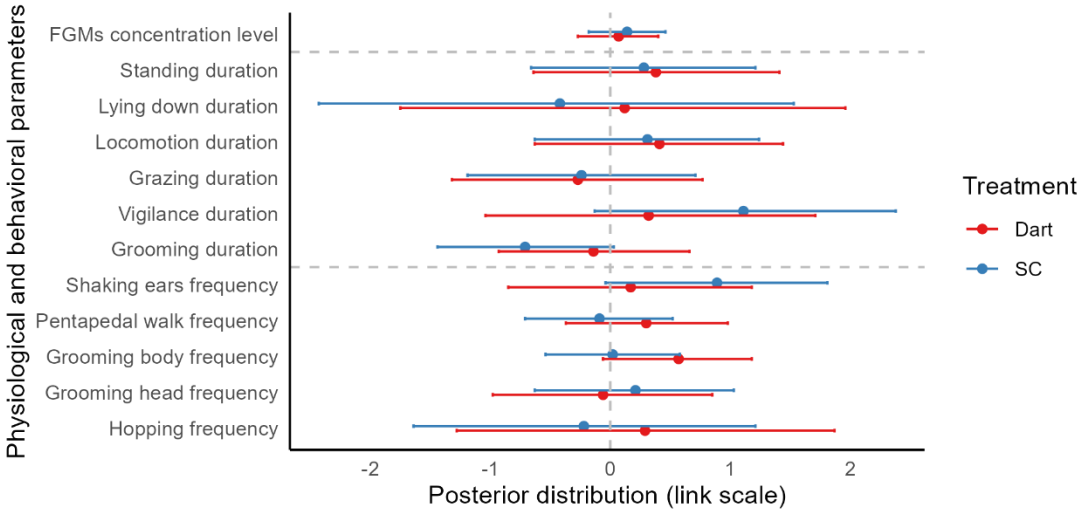

Figure 3.3. Posterior means (and 95% CrI) for the effects of Dart and SC delivered contraceptive treatment (β_3) on FGM concentrations and behavior of female eastern grey kangaroos in our Before-After-Control-Impact experiment in Look at Me Now Headland, Australia, 2018. Posterior means (and 95% CrI) for the effects of treatment (β_3) on six duration behaviors (standing, lying, locomotion, grazing, vigilance and grooming), and five frequency behaviors (shaking ears, pentapedal walk, grooming body, grooming head and hopping).

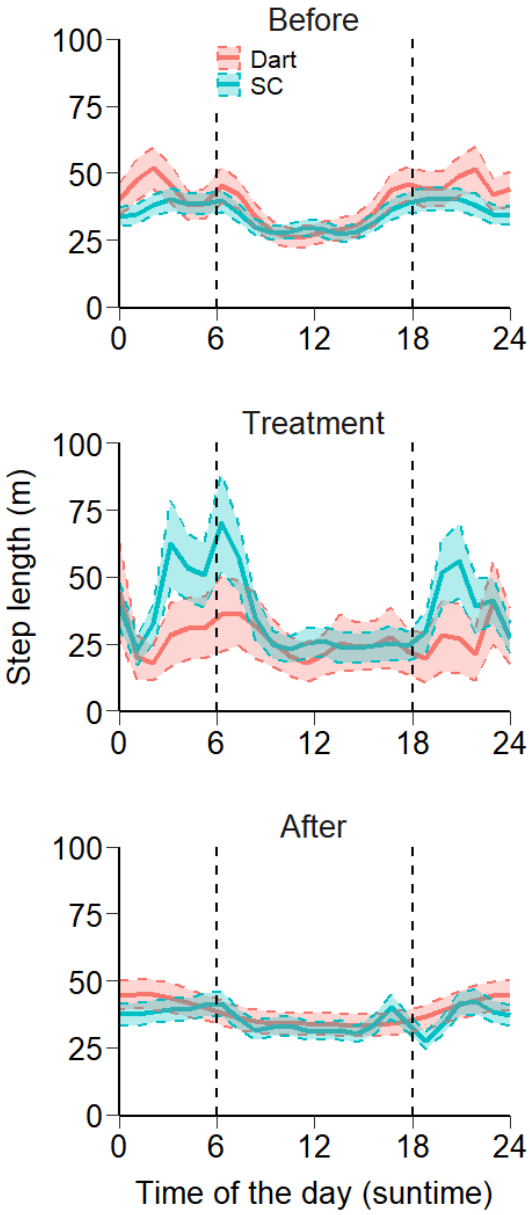

Figure 3.4. Expected mean (and 95% confidence intervals) diel cycle of movement activity (15 minutes step length) for female eastern grey kangaroos before (30 days), during (5 days) and after (30 days) contraceptive treatment. Dart (red, n = 4) and SC (blue, n = 4) groups in Look at Me Now Headland, Australia. Vertical dashed lines represent sunrise and sunset.

3.7 Supporting Information

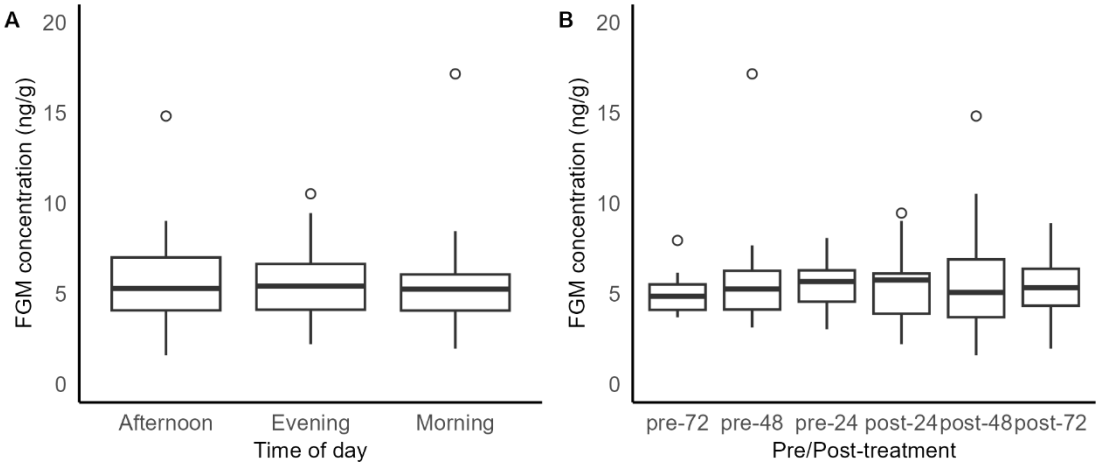

Figure 3.5. Supporting information. Exploratory analysis of fecal glucocorticoid metabolites (FGM) concentrations in 138 fecal samples collected from 31 individual kangaroos. A) Time of day: morning, afternoon, evening; B) Pre/Post-treatment: pre-72, pre-48, pre-24 and post-24, post-48, post-72.

Table 3.5. Supporting information. Posterior means for the model parameters investigating the effects of female eastern grey kangaroo weight (kg) and leg length (cm) between treatment groups (Dart (remotely delivered, n = 9), SC (subcutaneously delivered, n = 11) and Control (no treatment, n = 11) at the time of treatment, at Look at Me Now Headland, Australia. Estimates, 95% credible intervals (CrI), standard deviation of model residuals (sigma), Rhat statistic and effective sample sizes (ESS) are provided for each parameter.

	Estimate	l-95% CrI	u-95% CrI	Rhat	ESS
Weight (kg)					
sigma	0.3	0.03	0.59	1	20689
Intercept Control	27.87	25.64	30.12	1	14860
Dart	-2.45	-5.82	0.85	1	15661
SC	-4.21	-7.39	-1.02	1	14855
Leg length (cm)					
sigma	2.4	1.85	3.12	1	16971
Intercept Control	51.62	50.18	53.03	1	15645
Dart	-1.49	-3.63	0.64	1	15993
SC	-2.53	-4.52	-0.53	1	15537

Table 3.6. Supporting information. Posterior means for the model parameters assessing the age of pouch young at treatment between treatment groups (Dart, SC, Control), at Look at Me Now Headland, Australia. Estimates, 95% credible intervals (CrI), standard deviation of model residuals (sigma), Rhat statistic and effective sample sizes (ESS) are provided for each parameter.

	Estimate	l-95% CrI	u-95% CrI	Rhat	ESS
sigma	109.97	84.66	144.96	1	21112
Intercept					
Control	115.53	73.88	156.91	1	19454
Dart	3.72	-15.63	22.74	1	22544
SC	-1.1	-20.09	17.96	1	24284

Table 3.7. Supporting information. Posterior means for the behavior model parameters investigating the four mutually exclusive behaviors (grazing, lying down, locomotion and standing) in our BACI experiment at Look at Me Now Headland, recorded as the proportion of time allocated to each behavior throughout the duration of the video. β_1 = the expected change in behavior from before to after (BA) in absence of treatment type, β_2 = the difference in behavior between groups before the treatment (CI), β_3 = the expected true effect of treatment (interaction between the treatment types and the observation periods) on kangaroo behavior (BA:CI). Estimates, 95% credible intervals (CrI), standard deviation of model residuals (sigma), Rhat statistic and effective sample sizes (ESS), are provided for each parameter.

	Estimate	l-95% CrI	u-95% CrI	Rhat	ESS
Locomotion Intercept Control (before)	-6.13	-6.67	-5.59	1	32519
Lying Intercept Control (before)	-8.63	-10.17	-7.39	1	35296
Standing Intercept Control (before)	-6.09	-6.63	-5.55	1	31697
Grazing Intercept Control (before)	-6.51	-7.06	-5.97	1	46808
β_1 Locomotion Dart (before)	-0.21	-0.96	0.54	1	35231
β_1 Locomotion SC (before)	-0.06	-0.75	0.62	1	34264
β_2 Locomotion (after)	-0.23	-0.96	0.49	1	28830
β_3 Locomotion Dart (after)	0.41	-0.63	1.44	1	31704
β_3 Locomotion SC (after)	0.31	-0.63	1.24	1	30790
β_1 Lying down Dart (before)	0.43	-1.27	2.27	1	37724
β_1 Lying down SC (before)	-0.13	-1.84	1.63	1	39784
β_2 Lying down (after)	0.27	-1.09	1.69	1	36899
β_3 Lying down Dart (after)	0.12	-1.75	1.96	1	39884
β_3 Lying down SC (after)	-0.42	-2.43	1.53	1	42175
β_1 Standing Dart (before)	-0.14	-0.89	0.60	1	34468
β_1 Standing SC (before)	-0.07	-0.75	0.61	1	33589
β_2 Standing (after)	-0.20	-0.92	0.52	1	27950
β_3 Standing Dart (after)	0.38	-0.64	1.41	1	31049
β_3 Standing SC (after)	0.28	-0.66	1.21	1	30543
β_1 Grazing Dart (before)	0.24	-0.51	1.00	1	49693
β_1 Grazing SC (before)	0.13	-0.56	0.82	1	48328
β_2 Grazing (after)	0.15	-0.58	0.88	1	41641
β_3 Grazing Dart (after)	-0.27	-1.32	0.77	1	45298
β_3 Grazing SC (after)	-0.24	-1.19	0.71	1	43437
SD ID random effect (Locomotion)	0.08	0	0.25	1	60715
SD ID random effect (Lying down)	0.88	0.16	1.75	1	21037
SD ID random effect (Standing)	0.08	0	0.24	1	60204
SD ID random effect (Grazing)	0.09	0.00	0.25	1	66257

Table 3.8. Supporting information. Posterior means for the behavior model parameters investigating the two non-exclusive behaviors (grooming and vigilance) in our BACI experiment at Look at Me Now Headland, recorded as the proportion of time allocated to each behavior throughout the duration of the video. β_1 = the expected change in behavior from before to after (BA) in absence of treatment type, β_2 = the difference in behavior between groups before the treatment (CI), β_3 = the expected true effect of treatment (interaction between the treatment types and the observation periods) on kangaroo behavior (BA:CI). Estimates, 95% credible intervals (CrI), standard deviation of model residuals (sigma), Rhat statistic and effective sample sizes (ESS), are provided for each parameter.

	Estimate	l-95% CrI	u-95% CrI	Rhat	ESS
Grooming duration					
Intercept Control (before)	-2.78	-3.22	-2.3	1	45119
β_1 Dart (before)	-0.18	-0.85	0.48	1	46289
β_1 SC (before)	0.18	-0.43	0.77	1	45987
β_2 After without treatment	0.09	-0.47	0.65	1	47801
β_3 Dart (after)	-0.14	-0.93	0.66	1	52162
β_3 SC (after)	-0.71	-1.44	0.03	1	51249
SD animal ID random effect	0.3	0.03	0.59	1	20689
Vigilance duration					
Intercept Control (before)	-1.47	-2.16	-0.69	1	42992
β_1 Dart (before)	-0.29	-1.34	0.77	1	46500
β_1 SC (before)	-0.03	-0.99	0.91	1	45569
β_2 After without treatment	-0.83	-1.8	0.14	1	41292
β_3 Dart (after)	0.32	-1.04	1.71	1	45183
β_3 SC (after)	1.11	-0.13	2.38	1	43732
SD animal ID random effect	0.26	0.01	0.71	1	28863

Table 3.9. Supporting information. Posterior means for the behavior model parameters investigating the five discrete behaviors (hopping, head grooming, body grooming, pentapedal walk, and ear shaking) in our BACI experiment at Look at Me Now Headland, recorded as the number of occurrences during the length of the video. β_1 = the expected change in behavior from before to after (BA) in absence of treatment type, β_2 = the difference in behavior between groups before the treatment (CI), β_3 = the expected true effect of treatment (interaction between the treatment types and the observation periods) on kangaroo behavior (BA:CI). Estimates, 95% credible intervals (CrI), standard deviation of model residuals (sigma), Rhat statistic and effective sample sizes (ESS), are provided for each parameter.

	Estimate	l-95% CrI	u-95% CrI	Rhat	ESS
Hopping					
Intercept Control (before)	-4.69	-5.46	-3.8	1	50065
β_1 Dart (before)	-0.13	-1.32	1.05	1	51433
β_1 SC (before)	0.44	-0.65	1.47	1	51769
β_2 After without treatment	-0.28	-1.39	0.81	1	46833
β_3 Dart (after)	0.29	-1.28	1.87	1	51240
β_3 SC (after)	-0.22	-1.64	1.21	1	49148
SD animal ID random effect	0.25	0.01	0.72	1	34255
Grooming Head					
Intercept Control (before)	-6.09	-6.59	-5.6	1	53681
β_1 Dart (before)	-0.07	-0.77	0.63	1	58288
β_1 SC (before)	-0.14	-0.78	0.51	1	56045
β_2 After without treatment	-0.25	-0.88	0.38	1	53815
β_3 Dart (after)	-0.06	-0.98	0.85	1	60250
β_3 SC (after)	0.21	-0.63	1.03	1	57526
SD animal ID random effect	0.25	0.01	0.57	1	22647
Grooming Body					
Intercept Control (before)	-4.55	-4.94	-4.17	1	33781
β_1 Dart (before)	-0.62	-1.19	-0.05	1	34432
β_1 SC (before)	-0.22	-0.73	0.29	1	34821
β_2 After without treatment	-0.34	-0.76	0.08	1	38904
β_3 Dart (after)	0.57	-0.06	1.18	1	43270
β_3 SC (after)	0.02	-0.54	0.58	1	41585
SD animal ID random effect	0.34	0.15	0.55	1	26102

Pentapedal movement					
Intercept Control (before)	-4.66	-5.01	-4.28	1	52381
β_1 Dart (before)	-0.34	-0.87	0.18	1	56440
β_1 SC (before)	-0.06	-0.54	0.4	1	53536
β_2 After without treatment	0.09	-0.38	0.56	1	50809
β_3 Dart (after)	0.30	-0.37	0.98	1	56478
β_3 SC (after)	-0.09	-0.71	0.52	1	54265
SD animal ID random effect	0.17	0.01	0.39	1	23742
Shaking ears					
Intercept Control (before)	-4.59	-5.29	-3.89	1	33280
β_1 Dart (before)	-0.02	-1.03	0.99	1	35892
β_1 SC (before)	0.11	-0.79	1.03	1	34517
β_2 After without treatment	-1.01	-1.71	-0.32	1	45199
β_3 Dart (after)	0.17	-0.85	1.18	1	51566
β_3 SC (after)	0.89	-0.04	1.81	1	48000
SD animal ID random effect	0.7	0.39	1.08	1	26973

Table 3.10. Supporting information. Posterior means for the stress model parameters investigating the fecal glucocorticoid metabolites concentration in our BACI experiment at Look at Me Now Headland. β_1 = the expected change in FGM concentrations from before to after (BA) in absence of treatment type, β_2 = the difference in FGM concentrations between groups before the treatment (CI), β_3 = the expected true effect of treatment (interaction between the treatment types and the observation periods) on FGM concentrations (BA:CI). Pre_Post = collection periods – 3 days before, and 3 days after treatment; Day_time = morning, afternoon, and evening. Estimates, 95% credible intervals (CrI), standard deviation of model residuals (sigma), Rhat statistic and effective sample sizes (ESS), are provided for each parameter.

	Estimate	l-95% CrI	u-95% CrI	Rhat	ESS
shape parameter	39.56	7.96	187.85	1	1523
beta parameters					
Intercept (Control before)	1.64	1.47	1.81	1	14850
β_1 Dart (before)	0.03	-0.22	0.28	1	15170
β_1 SC (before)	0.04	-0.18	0.27	1	14671
β_2 After without treatment	-0.10	-0.35	0.14	1	12565
β_3 Dart (after)	0.07	-0.27	0.40	1	14413
β_3 SC (after)	0.14	-0.18	0.46	1	13742
Hierarchical parameters (SD)					
Animal ID	0.11	0.01	0.23	1	13844
Animal ID:Pre_Post	0.11	0.01	0.24	1	10379
Animal ID:Pre_Post:Day_time	0.23	0.02	0.36	1	1893

4. A tale of two stressors: Stress response of eastern grey kangaroos (*Macropus giganteus*) to culling and human disturbance in peri-urban landscapes

4.1 Abstract

Overabundant wildlife populations, whether native or introduced, can disrupt local ecosystems, as well as interfere with human activities and threaten biodiversity. Managing free-ranging populations in urban areas presents complex challenges and often ignites controversy, particularly in cases where lethal control methods are employed to manage charismatic native species. In this study, we investigated the physiological stress responses of eastern grey kangaroos to culling and disturbance at kangaroo management sites in the Australian Capital Territory (ACT). Using a Before-After Control-Impact design, we measured faecal glucocorticoid metabolite (FGM) concentrations as a proxy for stress in kangaroo populations across seven sites subjected to one of three treatments: 1. culling, 2. disturbance (no culling), and 3. control (no culling and no disturbance). Contrary to expectations, culling did not increase stress levels in surviving kangaroos. Instead, we observed an 11% decrease in FGM concentrations at 24 hours and an 18% decrease at 30 days post-culling. Kangaroos at culled sites had 44% higher baseline FGM concentrations before treatment, possibly reflecting pre-existing environmental stressors. Paradoxically, disturbed and undisturbed control groups showed no changes after 24 hours but exhibited higher FGM concentrations 30 days post-treatment. We found no evidence of sex-based differences in FGM levels, but juvenile kangaroos had baseline concentrations that were 11% higher than adults. These counterintuitive results could be attributed to various factors, including underlying physiological processes such as intraspecific competition, sampling artefacts or hypothalamic-pituitary-adrenal axis downregulation at high-density culled sites.

The absence of elevated stress hormone concentrations in culled populations challenges existing beliefs that culling causes physiological stress in kangaroos. Given the challenges associated with measuring stress responses in wildlife, future research should include multiple stress measures over longer temporal scales, and account for inter- and intra-individual variability to better understand the impacts of management practices on wildlife welfare. When tagging individuals is impractical, as in this study, researchers should focus on gathering a larger representative sample from diverse demographic groups.

4.2 Introduction

The management of wildlife populations can be controversial, particularly in urban areas where overabundant species (native or introduced) can significantly impact local ecosystems, human activities, and biodiversity, yet there can be strong opposition to some management techniques (Brunton 2018). The eastern grey kangaroo (*Macropus giganteus*) is an example of a species experiencing these tensions, as it often thrives in peri-urban settings, contributing to ecological pressures on parks and reserves and leading to conflicts with human populations, such as vehicle collisions with kangaroos, aggressive behaviour towards people and competition with livestock for pasture (Herbert et al. 2021). Lethal control of kangaroos is contentious, with public concerns about animal welfare during culling operations (Descovich et al. 2015). But how do we balance the needs of a growing human population with the welfare of these iconic native animals?

There is a growing public preference for non-lethal methods of population control, such as fertility control (Herbert 2004; Silva et al. 2025b; Wimpenny & Hinds 2018; Woodward et al. 2006) and translocation (Higginbottom & Page 2010; Thompson et al. 2022), which can be perceived as more welfare-friendly alternatives. Nonetheless, these non-lethal methods also present their own welfare challenges and require careful consideration and research before implementation (Pecorella et al. 2016; Thompson et al. 2022; Woodward et al. 2006). Where

possible, debates about the relative “humaneness” of wildlife management strategies should be informed by empirical measures of the impacts of different management techniques on animals at the individual and population level. Ultimately, effective wildlife management must balance the need for population control and human-wildlife conflict mitigation with ethical considerations regarding animal welfare, ensuring that practices are effective and humane (Descovich et al. 2015; Pecorella et al. 2016; Thompson et al. 2022).

At the heart of this dilemma lies a controversial practice: culling. This management strategy, aimed at regulating kangaroo population size, is contentious and serves as a catalyst for public debates and opposition to kangaroo management strategies (Croft & Witte 2021). Culling is frequently used to regulate kangaroo populations and minimise their impacts on agriculture, native vegetation, and human-wildlife conflicts (Descovich et al. 2016). In Australia, increased urbanisation and land clearing for agriculture have intensified conflicts between humans and kangaroos, creating debates about the efficacy and morality of culling as a management strategy (McLeod 2004; Olsen & Low 2006). Welfare concerns arise regarding the treatment of animals during culling operations, particularly the potential for suffering among individuals that are not killed instantly and the orphaning of dependent young (Descovich et al. 2015; Hampton & Forsyth 2016; McLeod 2004). Even when culling is conducted with precision and in accordance with relevant codes of practice, which promotes techniques to minimise suffering (Australian Government 2008), public concerns persist regarding the pain and suffering inflicted on culled individuals, the impacts on surviving conspecifics (McKinnon et al. 2018), and potential effects on non-target scavengers that utilise carcasses (Hampton et al. 2022). These discussions highlight the need for empirical evidence of the impacts of culling on the welfare of kangaroos during and after culling operations.

Culling can induce substantial stress among targeted individuals and their conspecifics. It can cause psychological stress in surviving individuals, disrupt social structures (Shannon et al. 2013), lead to shifts in the population's sex ratio and age structures affecting breeding success and survival rates (Beasley et al. 2013), and potentially exacerbate conflict with humans (O'Neill et al. 2017). Several studies have investigated the social structure and dynamics of animal populations following culling events, highlighting the potential consequences for survivors (Downing et al. 2023; O'Neill et al. 2017; Shannon et al. 2013). For instance, research conducted on dingoes (*Canis lupus dingo*) on Fraser Island, Australia, demonstrated that lethal control can destabilise social structures, resulting in increased aggression, stress and conflict among remaining individuals due to the loss of group organisation (O'Neill et al. 2017). Similarly, culling wild Canada geese (*Branta canadensis*) populations reinforced associations among survivors and also significantly influenced their social networks, suggesting that disruptions to social dynamics can affect both behavioural interactions and disease transmission within populations (Downing et al. 2023).

Human disturbances can also have important physiological and ecological implications for wild animals. Stress responses to culling may be triggered by the acute disturbance associated with the culling operation, and/or the longer-term stress associated with changes to social structure in culled populations (Baker et al. 2013; Carter et al. 2007). Gentsch et al. (2018) found that cortisol responses in wild ungulates vary widely depending on the type of trauma, indicating that hunting is not necessarily the most severe stressor. Other factors, including capture and handling, can induce equal or greater physiological stress than most hunting methods, for example, deer caught in fences experienced higher cortisol levels than animals injured by shooting (Gentsch et al. 2018).

Even in seemingly undisturbed areas, kangaroos may experience stress from unpredictable human activities or other environmental factors. As such, it is important to differentiate

between the stress induced by disturbance, which could occur in a variety of everyday scenarios, versus stress associated with culling per se. Stress responses in wildlife can be assessed through the non-invasive measurement of faecal glucocorticoid metabolites (FGM) (Pecorella et al. 2016; Sherwen et al. 2015), which has previously been demonstrated in a range of marsupial species such as brushtail possums (*Trichosurus vulpecula*) (Cope et al. 2022), bandicoots (*Perameles nasuta* and *Isoodon obesulus*) (Dowle et al. 2012), tammar wallabies (*Notamacropus eugenii*) (McKenzie & Deane 2005), bilbies (*Macrotis lagotis*), mountain pygmy-possums (*Burramys parvus*), Tasmanian devils (*Sarcophilus harrisii*), western grey kangaroos (*Macropus fuliginosus*) (Fanson et al. 2017), eastern grey kangaroos (Corvalan 2020; Fanson et al. 2017; Herbert et al. 2020; Sherwen et al. 2015), among others (Fanson et al. 2017).

The hypothalamic-pituitary-adrenal (HPA) axis plays a central role in the production and secretion of glucocorticoids (GC) (Touma & Palme 2005), steroid hormones released in response to stress (Sheriff et al. 2011). When exposed to a stressor, the hypothalamus releases corticotrophin-releasing hormone, stimulating the pituitary gland to secrete adrenocorticotropic hormone (ACTH), which then signals the adrenal cortex to produce and release GC (Touma & Palme 2005). Various factors can influence this process, including genetic predispositions, environmental conditions, social structure, age, and the individual's reproductive status (Dantzer et al. 2014; Touma & Palme 2005).

Stress refers to an animal's physiological reaction to adverse environmental factors or stressors that disrupt its internal regulatory balance, known as homeostasis (Novak et al. 2013). Stressors, whether acute or chronic, can lead to fluctuations in the responsiveness of the HPA axis and GC secretion (Touma & Palme 2005). The duration and intensity of stressors determine whether the response is acute (temporary) or chronic (prolonged) (Rao et al. 2024). Under normal conditions, GC play vital roles in energy regulation, glucose

metabolism, inflammation control, and reproduction (Fanson et al. 2017). During acute stress events, GC support energy mobilisation and enhance adaptive behaviours, such as increased vigilance or escape strategies (Möstl & Palme 2002). However, sustained elevations of GC levels induced by chronic stress can have detrimental effects on an individual's health, including weakened immune function, muscle deterioration, and reproductive challenges (Fanson et al. 2017).

Human disturbance and culling can both impact wildlife populations, but they do so in different ways and magnitudes. Research indicates that human activities, including habitat fragmentation, urban development, and recreational disturbances (e.g. camping in natural areas, dog walking, and hiking, among other leisure activities), can induce substantial stress in wildlife (Brunton et al. 2020). For instance, anthropogenic pressures, such as infrastructure development, population management interventions, clearing native vegetation for agriculture and urbanisation, can negatively affect the physiological health of various species, leading to chronic stress responses that can influence health, growth, reproduction, and overall survival (Brandimarti et al. 2021; Karaer et al. 2023). In some cases, the stress induced by human presence may surpass that induced by natural predators, as human disturbances can create a persistent state of fear and anxiety in wildlife (Fardell et al. 2020). Brunton et al. (2020) found that urban environments, characterised by high human population density and development, were associated with elevated levels of FGM in kangaroos. In contrast, kangaroos in less disrupted non-urban areas, exhibited lower stress levels, suggesting that the stress responses observed in urban settings may be more aligned with chronic stress due to the continuous nature of human disturbances (Brunton et al. 2020).

Our study aimed to assess the stress responses of kangaroo populations to culling. Culling may lead to acute stress (Pecorella et al. 2016) associated with human disturbance at the time of culling operations (e.g. movement of vehicles and people at night, gunshots, and the use of

high beam lights), but can also result in longer-term psychological stress responses induced by loss of conspecifics and resultant social dysfunction within populations (Shannon et al. 2013). By including a disturbance control, we sought to isolate the specific impacts of culling beyond the general presence of humans.

Kangaroo management in the Australian Capital Territory (ACT) is implemented through a structured conservation culling program aimed at maintaining ecological balance and protecting native flora and fauna (ACT Government 2018). Using the ACT as a case study, this research uses a Before-After Control-Impact (BACI) design to assess the effects of culling on stress levels in eastern grey kangaroo populations. FGM assessment was used as a proxy measure for stress. A procedural control, simulating the disturbance of culling without the removal (death) of individuals, is included to determine whether culling induces greater physiological stress in kangaroo populations than disturbance alone. This research will help to inform debates about the impact of culling on physiological stress responses in kangaroo populations.

4.3 Materials and Methods

This work was conducted with ethical approval from the University of Sydney's (USYD) Animal Ethics Committee under the permit number: # N00/7-2012/3/5791, 2015/917, 2016/1062. The kangaroo population estimates were covered by approval from the University of Canberra Animal Ethics Committee, approval CEAE 16-06. Faecal samples from culled animals were collected opportunistically post-mortem in accordance with the Nature Conservation (Eastern Grey Kangaroo) Controlled Native Species Management Plan 2017 (ACT Government 2017).

4.3.1 Study Sites

The study was conducted in 2018, at seven distinct sites located in the north-east of the ACT, Australia (Figure 4.1). In 2018, the ACT experienced a warm and dry year, with 472 mm of total annual rainfall at Canberra Airport (geographic coordinates: -35.3069 S, 149.1950 E) compared to an annual average of 605.7 mm (BOM 2018). The mean temperature recorded was 22 °C, with a mean maximum high of 40.6 °C and a mean minimum low of 6.9 °C.

Each study site is a designated Kangaroo Management Unit (KMU; Table 4.1), an area defined by the ACT government based on specific ecological, geographical (e.g. high speed roads and suburb edges), and management criteria (ACT Government 2018). Kangaroo densities are assessed annually and compared against target population densities to determine if kangaroo management is necessary. If so, the number of kangaroos to be culled is calculated using the Nature Conservation (Eastern Grey Kangaroo) Conservation Culling Calculator, which calculates the appropriate kangaroo densities necessary to preserve grassland conservation values, accounting for factors such as land tenure, vegetation composition, and conservation goals (ACT Government 2018). These sites contain areas of critically endangered yellow box-red gum grassy woodland and natural temperate grassland, and support a variety of threatened flora and fauna species, including the superb parrot (*Polytelis swainsonii*) and the striped legless lizard (*Delma impar*) (ACT Government 2018). All sites are peri-urban nature reserves, regularly frequented by humans during daylight hours. See Table 4.1 for more details.

4.3.2 Experimental design

We employed a BACI experimental design (Green 1979) to assess the effects of culling and human disturbance on FGM concentrations in free-ranging eastern grey kangaroo populations

(hereafter referred to as kangaroos). Each of the seven study sites was subjected to one of three treatments (Table 4.1):

- Culled: kangaroo culling was undertaken by the ACT Government as a conservation management strategy (ACT Government 2017) in accordance with the National Code of Practice for the Humane Shooting of Kangaroos and Wallabies for Non-Commercial Purposes (hereafter referred to as the Code) (Australian Government 2008). Shooting was conducted at night when the reserves were closed to the public, by a combination of contractors and ACT Government staff. The firearms and ammunition used were at the discretion of the contractor in accordance with the Code. Rifles were equipped with a telescopic sight and night vision technology (Australian Government 2008) and shooters aimed for head shots to ensure quick and humane kills in accordance with the Code.
- Disturbed: a disturbance was created by driving through the site at night (between 10 pm and midnight) at around 30 km per hour with a single 4-wheel drive vehicle, using high beam lights and making intermittent noise (e.g. sounding the car horn) to simulate the disturbance caused by the shooter teams during culling, without the associated death of conspecific animals. Nighttime was selected for consistency with the culling treatment and due to the reserve being closed to the public and the kangaroos being allegedly more active.
- Undisturbed: no disturbance was created at these sites.

Fresh kangaroo faecal samples were collected at each site to determine stress hormone concentrations before and after the treatment, designated as Pre- and Post-treatment. Pre-treatment samples were collected either the day before the simulated disturbance (disturbed sites) or from carcasses immediately after culling on the first night of operations. As

circulating GC are metabolised and excreted in faeces after a lag period (roughly equivalent to the gut transit time), GC concentrations measured in faeces from kangaroos reflect circulating stress hormone concentrations approximately 19 hours (range = 10-34 hours) earlier (Fanson et al. 2017). Hence, samples collected on the first night of culling reflect pre-treatment circulating GC concentrations. Post-treatment samples were collected from each site within 12 to 48 hours after treatment (referred to as "Post 24 hours") and again up to 30 days post-treatment (referred to as "Post 30 days"). At undisturbed sites, samples were collected during the day for one day ("Pre") and again 30 days later ("Post 30 days") to avoid any effect of a possible disturbance caused by us when collecting the Post samples.

Samples were collected early in the morning and late afternoon and disturbance was minimised by observing kangaroos from a distance (between 10 and 15 metres) and then calmly walking towards them to collect voided samples. Whenever possible, samples (all pellets per defecation) were collected after observing defecation from individuals of known age class (adult or juvenile) and sex, before placing them in a zip lock bag and recording age class, sex, site, date and time. Sample freshness was determined by the warmth of the pellets when touching with gloved hands, and samples were considered independent if they were separated by >1m and/or were distinctly different in size. Samples were stored in an ice box at the site and subsequently frozen at -20 °C for future FGM analysis.

4.3.3 Assessment of faecal glucocorticoid metabolites levels

The FGM assays were conducted in the Gunn Building laboratory at The University of Sydney, Sydney, Australia. Samples were thawed and dried for about 18 hours at 65 °C in a Qualtex Thermostat laboratory oven. After drying, the samples were homogenised with a mallet, passed through a fine mesh sieve, and the fine faecal powder was stored in vials at -20 °C. For the extraction, approximately 0.20 g of the powder was weighed and placed into test

tubes, with the exact weight recorded. Each tube received 2.5 mL of 80% methanol (Sherwen et al. 2015). The tubes were vortexed for 15 seconds and then shaken at 210 rpm on a Thermo Fisher Scientific orbital shaker for one hour at room temperature, followed by centrifugation using a Sorvall RC-3 automated refrigerated centrifuge at 2500 g for 15 minutes. The clear supernatant was carefully transferred into labeled glass vials and stored at -20 °C for later analysis.

FGM concentrations were determined using the 37e enzyme immunoassay, which was initially developed for wild mice (*Mus musculus* (Touma et al. 2003) and later validated for eastern grey kangaroos (Fanson et al. 2017). We also biochemically validated the assay in our laboratory by demonstrating parallelism between a serially diluted sample pool and the standard curve. This assay is suitable for broad-spectrum FGM analysis, measuring GC metabolites with a 5α-3β, 11β-diol structure (Fanson et al. 2017). Standards, antibodies, and labels used in the assay were acquired from R. Palme from the Institute of Biochemistry at the University of Veterinary Medicine in Austria (Touma et al. 2003).

For the assay, microtiter plates (Costar 96 well, high binding assay plate, Corning Inc.) were pre-coated with goat anti-mouse immunoglobulin G (GAMG) coating antibody. The plates were thawed and washed three times before use. We added 0.05 mL of steroid standard, diluted faecal extract (1:20 in Trizma buffer) or a control to each well, then added 0.1 mL of biotinylated steroid and 0.1 mL of primary antibody. Equilibrium binding took place during an overnight incubation at 4 °C. After washing the plates three times, we added 0.25 mL of streptavidin-POD conjugate to each well and incubated for 45 minutes at 4 °C. The plates were then washed six times and 0.25 mL of TMB (3,3′,5,5-Tetramethylbenzidine) substrate was added, with the reaction being stopped by adding 0.05 mL of sulfuric acid. The optical density was measured at 450 nm with a reference wavelength of 630 nm using an ELx808 BioTek microplate reader with Gen5 Software. The results were uploaded to MyAssays.com

and analysed using four-parameter logistic curve fitting. The assay demonstrated reliability with intra-assay coefficients of variation below 10% and inter-assay coefficients below 15% for all assays conducted.

4.3.4 Statistical Analysis

All data analyses were conducted using R version 4.2.1 (R Core Team 2023) with models fitted using the 'brms' (Bürkner 2017) package.

Due to inconsistent sampling times across sites (12-, 24-, 36- or 48-hours post-treatment), we consolidated all samples collected within 48 hours into a single "Post 24 hours" group to increase the sample size at each site for a more robust analysis.

We assessed the effect of treatments (Culled, Disturbed, Undisturbed) on FGMs at the site level, using a Bayesian gamma generalised linear mixed model (GLMM) based on the following BACI design (Comte et al. 2023; Pardini et al. 2018):

$$Y_i = \alpha + \beta_1 BA + \beta_2 BA + \beta_3 BA{:}CI + \theta_j,$$

Where, Y_i represents the dependent variable (concentration of FGM), α is the intercept of the model representing the baseline FGM (Undisturbed group) before treatment; β_1 is the expected change in FGM concentrations from before to after (BA) in absence of treatment, β_2 is the difference in FGM concentrations between the two treatment groups and the control group before treatment (CI), β_3 is the expected true effect of treatment (interaction between the treatment types and the before and after periods) on FGM concentrations (BA:CI), and θ_j represents random effects, accounting for variations between sites that are not explained by the fixed effects.

We used weak normal priors N (0,10) for all model parameters. We ran four Markov Chain Monte Carlo (MCMC) chains, with 80,000 iterations and 40,000 warm-ups. We adjusted the Hamiltonian Monte Carlo (HMC) sampling with an adapt delta of 0.999, thin of 10, and a

maximum tree depth of 15 to minimise divergent transitions and improve the accuracy of our estimates. Good mixing of the chains was assessed visually and using the Gelman-Rubin statistics (Brooks & Gelman 1998).

To minimise human disturbance effects in the undisturbed sites, no Post 24 hours samples were collected from these sites. For this treatment, samples were collected on a single day and randomly split into Pre- and Post- 24 hours samples, based on the assumption that FGM concentrations should remain consistent in the absence of disturbance. To validate this assumption, we ran three models using data split in different random ways. All models produced similar parameter estimates and model fits. We, therefore, retained the first random split for subsequent analyses.

Initial exploratory analysis indicated differences in FGM concentrations between juveniles and adults. Consequently, we employed a Bayesian gamma GLMM to assess how sex and age class interactively influenced FGM concentrations in Pre- samples where we had sex and age class specified. We used similar priors and four MCMC chains, with 40,000 iterations and 10,000 warm-ups. We adjusted the Hamiltonian Monte Carlo (HMC) sampling with an adapt delta of 0.999 and maximum tree depth of 18. Good mixing of the chains was assessed as previously described.

4.4 Results

A total of 714 faecal samples were collected from kangaroos pre- and post- treatment, including 235 Pre- (Culled n = 99, Disturbed n = 101, Undisturbed n = 35), 286 Post-24 hours (Culled n = 115, Disturbed n = 136, Undisturbed n = 35), and 193 samples Post- 30 days (Culled n = 111, Disturbed n = 49, Undisturbed n = 33) (see Table 4.5. Supporting information for further details). The mean FGM concentration before treatment was 8.7 ng/g

(95% CI: 8.2 – 9.2 ng/g) across the dataset. Mean FGM concentrations ranged from 4.7 to 16.6 ng/g across the different sites before treatment (Table 4.2).

4.4.1 Baseline FGM concentrations

The baseline FGM concentration in undisturbed sites was 1.75 ng/g (95% CrI: 1.20 to 2.31 ng/g; β_1). Before treatment, there was no evidence of a difference in FGM concentration in the disturbed sites (0.36 ng/g, 95% CrI: -0.44 to 1.14 ng/g; β_1), but the FGM levels in the culled sites was 0.76 ng/g higher than the undisturbed site (95% CrI: 0.03 to 1.48 ng/g; β_1) (Table 4.3).

4.4.2 Changes in FGM concentrations over time

In the absence of treatment (β_2), FGM concentrations remained stable after 24 hours (-0.04 ng/g, 95% CrI: -0.21 to 0.12 ng/g) but increased by 0.47 ng/g (95% CrI: 0.30 to 0.64; β_2) after 30 days. There was no evidence that the treatment in the disturbed sites influenced FGM concentrations after 24 hours (0.14 ng/g, 95% CrI: -0.04 to 0.33 ng/g; β_3) or after 30 days (0.09 ng/g, 95% CI: -0.11 to 0.30 ng/g; β_3). In the culled sites, FGM concentrations were 11% lower after 24 hours (-0.23 ng/g, 95% CrI: -0.42 to -0.04 ng/g; β_3) compared to pre-treatment levels, and 18% lower after 30 days (-0.91 ng/g, 95% CrI: -1.1 to -0.71 ng/g; β_3), accounting for the observed increased in the undisturbed sites (Table 4.3).

4.4.3 FGM concentrations between sex and age groups

There was no evidence of differences in FGM concentrations between males and females before treatment (-0.05, 95% CrI: -0.19 – 0.09). Juvenile animals, however, had FGM concentrations 11% higher than adults (0.27, 95% CrI: 0.09 – 0.45), for both sexes (-0.09, 95% CrI: -0.35 – 0.18) (Table 4.4).

4.5 Discussion

In the absence of disturbance or culling, FGM concentrations in kangaroos at peri-urban control sites were stable in the short term (24h) but increased after 30 days. We found no evidence that disturbance (without shooting) influenced FGM concentrations at either 24 hours or 30 days. In contrast to undisturbed and disturbed sites, kangaroos at culled sites had 43% higher FGM concentrations before treatment. Culling was associated with an 11% decrease in FGM concentrations after 24 hours and an 18% decrease 30 days later. Additionally, we found no evidence of differences in baseline FGM concentrations between sexes, however, juvenile animals had 11% higher FGM concentrations than adults. By assessing changes in FGM concentrations associated with culling and human disturbance across multiple sites, our study provides robust empirical insights into the impacts of management practices on kangaroo welfare, specifically focusing on stress responses at the population level.

Contrary to our initial expectations, culling did not intensify stress levels in surviving kangaroos. Instead, we measured a significant decrease in FGM concentrations, both 24 hours and 30 days post-culling. Several factors could explain this apparent decline in stress levels post-culling, whilst the control sites paradoxically showed an increase in stress hormone concentrations after 30 days: i) the higher baseline FGM concentrations in the culled sites could be an indicator of high baseline stress levels due to intraspecific competition for food at high density sites; ii) removal of a stressor from the environment resulted in a reduction in circulating GC concentrations at culling sites; iii) the sampling technique was not powerful enough to detect consistent changes in FGM at population levels; or iv) the kangaroos at culled sites were distressed by treatment, but FGM concentrations did not rise because of underlying physiological processes.

The relationship between culling, resource availability, and stress in kangaroo populations is intricate. While reduced kangaroo grazing pressure has been shown to increase vegetation cover and soil nutrient concentrations, contributing to healthier plant communities (Morris & Letnic 2017), this recovery is a gradual process (Finlayson et al. 2021; Yayneshet et al. 2009). Some perennial flora remain resistant to recovery even after four years of high rainfall and reduced grazing pressure (Gordon et al. 2021). Therefore, it is unlikely that substantial changes in food availability would manifest in reduced stress within 30 days of culling. In high-density populations with limited food resources, aggressive interactions between individuals tend to increase as they compete for space and resources (Li et al. 2007). Studies have shown that kangaroo density is inversely related to per capita green herbage mass, with higher-density sites having less available food per individual (Portas & Snape 2018). Culling could lead to immediate reductions in competitive behaviours by lowering population density and increasing per capita food availability (Portas & Snape 2018), thereby reducing stress levels in surviving conspecifics.

The stress response to culling can vary between species and over time. For example, in fallow deer (*Dama dama*), culling resulted in increased faecal cortisol levels 24 hours post-culling, but these levels decreased after 72 hours to lower than those in the non-culled area (Pecorella et al. 2016). This pattern suggests an initial acute stress response followed by a rapid adaptation, likely indicating an adaptive short-term endogenous response to stress (Pecorella et al. 2016). However, the method of shooting used was not detailed in the fallow deer study, and shooting methods such as the ACT technique used in peri-urban kangaroos culling employ suppressors and low-light conditions, which have been demonstrated to have a low impact on conspecifics, potentially reducing acute stress responses (Hampton & Forsyth 2016). In our study, FGM levels decreased 24 hours after culling and further decreased 30 days later without detecting an immediate acute stress response. While the underlying

mechanisms may differ from those observed in fallow deer, these results suggest that culling can have complex and species-specific effects on stress levels in surviving animals. The decrease in FGM levels post-culling could be attributed to reduced competition for patches of food (Ramp & Coulson 2004), with lower population density leading to fewer aggressive interactions (Fattorini et al. 2018), or other factors not immediately apparent. In contrast, the increase in FGM concentrations demonstrated in the non-culled groups after 30 days could be due to a reduction in food availability associated with the start of winter and subsequent increase intraspecific competition. These results could be interpreted as the culled cohort experiencing lower intraspecific competition pressures even during winter.

Our findings highlight the complexity of measuring stress in free-ranging populations and emphasise the intricacies of the HPA axis as previously documented in animal research (Karaer et al. 2023; Lyons et al. 2010; Rice et al. 2008) and human studies (Goldman-Mellor et al. 2012). For example, factors like early life history, which is usually unknown in free-ranging animals, can significantly impact GC production in adulthood. Early life stressors can lead to a downregulated response in the HPA axis to subsequent stressors. Specifically, long-term or chronic stress can create a negative feedback loop in the HPA axis, resulting in reduced cortisol responses to future acute stressors (Karaer et al. 2023). This suggests that individuals who have experienced early-life stress (e.g. food shortage) may not exhibit the same intensity of cortisol increase in response to stress compared to those who have not faced such stressors (Karaer et al. 2023). In some species, individuals who are chronically stressed may reach a physiological threshold where further increases in GC levels are not observed (Cyr & Romero 2008). In our study, the initial high levels of FGM concentrations at the culled sites could indicate high chronic stress due to high-density populations (Gordon et al. 2021), unknown environmental stressors, or being subjected to frequent management such as culling (ACT Government 2018). In this context, kangaroos at culled sites could have been

experiencing chronic stress and may not have been able to display an acute response to culling. However, we do not have historical data on the physiology of these populations over longer temporal scales, and therefore, additional studies are required to differentiate these effects.

Assessment of FGM concentrations has widely been used as a proxy for stress measurement in wildlife (Dantzer et al. 2014). The assay used in this study was previously validated in the study species (Fanson et al. 2017) and therefore, should be capable of detecting a change in GC stimulated by the HPA axis in response to stress in kangaroos. However, the sampling approach at the site level may have limited our capacity to detect consistent changes in GC at the population level. As GC production is highly variable within and between individuals (Touma & Palme 2005), the random sampling of individuals at discrete time points, rather than longitudinal sampling of known animals, may have reduced our power to detect changes over time. In a study of eastern grey kangaroos in New South Wales, considerable variability in FGM concentrations was observed within and between individuals, with, for example, concentrations in one individual fluctuating from 17.16 ng/g to 3.96 ng/g within a single day (Silva et al. 2025a). Such variability can be influenced by intrinsic factors like age, reproductive status, and past experiences, as well as extrinsic factors such as season, time of day, food availability, weather, and anthropogenic disturbances (Palme et al. 2005). Past experiences can either sensitise or desensitise an individual's HPA axis in response to current or future environmental challenges, shaping how they respond to stressors over time (Dantzer et al. 2014). This individual physiological plasticity can complicate the interpretation of how environmental challenges or anthropogenic disturbances impact population-level stress responses (Dantzer et al. 2014). Although we accounted for site variability in our analyses, we were not able to identify individual kangaroos in order to keep the same cohort at each timepoint. Further studies using tagged individuals and a more frequent sampling strategy

would further develop our understanding of the stress response of kangaroos subject to human disturbances and culling.

The relationship between GC and stress responses can also vary across different life stages, indicating that age plays an important role in biological stress management (Palme et al. 2005). In our study, we found significant differences in FGM concentrations between age classes, with juvenile kangaroos exhibiting higher FGM levels compared to adults. These findings align with previous research showing that GC responses to stress are often more pronounced in younger animals, reflecting their developmental stage and environmental challenges (Crespi et al. 2013). Studies across other species have demonstrated that juvenile individuals may exhibit higher baseline GC levels compared to adults (Bonier et al. 2009; Pryce et al. 2002), suggesting a heightened stress response during early life stages. For instance, Pryce et al. (2002) found that cortisol levels were higher in neonatal and infant marmoset monkeys (*Callithrix jacchus*), and they gradually decrease as they reach adulthood. Higher levels of stress hormones in young marmosets are due to a combination of hyperactivity of the HPA system, a reduced negative feedback mechanism, and potential adaptive advantage for growth and development (Bonier et al. 2009; Pryce et al. 2002). We speculate that the elevated FGM levels observed in juvenile kangaroos may serve a similar physiological purpose, potentially assisting in their adaptation to environmental challenges such as limited resources during droughts and nutritional stress in dry years due to reduced fat reserves (Portas & Snape 2018). Higher GC levels during early life could be beneficial for growth and development, preparing young for future stressors (Pryce et al. 2002).

These differences in GC concentrations between age classes may also have impacted our study's findings. Potential differences in the number of unknown adults and juveniles sampled at each time point and site may have introduced random bias into the results. For example, approximately 29% of the pre-treatment samples from our culled sites were from

juvenile animals, but age class information was not available for the control sites. If the control sites had a lower proportion of juvenile samples, this discrepancy could have skewed the results for baseline concentrations since juveniles exhibited higher stress levels. These samples could not be excluded from the statistical analyses due to most samples having unknown age classes. Given the limited number of samples from known juvenile animals in our study, especially post-treatment, further investigation is needed to fully understand the effects of treatment on FGM concentrations and the role of GC in the growth and development of juveniles, as well as their mechanisms for coping with stress.

Other measurement proxies could be considered when measuring stress in kangaroos in the future, such as the downstream effects of chronic stress (Breuner et al. 2013). Examples of these are measurements of glucose, free fatty acids, body mass, immune response, reproductive inhibition and oxidative stress, which could increase or decrease in chronically stressed animals (Dantzer et al. 2014). However, most of these downstream metrics require the analysis of blood samples (Dantzer et al. 2014). In a free-ranging population, collecting blood samples would necessitate the capture and handling of individuals, which would almost certainly induce stress and lead to biased results. In such contexts, FGM measurements remain the most effective monitoring technique.

Combining longitudinal sampling strategies with other stress measures, such as behavioural assessments and camera traps, to identify unknown stressors would improve the measurement and interpretation of stress responses to wildlife management. In addition, ensuring a balanced representation of demographic groups within the populations studied is also important to minimise biases and enhance the statistical robustness of the findings.

4.5.1 Implications for wildlife management

Our study did not detect a significant increase in stress hormone concentrations in kangaroo populations in response to culling. These findings suggest that culling may not inherently increase stress for surviving kangaroos as previously assumed and, conversely, could reduce stressors in food limited populations.

Additional research is required to confirm these observations. Monitoring FGM concentrations as the only proxy for stress response may be susceptible to biases, especially for unreplicated and limited sample strategies. Robust analytic frameworks, such as the BACI design we used in this study, should be used when feasible. Managers should also consider using a combination of physiological, ecological, and behavioural monitoring when assessing the impacts of wildlife management strategies on stress at the population level.

Notwithstanding the potential limitations, this study provides the first insight into stress responses to culling in kangaroo populations. These empirical results will hopefully influence current perceptions of kangaroo management practices and potentially other wildlife species subjected to similar interventions. Management decisions must be supported by robust scientific evidence that considers the immediate effects of interventions such as culling, and also the long-term impacts on animal welfare and population dynamics (Shannon et al. 2013).

To improve the effectiveness of culling practices and the welfare outcomes for animals subjected to culling, management practices should focus on optimising operational methods, including minimising the duration and visibility of culling activities, ensuring precision in targeting when shooting, employing professional contractors trained in humane killing techniques, and implementing measures to reduce disruptions to surviving conspecifics (Hampton & Forsyth 2016; Wilson & Edwards 2019). These refinements can help reduce the

potential for acute stress responses in kangaroos and improve public perceptions of culling as a humane and necessary management tool.

By considering these factors, wildlife managers can more effectively align their strategies with conservation objectives and welfare considerations, ensuring that interventions such as culling are conducted responsibly and with due attention to animal welfare. This study highlights the importance of an adaptive wildlife management framework, where decisions are continually informed and refined by empirical research and ecological understanding.

4.6 Tables and Figures

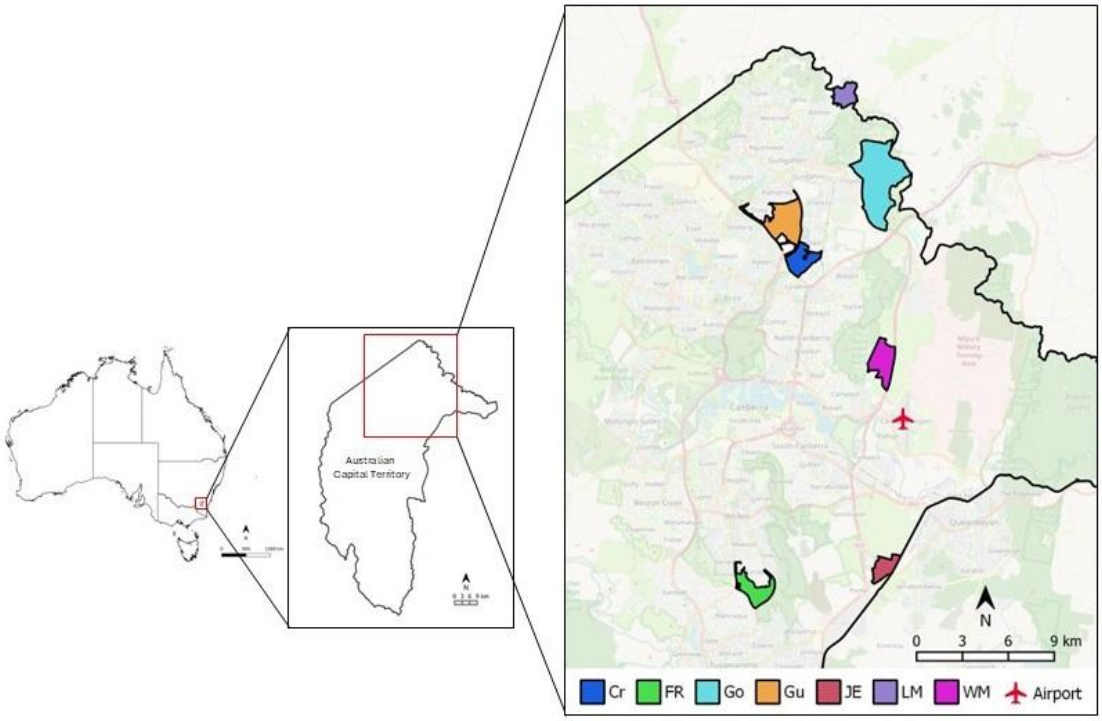

Figure 4.1. Study sites in the Australian Capital Territory (ACT), Australia. Each site is part of a distinct Kangaroo Management Unit (KMU) within the ACT: Cr = Crace KMU, FR = Farrer Ridge KMU, Go = Goorooyarroo KMU, WM = West Majura grasslands KMU, JE = Jerrabomberra East KMU, Gu = Gungaderra KMU, LM = Little Mulligans KMU. The map provides a visual reference for the spatial distribution of the reserves used in the study, highlighting their proximity to Canberra Airport and the Territory boundary (solid black line).

Table 4.1. Summary of treatment type, population density, culling recommendations, and management outcomes in 2018 at study sites in the Australian Capital Territory (ACT), Australia. Site = reserve where samples were collected; Treatment = Culled, Disturbed (no culling) and Undisturbed (no culling or disturbance).

Site	Area (ha)	Geographic coordinates	Treatment	Population density (ha^{-1}) *	Recommended number to cull in 2018	Number culled in 2018	Habitat description	Management history	Previous culling
West Majura Grasslands	231	149.18173, -35.27228	Culled	5.41	895	860	Native grassland and woodland	Targeted culling and ongoing monitoring	No previous culling
Jerrabomberra East	127	149.17750, -35.37807	Culled	4.43	281	281	Native grassland	Kangaroo exclosure, strategic burns, and ongoing monitoring	2017
Goorooyarroo	795	149.17962, -35.17354	Culled	0.95	357	355	Native woodland	Site surrounded by predator proof fence, two kangaroo exclosures that aim to maintain a lower kangaroo density, strategic livestock grazing, Targeted culling and ongoing monitoring	2017
Farrer Ridge	202	149.10924, -35.38697	Disturbed	2.12	0	0	Native woodland	Ongoing monitoring	No culling
Gungaderra	364	149.12651, -35.20150	Disturbed	1.18	0	0	Native grasslands and woodlands	Targeted culling, strategic burning and livestock grazing and ongoing monitoring	2016
Little Mulligans	125	149.16137, -35.14450	Undisturbed	2.31	0	0	Native woodland	No regular monitoring	No culling
Crace	180	149.14117, -35.22545	Undisturbed	1.46	0	0	Native grassland and planted woodland	Targeted culling, strategic burning and livestock grazing and ongoing monitoring	2015

*Estimated population densities for 2018 except for Little Mulligan, which was surveyed in 2017. (ACT Government, 2018)

Table 4.2. Mean baseline concentrations of faecal glucocorticoid metabolites (FGM, ng/g) with upper and lower 95% confidence intervals (CI) across seven sites in the Australian Capital Territory (ACT), prior to treatment interventions. Site = reserve where samples were collected; Treatment = Culled, Disturbed (no culling) and Undisturbed (no culling or disturbance); n = number of faecal samples analysed from each site.

Site	Treatment	n	Mean FGM (ng/g)	l-95% CI	u-95% CI
Majura	Culled	9	16.6	9.8	23.4
Jerrabomberra	Culled	40	12.0	11.2	12.8
Goorooyarroo	Culled	50	9.7	8.9	10.5
Farrer Ridge	Disturbed	60	9.1	8.2	10.0
Gungaderra	Disturbed	41	7.1	6.3	7.9
Crace	Undisturbed	36	6.6	5.8	7.4
Little Mulligans	Undisturbed	34	4.7	4.1	5.4

Table 4.3. Posterior means for the stress model parameters investigating faecal glucocorticoid metabolite (FGM) concentrations in our BACI experiment with three treatments (Culling, Disturbed (no culling) and Undisturbed (no culling or disturbance)) across seven sites in the Australian Capital Territory. β_1 = the expected change in FGM concentrations from before to after (BA) in absence of treatment type, β_2 = the difference in FGM concentrations between groups before the treatment (CI), β_3 = the expected true effect of treatment (interaction between the treatment types and the observation periods) on FGM concentrations (BA:CI). Pre = samples collected 12-24 hours pre-treatment; Post 24 = samples collected 12-48 hours post treatment; Post 30 = samples collected 30 days post treatment. Estimates, 95% credible intervals (CrI), standard deviation of model residuals (sigma), Rhat statistic and effective sample sizes (ESS) are provided for each parameter.

Model parameters	Estimates	l-95% CrI	u-95% CrI	Rhat	ESS
Intercept (Undisturbed - Pre)	1.75	1.20	2.31	1.00	15696
β_1 Culled - Pre	0.76	0.03	1.48	1.00	15165
β_1 Disturbed - Pre	0.36	-0.44	1.14	1.00	15468
β_2 Post 24	-0.04	-0.21	0.12	1.00	15748
β_2 Post 30	0.47	0.30	0.64	1.00	15982
β_3 Culled – Post 24	-0.23	-0.42	-0.04	1.00	15530
β_3 Disturbed - Post 24	0.14	-0.04	0.33	1.00	15776
β_3 Culled - Post 30	-0.91	-1.10	-0.71	1.00	15845
β_3 Disturbed - Post 30	0.09	-0.11	0.30	1.00	15872
Shape	8.30	7.46	9.16	1.00	16036

Table 4.4. Posterior means for the model parameters assessing differences in FGM concentrations by age class and sex in eastern grey kangaroos (baseline samples collected 12-24 hours pre-treatment across seven sites in the Australian Capital Territory). Estimates, 95% credible intervals (CrI), standard deviation of model residuals (sigma), Rhat statistic and effective sample sizes (ESS), are provided for each parameter.

Model parameters	Estimates	l-95% CrI	u-95% CrI	Rhat	ESS
Intercept (Adult Female)	2.36	2.28	2.45	1.00	70580
Sex Male	-0.05	-0.19	0.09	1.00	62956
Age_class Juvenile	0.27	0.09	0.45	1.00	56579
Sex Male : Age class Juvenile	-0.09	-0.35	0.18	1.00	51159
Shape	10.92	8.17	14.04	1.00	73978

4.7 Supporting Information

Table 4.5. Supporting information. Summary of mean faecal glucocorticoid metabolite (FGM) concentrations (ng/g) and 95% confidence intervals (CI) by treatment type, age class, sex, and periods (Pre- (samples collected 12-24 hours pre-treatment), Post- 24 (samples collected 12-48 hours post treatment), Post- 30 (samples collected 30 days post treatment), including sample counts and 95% confidence intervals. NA represents samples from animals of unknown age class and/or sex. F, female; M, male.

Treatment	Age class	Sex	Period	n	Mean FGM (ng/g)	l-95% CI	u-95% CI
Culled	Adult	F	Pre	45	10.7	4.3	21.6
Culled	Adult	F	Post 24	21	11.5	7.7	17.4
Culled	Adult	F	Post 30	1	9.6	-	-
Culled	Adult	M	Pre	25	10.4	6.3	18.4
Culled	Adult	M	Post 24	12	10.2	6.9	15.5
Culled	Juvenile	F	Pre	14	13.8	8.6	37.8
Culled	Juvenile	F	Post 24	15	12.0	7.2	18.9
Culled	Juvenile	M	Pre	15	12.1	8.7	19.7
Culled	Juvenile	M	Post 24	13	9.8	6.5	13.0
Culled	NA	NA	Post 24	54	9.0	3.7	18.3
Culled	NA	NA	Post 30	110	8.0	1.1	25.7
Disturbed	Adult	F	Pre	5	9.7	6.4	12.8
Disturbed	Adult	F	Post 24	24	8.6	6.5	12.6
Disturbed	Adult	F	Post 30	8	14.7	8.7	20.1
Disturbed	Adult	M	Pre	3	7.9	6.9	9.0
Disturbed	Adult	M	Post 24	7	9.1	4.5	15.0
Disturbed	Adult	M	Post 30	11	14.2	8.0	18.4
Disturbed	Juvenile	F	Post 24	1	10.5	-	-
Disturbed	Juvenile	F	Post 30	2	14.4	13.6	15.2
Disturbed	Juvenile	M	Post 24	2	10.9	10.2	11.7
Disturbed	Juvenile	M	Post 30	3	16.0	13.0	20.5
Disturbed	Juvenile	NA	Post 24	1	9.0	-	-
Disturbed	NA	NA	Pre	93	8.2	2.1	23.1
Disturbed	NA	NA	Post 24	101	9.1	4.9	14.6
Disturbed	NA	NA	Post 30	25	14.1	8.2	22.7
Undisturbed	Adult	F	Post 30	2	6.0	5.2	6.7
Undisturbed	Juvenile	F	Post 30	1	8.1	-	-
Undisturbed	Juvenile	M	Post 30	1	10.1	-	-
Undisturbed	NA	NA	Pre	35	5.8	2.4	12.5
Undisturbed	NA	NA	Post 24	35	5.6	2.9	11.7
Undisturbed	NA	NA	Post 30	29	9.2	5.0	16.1

5. General Discussion

5.1 Overview

The eastern grey kangaroo (hereafter kangaroo) is a common marsupial found along Australia's eastern coast and frequently inhabits peri-urban areas such as golf courses and reserves (Herbert et al. 2021). Kangaroo populations can become overabundant in certain areas, prompting management actions such as culling or fertility control. (Adderton 2004). This thesis contributes to the knowledge of effective kangaroo management by developing an efficient remote delivery system for a long-lasting contraceptive implant, thereby providing an additional tool for population control. This remote delivery technique brings fertility control closer to mainstream operationalisation for kangaroos and other wildlife. By emphasising the need to balance management strategies and conflict mitigation with considerations for animal welfare, this thesis highlights the importance of using scientific evidence to evaluate different management approaches and ensure they are effective, humane, and promote positive welfare outcomes (Descovich et al. 2016; Hampton et al. 2015b).

Until now, there has been limited research on the remote delivery of deslorelin contraceptive implants and the behavioural and stress responses to remote contraceptive delivery. Similarly, the stress induced by culling on surviving kangaroos has remained largely unexplored. This thesis addressed knowledge gaps on the potential efficacy of remotely delivered deslorelin contraceptive implants in kangaroos; the physiological and behavioural impacts of different contraceptive delivery methods in kangaroos; and the stress responses of conspecific kangaroos following culling for conservation.

The efficacy of the deslorelin agonist contraceptive, Suprelorin® was evaluated in three wild kangaroo populations in New South Wales, including treatment via a prototype dart (remote

treatment) and hand injection at two dosages following capture by chemical immobilisation (Chapter 2). A significant finding of this study was that the duration of contraception was similar between females in the remote treatment group and those who received hand-injected implants. In addition, similar contraceptive efficacy was achieved with lower doses of Suprelorin® without affecting the duration of contraception. As a result, remote delivery was demonstrated to be an effective and efficient strategy for contracepting kangaroos in peri-urban populations, overcoming previous logistical challenges in contraceptive administration.

The potential of remotely delivering Suprelorin® to wild kangaroos was further highlighted in Chapter 3, where the lower dose was administered in a fourth free-ranging population to evaluate the physiological and behavioural impacts of both delivery methods. Neither remote delivery, nor capture and hand-injection method had a discernible impact on kangaroo behaviour, faecal glucocorticoid metabolite concentrations or movement patterns that could indicate pain or stress after treatment. The remote delivery method demonstrated comparable effectiveness to the traditional subcutaneous injection capture-and-treatment method, with reduced risks associated with capture and handling. These findings have direct management implications for managing free-ranging populations in peri-urban areas, where animal capture may be impractical due to safety risks to animals and humans.

Although non-lethal methods are often considered more welfare-friendly by the general public, this approach still encounters logistical challenges, particularly in managing large open populations where kangaroos can move in and out. Therefore, when feasible, culling is still the most common management practice implemented for population control for environmental conservation purposes. Chapter 4 explored the physiological stress responses of kangaroo populations to culling, using FGM as a proxy to measure stress in kangaroos before and after a cull. Contrary to expectations, culling did not increase stress levels in surviving kangaroos at culled sites. Instead, a decrease in FGM concentrations was observed

post-culling, challenging existing beliefs regarding the stress caused by culling and emphasising the need for further research to better understand the impacts of management practices on free-ranging populations.

Collectively, these results highlight the importance of evaluating assumptions about the stress and welfare implications of current management strategies. They also underscore the effects of localised overabundance on one of Australia's most cherished species.

5.2 Assessing Suprelorin® as a tool for managing kangaroos

The selection of a management strategy to control the overpopulation of kangaroos in peri-urban areas can be contentious and is frequently shaped by social and political factors (Herbert et al. 2021). Fertility control has emerged as an alternative to lethal methods, aiming to reduce overabundant populations by inhibiting reproduction. A review by Wimpenny et al. (2021) highlighted key desirable characteristics for effective wildlife fertility control: (1) the agent's specificity to the target species; (2) high efficacy with a preference for a single, fast-acting and long-lasting treatment; (3) safety during gestation and lactation, and for animals receiving multiple treatments; (4) humaneness with minimal impact on social behaviour and animal welfare; and (5) cost-effectiveness of the agent and its delivery method. This thesis specifically addressed these key characteristics in the context of kangaroo management, exploring how fertility control can be improved to meet these criteria and provide a humane and effective tool for population control.

5.2.1 Efficacy, duration and species specificity

Suprelorin® has shown promise as a contraceptive implant for macropods (Wimpenny et al. 2021). The effectiveness of the implant for kangaroos has been successfully investigated in captive (Herbert et al. 2006; Woodward et al. 2006) and free-ranging kangaroo populations (Wilson et al. 2013), where it effectively inhibited fertility for extended periods. The active

compound deslorelin (a GnRH agonist) is slowly released from the implant resulting in the downregulation and desensitisation of pituitary GnRH receptors, which inhibits the production and release of LH and FSH, consequently disrupting follicular development and oestrous cycles in females (Herbert et al. 2005).

Some studies have demonstrated a dose-response relationship associated with Suprelorin® implants in different species, where higher doses resulted in longer periods of infertility in males and females (Cope et al. 2019; Eymann et al. 2007; Junaidi et al. 2009). For example, in female Tasmanian devils (*Sarcophilus harrisii*), a double dose (2 × 4.7 mg) increased the duration and efficacy of contraception compared to a single dose (Cope et al. 2019). Similarly, in brushtail possums (*Trichosurus vulpecula*), females given a double dose (2 × 4.7 mg) experienced longer contraception compared to those receiving a single dose (Eymann et al. 2007). Male domestic dogs also showed extended reproductive suppression with increased dosage, with a 12 mg dose resulting in longer suppression than a 6 mg dose (Junaidi et al. 2009).

The dose-response relationship to deslorelin implants has been less extensively studied for kangaroos. In Herbert et al. (2006), deslorelin successfully inhibited reproduction in female kangaroos in a captive population for 7.5 months with one 10 mg implant and for 19 months with two 10 mg implants. It should be noted however, that Herbert et al. (2006) used pre-commercial implants with higher doses than those used in the current commercially available product. Although research suggests that higher doses may lead to a longer contraceptive effect in other species, the efficacy of a single dose of the commercially available 4.7 mg Suprelorin® implant had not yet been investigated in kangaroos until now. In this thesis (Chapter 2), there was only a small (36 days), non-significant increase in reproductive suppression when a double dose of the 4.7 mg implant was used. While the low-dose group exhibited a lower degree of suppression of the pituitary than the high-dose as evidenced by

the magnitude of the hormonal response to a GnRH challenge, doubling the dose did not proportionally enhance the contraceptive effect. The absence of a linear dose-response relationship suggests that the low and high doses had similar efficacy. Additionally, Suprelorin® extended interbirth intervals by 155 days in the low-dose and 208 days in the high-dose group compared to the control group; however, this difference was not statistically significant. Measuring the interval between births is a more relevant metric in field management, especially for kangaroos with a complex reproductive strategy, as it accounts for the animal's reproductive status at the time of treatment.

These results demonstrated the efficacy of Suprelorin® in suppressing reproduction in kangaroos and delaying births with a single dose for extended periods. These results are significant, as a lower dosage would increase the likelihood that Suprelorin® implants could be safely delivered by remote dart injection to kangaroos.

5.2.2 Safety during gestation and lactation

This study corroborates previous research showing that Suprelorin® implants can be safely used in pregnant kangaroos, with successful gestation and lactation continuing post-treatment (Wilson et al. 2013). One key outcome with significant implications for kangaroo management is the impact of embryonic diapause on fertility control efficacy. The anti-reproductive effects of Suprelorin® implants can be masked by subsequent births from pre-treatment conceptions, whether the female was experiencing embryonic diapause or an active pregnancy at the time of treatment (Herbert et al. 2006; Wilson & Coulson 2016). Thus, a high proportion of females with blastocysts in diapause can reduce the effectiveness of fertility control agents in the first year of treatment. There were varying rates of embryonic diapause detected across the study sites, ranging from 12% to 30% at sites described in Chapter 2 and 5% at the LAMN site in Chapter 3. The higher prevalence of diapause at sites

in Chapter 2, particularly at Darlington Park (DP), aligns with findings from Wilson et al. (2013), who attributed higher rates to superior habitat quality and favourable environmental factors. DP's mixed environment, comprising coastal bushland, a caravan park, a residential area, private farmland and a golf course, provides abundant food resources and ample water to local kangaroos. LAMN had a lower prevalence of blastocysts in diapause, likely due to grazing pressures, high population density and compromised kangaroo health (Brandimarti et al. 2021).

The timing of implant administration and environmental factors must be thoughtfully evaluated to improve contraceptive effectiveness. In this species, births of young peak in summer and thus, it has been suggested that the optimal implanting window should be between April and May, when most females will have given birth and have pouch young under 100 days old (Poole 1983). This will minimise the probability of a female already being pregnant at the time of treatment (Wilson et al. 2013), but this could vary between populations. In this thesis, the assessment of pouch young age at the time of treatment and incidence of diapausing blastocysts or active pregnancies suggest that contraceptive treatment should ideally be conducted from February to March for better contraceptive effects at our study sites. Ideally, an assessment of the reproductive patterns of the target population should be conducted before planning and implementing contraceptive programs at new sites. This will minimise the likelihood of pregnancies and blastocysts in diapause delaying the net benefits of reproductive suppression.

5.2.3 Repeated treatments

While Suprelorin® effectively reduced fertility in the populations studied in Chapters 2 and 3, its relatively short duration of action remains a primary limitation. Research indicates that implants containing deslorelin typically provide contraception for only one to two years, with

the contraceptive effect waning in year 2 (Wilson & Coulson 2016; Woodward et al. 2006). This means repeated treatments are required to maintain infertility over the longer term. Also, the first year of treatment may not achieve a significant reduction in reproductive output as some females may already be pregnant or have a blastocyst in diapause that will continue development and mask the effects of the treatment.

An annual treatment regime was implemented for the cohort of contracepted female kangaroos at LAMN (Chapter 3) when concurrent health assessments revealed that the population was experiencing chronic nutritional stress, nonregenerative anaemia, nutritional deficiencies and a heavy burden of parasitic infections (Brandimarti et al. 2021). The objective was to maintain reproductive suppression through downregulation of the pituitary gland, allowing females the opportunity to recover their health and to assess the long-term efficacy of an annual Suprelorin® treatment regime. As the experiment in Chapter 2 demonstrated a contraceptive effect of 390 days following treatment with a single 4.7 mg implant, it was hypothesised that annual treatments would effectively suppress reproduction and extend interbirth intervals indefinitely if each female received annual treatment. At LAMN (Chapter 3), a reduction in fertility throughout the annual regime was demonstrated. At the time of the first treatment, 85% of treated females had pouch young (PY), but this dropped to 11% at the end of year 1 and 27% at the end of year 2 (compared with 100%, 63% and 63% over the successive years in the control group). The slight increase in fertility of treated females in the final year was likely due to a 4-month delay in re-treatment due COVID-19 travel restrictions. This further highlights the need for an annual (~12 month) retreatment regime to maintain reproductive suppression in kangaroos when Suprelorin® is used. A decline in fertility was observed over the years in both treated and control groups at LAMN, possibly attributable to a decline in the health of kangaroos at this site, as identified by the Brandimarti et al. (2021) health assessment study.

These results confirm previous studies indicating that repeated treatments are necessary to maintain long-term suppression in kangaroos (Herbert et al. 2006; Wilson & Coulson 2016; Wilson et al. 2013). However, the variation in contraceptive efficacy within individuals and populations presents challenges for effective contraception, as factors such as body condition, age, and reproductive status can affect contraceptive efficiency (Wimpenny et al. 2021). Therefore, additional research is needed to explore individual variability in contraceptive response.

Female kangaroos can adopt a conservative reproductive strategy by ceasing lactation and redirecting energy towards their own growth and survival during times of resource scarcity (Toni et al. 2020). By suppressing their reproductive activities and redirecting resources that would normally be allocated to nurturing offspring, they can boost their overall health and reproductive success over the long term (Forrester et al. 2024). Therefore, ongoing contraceptive treatments at LAMN would potentially provide individuals with an opportunity to redirect energy towards improving their health, particularly when facing environmental stressors such as those present at this site. Future research should investigate the long-term effects of contraception on individual kangaroo health and body condition, as well as the potential for improved reproductive success following periods of reproductive suppression.

One challenge encountered during the long monitoring phase of the experiment, was the difficulty in finding the females across the years. For example, only 41% of treated females and 49% of control females could be located by the third year at the sites in Chapter 2. Similar, at LAMN about 25% of the females had disappeared before the third treatment could be administered. This decline was likely attributed to mortality unrelated to the treatment, short-term absence from the site or lack of visibility at the time of resighting (made more difficult by COVID-19 travel restrictions), or natural dispersal. Nonetheless, this represents a challenge when trying to assess the long-term efficacy of fertility control treatments in wild

populations. Future studies should incorporate methods and mathematical models to account for these losses when trying to assess fertility rates and the long-term efficacy of contraception.

5.2.4 Delivery method

Contraceptives were administered to kangaroos using two methods: by subcutaneous injection (hand injected after capture), or remotely delivery via intramuscular darting. The efficacy and duration of the implants delivered remotely were comparable to that of the hand-injection method for the same dose (Chapters 2 and 3), suggesting remote delivery can be as effective as hand-injected delivery in managing kangaroo fertility. Both delivery methods demonstrated similar suppression of pituitary LH responsiveness (in response to a 4.7 mg treatment dose), indicating that both treatment methods achieved comparable physiological responses, effectiveness and release rates.

The 4.7 mg implant measures 2.3 mm in diameter and 12 mm in length, while the 9.7 mg implant is larger. The small size of the 4.7 mg implant makes it suitable for remote delivery darts, as a larger size would encounter greater logistical challenges, such as the risk of implant breakage upon impact, which could result in an increased release rate of the active compound (Stewart et al. 2020), thereby reducing the contraceptive longevity. In addition, the larger implant would require darts with a longer needle to accommodate the implants and increase weight of the darts. In cadaver pilot studies, longer needles resulted in an increased depth of penetration, which would likely increase the risk of dart-related injuries (Herbert unpublished).

5.2.5 Welfare implications

Humaneness is one key desirable characteristic of an effective wildlife fertility control agent, whereby there are minimal impacts on behaviour and animal welfare (Wimpenny et al. 2021).

From a welfare perspective, fertility control could potentially be seen as more harmful than lethal strategies, due to capture and handling stress imposed by manually handling, darting or anaesthetising animals (Hampton et al. 2015b; Hampton et al. 2019) and/or depriving animals from engaging in natural behaviours such as mating, rearing offspring and dispersal (Hampton et al. 2019; Mellor & Beausoleil 2015). However, in urban and peri-urban areas, lethal management of overabundant populations may be unsafe due to the proximity to residential areas (Wimpenny et al. 2021). In this context, fertility control provides a management tool to minimise adverse welfare outcomes, as unmanaged overabundant populations are vulnerable to starvation, mortality from competition for limited resources, and anthropogenic injuries such as vehicle collisions (Hampton et al. 2019).

The success of contraceptive methods hinges on their ability to suppress reproduction effectively while minimising negative physical, physiological, or behavioural effects (Gray & Cameron 2010). The longer-term effects of Suprelorin® have been assessed in kangaroos and other species, including Tasmanian devils and tammar wallabies (*Macropus eugenii*), with no adverse side effects (Herbert et al. 2005) or significant behavioural changes (Cope et al. 2018a; Woodward et al. 2006) detected. However, the method of delivery might result in pain, behavioural and physiological changes (Brivio et al. 2015; Cattet et al. 2008). Darting, capture and handling procedures can disturb natural wildlife behaviours, altering movement patterns and landscape use (Trondrud et al. 2022). Herbert et al. (2020) have demonstrated that such interventions induce acute stress in kangaroos, as evidenced by elevated FGM concentrations after capture and GPS collaring. Therefore, a critique of the humaneness of Suprelorin® contraceptive treatment must include an assessment of the impacts of implant delivery systems on animal welfare.

A prototype dart (Figure 2.1) was used in the remote delivery experiments, which was similar to the standard dart used for immobilising kangaroos but had a larger calibre needle to

accommodate the implant. In previous field trials, 20-25% of kangaroos were observed limping within 48 hours of dart delivery (Herbert, unpublished). This degree of impact on individual animals was viewed as unacceptable by the researchers and did not align with the desirable characteristics of a fertility control agent. In the study described in Chapter 3, a non-steroidal anti-inflammatory/analgesic drug (Meloxicam) was added to the dart to minimise pain associated with remote delivery. Observations conducted in the 24-48 hours after treatment detected no evidence of limping in kangaroos following either delivery method. Kangaroos exhibit minimal reactions to dart impact (Tribe et al. 2014), suggesting that, in general, darting does not inflict 'visible' signs of pain. However, due to their stoic nature (NSW Government 2021), an additional method for assessing pain involves evaluating the effects of physiological stress through fluctuations of glucocorticoid metabolites excreted in faeces (i.e. faecal glucocorticoid metabolites, FGM) in response to stressors.

Chapter 3 examined the stress and pain related to the implant delivery methods through proxies, including behavioural observations, FGM, and alterations in movement patterns using GPS, representing the first systematic study investigating the pain and stress effects of Suprelorin® contraceptive implants delivery methods in a free-range kangaroo population. The results revealed no significant behavioural changes or variations in FGM levels that would suggest compromised welfare due to either delivery method. Furthermore, movement patterns showed no changes that might indicate pain following treatment with either method. However, an important limitation of this study was the small sample size of GPS collared animals. Due to the availability of collars, only nine treated females were tracked, and no control animals were fitted with collars. Future studies should increase sample sizes and incorporate control animals to strengthen the robustness of movement-based welfare assessments.

Throughout the study, no mortalities associated with capture or animal handling were recorded during the treatment period at any of the study sites. At sites in Chapter 2, five females died months or years later due to causes unrelated to the delivery method or treatment (e.g. vehicle collisions). In contrast, Tribe et al. (2014) reported a mortality rate of 5 to 10% in their study, with deaths attributed to myopathy (19%), dart-related injuries (19%) and during anaesthetic recovery (22%), among others. Although acute stress can trigger capture myopathy, these rates seem alarmingly high. This difference in mortality rates between studies highlights the importance of careful consideration of capture and recovery methods. In this thesis, several precautionary measures were implemented, including: training the shooter's accuracy in hitting the target by practising with darts aimed at targets placed in trees before the work started; prior to darting a kangaroo, the landscape, potential hazards and proximity of other animals or young sticking out of the pouch were assessed, and the shot was not taken if not deemed safe; after capture and treatment administration, the kangaroo was left to recover in calm, shaded areas; capture bags were placed over the kangaroo's heads to ensure a calm recovery; continuous monitoring was maintained until the kangaroo was safely recovered. Additionally, the high rate of dart injuries reported by Tribe et al. (2014) emphasises the need for skilled operators who can anticipate and respond to unexpected animal behaviour, ensuring that the dart is accurately delivered to the intended target area (Hampton et al. 2021b) and that darting is not attempted on flighty or distressed individuals or group of animals. Shooter's proficiency should be assessed using accuracy tests at variable distances to minimise the risks of injuries and ensure the welfare of the animal (Hampton et al. 2021b). Animal welfare was prioritised in the protocols used in this thesis, focusing on reducing stress and minimising injuries during darting, capture, handling, and recovery stages. Preventing factors that lead to capture myopathy and adhering to protocols that reduce

animal welfare risks should be adopted in any study that involves the use of animals to ensure their safety and well-being during procedures (Hampton et al. 2021a).

Given the similarity in effectiveness and efficiency between the two methods, combined with the absence of evidence that either delivery method induced stress or resulted in pain in kangaroos, the remote delivery method offers a more practical solution for managing overabundant peri-urban populations, minimising risks associated with capture and handling, economic costs and increasing scalability of delivery (Massei 2023; Wimpenny et al. 2021). However, there remain logistical challenges associated with the recognition of treated individuals (see section 5.2.7).

5.2.6 Cost efficiency

Remotely delivered contraception is more cost-effective as it eliminates costs associated with immobilisation drugs, reduces the time required for contraceptive application and the economic costs associated with the personnel needed for animal capture, handling and post-immobilisation recovery (Wimpenny et al. 2021). The studies presented in this thesis have demonstrated that remote delivery achieved equivalent contraceptive efficacy to the hand-injected method while using half the dose and reduced costs associated with labour, with significantly improved ease of administration and minimal welfare impacts.

The short duration of action of Suprelorin® means that repeated annual treatments are needed to maintain reproductive suppression in female kangaroos, which could influence the cost-effectiveness of using Suprelorin® versus other contraceptive approaches that have a longer contraceptive duration. The remote delivery system developed could potentially be adapted for use with other long-term contraceptives, such as GonaCon™, facilitating population management strategies while minimising animal welfare issues. Future research should investigate the potential and impacts of using this system to deliver other contraceptives.

Nonetheless, remote delivery presents an effective approach for managing overabundant peri-urban populations, improving the scalability of contraceptive distribution, addressing logistical challenges, and reducing economic costs and major risks associated with capture and handling (Massei 2023).

5.2.7 Advantages and disadvantages of Suprelorin® contraceptive

The Suprelorin® contraceptive has a few advantages and disadvantages that need to be considered when evaluating the likely efficacy of this approach versus other fertility control techniques for the non-lethal management of kangaroo populations.

One disadvantage is the relatively short duration of infertility induced by the implant. While Suprelorin® effectively suppressed reproduction with a single dose of the implant, the relatively short effect means that annual repeats would be required to maintain infertility long-term. Other contraceptive approaches, such as the use of GonaCon™ or levonorgestrel implants, have a longer contraceptive duration (Wimpenny et al. 2021). Therefore, any efficiency gains achieved through the capacity to deliver Suprelorin® to kangaroos remotely must be weighed up against the costs of delivering these other contraceptives across the lifetime of a female kangaroo.

The effectiveness of Suprelorin® treatment in the first year may be obscured by pre-existing pregnancies and embryonic diapause. Since Suprelorin® does not induce abortion in kangaroos, blastocysts conceived prior to treatment can continue to develop (Wilson et al. 2013). The birth of a PY conceived before treatment can give a false impression that the treatment failed or was ineffective when, in reality, it effectively prevented new conceptions, but due to the short duration of action of the implant, the effects cannot be perceived. The prevalence of embryonic diapause can be high in superior habitats that offer kangaroos abundant food and reliable water sources (Wilson et al. 2013), such as the DP site described

in Chapter 2. In such instances, the true effects of the contraceptive might only be seen in the second year if treatment is repeated in a timely manner. It should be noted that the other contraceptives used in kangaroos face the same challenge, with the birth offspring conceived before treatment being reported for both GonaCon™ (Wimpenny & Hinds 2018) and levonorgestrel (Nave et al. 2002).

Another challenge is the two-phase endocrine response of GnRH agonist contraceptives. In female kangaroos, this biphasic response can complicate fertility control efforts as the initial hormone surge during the acute phase could induce oestrus and successful conception before the pituitary gland has been desensitised to GnRH (Gong et al. 1996). Three females in Chapter 2 gave birth approximately 40 days after receiving the implant, calculated using established age growth curves (Poole et al. 1982). As explained in section 2.3.7, these pregnancies could either have been conceived before or immediately after treatment, i.e. during the acute response to treatment. In Wilson et al. (2013), some females who had PY removed during treatment also became pregnant shortly after receiving Suprelorin® implants, which the authors similarly suggested could have been the result of oestrus induced by the acute phase following treatment.

The fact that Suprelorin® does not permanently inhibit infertility could be perceived as a limitation, but given its reversibility, it can be a valuable tool for conservation programs, zoo housing and game parks (Bertschinger et al. 2008; Cope et al. 2018b). Contraception can be selectively employed in conservation efforts to increase genetic quality and behavioural integrity in animal populations (Cope et al. 2018b). For example, contraception for Tasmanian devils provides conservation managers with precise control over breeding at the individual level (Cope et al. 2018a). In insurance populations (i.e. captive or managed groups established as a safeguard against species extinction), genetic assessment is utilised to determine relatedness between individuals and to allocate breeding recommendations on an

annual basis. Thus, selected females can be contracepted while still being housed in a mixed-sex enclosures, allowing for the maintenance of natural behaviours (Cope et al. 2018a). In peri-urban areas, free-ranging kangaroo populations can become enclosed by anthropogenic infrastructure development, including roads, houses and wildlife exclusion fences. Herbert et al. (2021) suggested that road construction contributes to the unsustainably high densities of kangaroo populations due to isolation and lack of connectivity. Paradoxically, roads can also contribute to population declines, as kangaroo-vehicle collisions significantly impact kangaroos. For instance, one of the populations treated with contraceptives in Chapter 2 (Nelson Bay Golf Course), initially had a high kangaroo density of 3.4 per hectare (ha). Following contraceptive treatment in 2013, subsequent counts revealed a declining population trend (Herbert et al. 2021), with density declining to 1.0 ha by 2019. This decline was attributed to an increase in road kills around the golf course and dog attacks on juveniles, which prevented recruitment into the population (Herbert unpublished). If Suprelorin® had caused permanent infertility, that population would have faced the risk of extinction. Therefore, in cases where the future of populations is uncertain due to urbanisation, habitat fragmentation, health or grazing pressures, a reversible contraceptive may be beneficial as fertility returns when treatment is ceased, allowing for more flexible management of the population if mortality rates change over time. After the population recovers, contraception can then be restarted to maintain the desired population density.

Suprelorin® implants are small and suitable for remote delivery and their efficacy and efficiency in delivering contraception without inducing significant stress or pain to kangaroos has been demonstrated here. A key challenge with remote contraceptive delivery is being able to identify individuals who have been treated during the treatment window (Massei 2023). Knowing which animals have been treated and which have not is imperative in population control programs. In Chapters 2 and 3, all females were initially captured and

identified with unique ear tags, but this would not be practical for large free-ranging populations. While solutions for this problem have not been investigated here, future studies should account for this. One possible solution could be to include a temporary dye marker in the contraceptive dart using double-chambered darts that can release both the drug and a dye upon impact, marking animals temporarily (Turner & Rutberg 2013). Previous studies have investigated darts that leave a paint mark on white-tailed deer (*Odocoileus virginianus*), but the dyes used were not entirely effective in marking the animal (Naugle et al. 2002), requiring further refinements. A similar concept is currently being trialled on eastern grey kangaroos elsewhere with a dart that injects GonaCon™ and simultaneously dispenses a marking paint on the animal's fur, enabling the identification of those that have been treated (Wimpenny et al. 2021). A concern with dye-containing darts is their increased weight, which may enhance the risk of damage in dart penetration and dart-related injuries (Herbert unpublished). Combining the contraceptive implant and the marker into a single, lightweight dart would be ideal for remotely delivering contraception on a larger scale. Further research and improvements are necessary to enhance the remote delivery system, enabling a more effective and broader application of contraceptives in wild kangaroos or other species in urban and peri-urban areas, where lethal management methods may be impractical or less acceptable.

Ongoing management efforts for population control can be economically costly and resource-intensive. Therefore, a contraceptive that induces infertility for multiple years would be more cost-effective in the long term. The remote delivery system described in this thesis could be modified to be used to deliver longer-lasting contraceptives such as the GonaCon™ vaccine. GonaCon™ is currently undergoing trials for remote delivery in kangaroo populations in the ACT (Wimpenny et al. 2021). However, GonaCon™ has a viscous and oily texture, which could present additional challenges for efficient delivery. GonaCon™ can also cause

injection site reactions, including granuloma formation, in various species, such as tammar wallabies, kangaroos and white-tailed deer (Evans et al. 2015; Snape 2012; Wimpenny & Hinds 2018). Evans et al. (2015) tested a syringe-dart configuration for remote delivery, achieving comparable results in drug dispensing to the hand-injected method but with a low level of vaccine efficacy in white-tailed deer. In wild horses, a remote delivery system was successfully tested with efficacy similar to hand injecting (Baker et al. 2023). However, side effects such as intramuscular swellings and the presence of draining abscesses at the vaccination site were reported. Additional boosters were required for long-term effects (Baker et al. 2023). Both Suprelorin® and Gonacon™ have inherent merits, deficiencies and risks, and the appropriate method selection depends on the requirements and particular aspects of individual populations. If validated, a single-dose, long-lasting remote delivery GonaCon™ vaccine could offer an alternative for well-established populations not facing the risk of population crashes due to anthropogenic or natural factors. For smaller populations facing these challenges, a reversible contraceptive such as Suprelorin® might be the best alternative in the event of unforeseen population declines, as observed in the Nelson Bay Golf Course population studied here. Given that GonaCon™ and Suprelorin® target the GnRH receptors of the pituitary gland, a strategic combination of these contraceptives could offer a comprehensive approach to population management. This approach might be particularly valuable in areas experiencing rapid urban development, including the construction of road infrastructure, which can lead to unpredictable population declines (Herbert et al. 2021). Comprehensive studies are essential to evaluate this proposed strategy, focusing on assessing the efficacy and safety of the sequential use of these methods, examining potential interactions between the two, and exploring the long-term impacts on individual health and population dynamics.

One important advantage of Suprelorin® is that because it is a veterinary product registered for use in dogs, it is widely available in Australia, while GonaCon™ is only approved under research permits (Wimpenny & Hinds 2018) and is not registered for widespread applications in the country yet (Centre for Invasive Species Solutions 2017). The ACT Government has been conducting trials of GonaCon™ on kangaroos since 2015, and in 2022 announced plans to integrate it into their kangaroo management program (ACT Government 2022) but until GonaCon™ can be commercialised in Australia, Suprelorin® may be the only practical approach if remote delivery is desired.

Remote delivery of contraceptives offers a more efficient and widespread implementation of fertility control strategies for kangaroos in peri-urban areas, where culling may be impractical or undesirable (Wimpenny et al. 2021). However, future studies are required to assess the long-term impacts of contraceptives on population dynamics, as well as the behavioural and stress impacts on the overall health and welfare of treated individuals over longer periods of time. Wildlife populations managed solely with fertility control may remain at high densities for several years after initiating a contraception program, a lag which may be amplified by the reproductive strategy of female kangaroos (Coulson & Wilson 2024). This delay in population reduction can be problematic in situations where more immediate management action is required. Therefore, a more effective approach might involve initially reducing the population to a desired number using other management strategies, such as culling, and then using fertility control to maintain desired population levels (Fagerstone 2002).

5.3 Assessing welfare implications of culling

Culling, while frequently used to manage kangaroo populations and mitigate human-wildlife conflicts, remains a contentious topic due to significant welfare concerns regarding the potential of suffering in animals not killed instantly, orphaning of dependent young, and impacts on other conspecifics related to changes in social structure (Descovich et al. 2015;

Hampton & Forsyth 2016; McKinnon et al. 2018). The welfare implications for orphaned joeys have been previously assessed (Sharp 2015; Sharp & McLeod 2016), as have the welfare outcomes of animals shot in culling programs (Hampton & Forsyth 2016). However, there is considerable speculation regarding the psychological and physiological impacts endured by surviving conspecifics, yet knowledge remains scarce. Public opinion is that surviving such events induces significant stress (McKinnon et al. 2018), potentially manifesting through elevated stress hormone levels and consequent welfare issues. However, no peer-reviewed research is known to have measured the welfare effects on surviving kangaroos in peri-urban areas after a cull. The assessment framework for animals that have been shot during a cull generally focuses on parameters directly related to the culling process, such as percentage of animals shot but not killed, instantaneous death rate, time to death, and locations of bullet wounds in the carcasses (Hampton et al. 2015a; Hampton & Forsyth 2016). However, this framework cannot be used to assess the welfare implications for conspecific animals following a cull.

To effectively evaluate the welfare implications for surviving conspecifics following culling operations, one must quantify the stress-related costs experienced by these animals and their capacity to respond to such stressors (McLaren et al. 2007). The main question is to determine whether the biological stress incurred results in a significant cost that might compromise the animal's health and survivorship (Moberg 2000) or reflect a psychological response to the loss of conspecifics in the short term.

Measuring FGM concentration offers a practical and non-invasive approach to measuring stress hormone levels in animals (Touma & Palme 2005), thus serving as a valuable tool for assessing physiological stress responses as an indicator of their welfare status. This approach allows researchers to identify if an intervention, such as culling or other disturbances, triggers stress responses in animals, and to differentiate between acute and chronic stress. Chapter 4

specifically addressed concerns about the stress induced by culling on surviving conspecifics by using FGM as a proxy to measure stress at the population level, using a Before-After-Control-Impact (BACI) design to enhance robustness. By including a disturbance control, the specific impacts of culling were isolated beyond the mere presence of humans and/or disturbance associated with humans.

This chapter investigated the physiological stress responses of kangaroo populations to culling and disturbance at seven peri-urban sites of the Australian Capital Territory (ACT) subjected to one of three treatments: 1. culling, 2. disturbance (no culling), and 3. control (no culling and no disturbance). Contrary to expectations, culling did not increase stress levels in surviving kangaroos. Instead, a decrease in FGM concentrations at 24 hours and 30 days post-culling was observed in culled populations. Paradoxically, disturbed and undisturbed control groups remained stable 24 hours later, but exhibited an increase in FGM concentrations after 30 days. In fallow deer (*Dama dama*), culling led to higher faecal cortisol levels 24 hours after the culling, but these levels decreased after 72 hours to become lower than those in the non-culled area (Pecorella et al. 2016). While fallow deer exhibited an initial acute stress response followed by a rapid adaptation, likely indicating a short-term endogenous response to stress (Pecorella et al. 2016), an acute stress response was not detected in kangaroos at culled sites indicating that culling may have complex and species-specific effects on the stress levels of surviving animals. These findings challenge the beliefs that culling induces stress in kangaroo populations, indicating instead that a complex interplay of factors may impact stress responses.

In the ACT, culling is implemented to reduce the effects of overgrazing caused by high kangaroo densities aiming to protect native flora and fauna (ACT Government 2018). Culled sites had higher kangaroo densities than the disturbed and undisturbed control sites, as evidenced by regular counts conducted by the ACT kangaroo program. In fact, culled sites

showed 43% higher baseline (pre-cull) FGM concentration than the other sites. Kangaroo density is inversely related to per capita green herbage mass, with areas of higher density offering less available food per individual (Portas & Snape 2018). Based on this, it is tempting to assume that the decrease in FGM levels means that culling reduced stress in kangaroos at culled sites by reducing competition for limited resources. However, interpreting these results needs careful consideration, as multiple factors can influence the production and excretion of glucocorticoid metabolites in response to a stressor. One possible explanation is a reduction in intraspecific competition (i.e. agonistic interactions) for resources rather than an increase in available food resources. While vegetation recovery after removal of grazing pressures is a slow process (Finlayson et al. 2021; Yayneshet et al. 2009) unlikely to be perceived by kangaroos within 30 days, a decrease in aggressive behaviour among individuals due to reduced competition might be immediately perceived. This suggests that the observed reduction in stress levels might be more closely related to changes in behavioural competition rather than to rapid changes in the quantity of food available. In high-density areas with limited food availability, competition for resources tends to increase aggression between individuals (Li et al. 2007), and removing kangaroos could lead to an immediate reduction in competitive behaviours by lowering population density and increasing food availability per patch (Ramp & Coulson 2004).

One limitation of using FGM to measure stress responses is the variability within individuals. This was highlighted in Chapter 3, with the example of one female at LAMN who had FGM concentrations of 17.16 ng/g before treatment, which then decreased to 3.96 ng/g on the same day and fluctuated thereafter up to 14.83 ng/g 48 hours post-treatment. Chiu-Werner et al. (2025) also observed considerable variation within individuals in brushtail possums and spotted-tailed quolls (*Dasyurus maculatus*). Factors such as age, reproductive status, and past experiences, along with external influences like season, time of day, food availability,

weather, and human activities, can affect how an animal perceives and reacts to stressors over time (Dantzer et al. 2014). This physiological adaptability can result in different stress responses within the same individual and among individuals in a population (Dantzer et al. 2014). The variation in how individuals adapt to stressors adds complexity to understanding the overall effects of these disturbances at a population level. Consequently, it is possible that the findings in Chapter 4, could be reflecting the variability in FGM concentrations among individuals (related to the sampling approach) rather than a direct reaction to culling or disturbances. Although population variability was accounted for in the statistical analyses, the model could not account for individual variabilities as the animals could not be individually identified.

Age and sex are also known to affect FGM responses (Palme et al. 2005). Although a difference between sexes was not observed in this experiment, juveniles showed higher stress hormone levels than adults. These findings align with research across other species, which reported elevated stress in juvenile animals, suggesting that higher stress could be a potential adaptive advantage for growth and development (Bonier et al. 2009; Pryce et al. 2002). Juvenile kangaroos are particularly susceptible to nutritional stress and starvation, especially during colder months when food availability is low (Portas & Snape 2018). This vulnerability stems from their high energy requirements for growth coupled with a limited gastrointestinal tract capacity relative to their body size (Munn & Dawson 2006).

The high baseline FGM levels observed (pre-culling) in the culled areas may indicate chronic stress from high population densities (Gordon et al. 2021), unknown environmental factors, or frequent management practices (ACT Government 2018). In some species, individuals experiencing chronic stress may reach a physiological threshold beyond which no further increases in GC levels are observed (Cyr & Romero 2008). Individuals who have experienced early-life stress (e.g. food shortage, management interventions or intraspecific

competition for resources) may not have the same reaction or exhibit the same intensity of hormonal response to stress compared to those who have not faced such stressors (Karaer et al. 2023). Kangaroos at culled sites could have been experiencing chronic stress and may not have been able to display an acute response to culling. The increase in FGM concentrations in the non-culled groups after 30 days could be attributed to a reduction in food availability and increased intraspecific competition for food due to the start of winter.

While interpreting results requires careful consideration of influencing factors on stress levels, there was no detectable increase in stress induced by culling in this study. Instead, a decrease was shown, as opposed to non-culled populations, and therefore, these results could be interpreted as the culled cohort experiencing lower intraspecific competition pressures even during winter. These findings, therefore, showed no evidence to suggest that culling induced an increase in stress in remaining conspecifics and can be an effective and humane management strategy for controlling kangaroo populations.

To improve the effectiveness and welfare outcomes of culling, management practices should focus on improving operational methods. This includes reducing the duration and visibility of culling activities, ensuring precision in targeting, employing contractors who are professionally trained in humane killing techniques in accordance with the Codes of Practice (Australian Government 2008), and implementing measures to limit disruptions to surviving individuals (Hampton & Forsyth 2016; Wilson & Edwards 2019). The empirical results demonstrated here will hopefully influence current public opinions on kangaroo management practices and potentially other wildlife species subjected to similar interventions.

5.3.1 Sampling limitations

The main limitation of this study was the inability to identify individual animals and, therefore, account for intra-specific variability. As GC production can vary within the same

individual, collecting random faecal samples may introduce bias, as there could be multiple samples from the same individual, potentially affecting the results (Dantzer et al. 2014), or particular age-cohorts with higher baseline levels may inadvertently be over-sampled. With approximately one-third of samples from culled sites identified as coming from juveniles but lacking comparable data for control sites, potential bias in baseline stress levels may have been introduced, particularly as juveniles exhibited higher stress levels. Statistical analyses were limited by the prevalence of unknown age classifications, which could have skewed the results in the baseline concentrations, indicating the higher baseline stress levels at culled sites.

Glucocorticoid levels exhibit daily variations influenced by natural circadian rhythms. (Dantzer et al. 2014). These variations are important to consider when measuring physiological stress as they can affect baseline levels of stress hormones in individuals throughout the day. Diurnal variation can obscure interpretations of stress responses because measurements taken at different times of the day may yield different stress levels, even in the absence of any environmental stressors (Dantzer et al. 2014; Millspaugh & Washburn 2004). Diurnal animals generally display peak hormone secretion towards the end of the dark period, while nocturnal animals exhibit peaks towards the end of the light period (Touma & Palme 2005). This diurnal variation could not be accounted for as baseline faecal samples from culled sites were collected opportunistically immediately after the animal was culled at night, while samples from other groups were collected during the day.

5.3.2 Considerations for future studies

For a more comprehensive understanding of stress responses induced by culling in kangaroos, future research should consider integrating physiological analysis with behavioural observations and long-term monitoring across different times of the day, seasons

and life stages, ideally using a larger sample size. Incorporating longitudinal sampling strategies with other measures of stress would improve the measurement and interpretation of stress for wildlife management. Additionally, collecting a larger number of samples from the same individuals over extended periods of time would be beneficial to detect variabilities within individuals and populations, although this would only be possible in populations where animals have been previously tagged and can be identified.

Future studies should also consider collecting a balanced representation of demographic groups, if possible, as this helps reduce biases and strengthens the statistical reliability of the results. These recommendations would enhance knowledge and ensure more robust empirical research to inform kangaroo management and conservation strategies, particularly regarding the impacts of culling.

5.4 Real-world applications

Fertility control is commonly preferred by the public over lethal wildlife management methods (Descovich et al. 2015), especially for highly valued species like kangaroos. However, population models indicate that fertility control is usually less effective at decreasing abundance than culling (McLeod & Saunders 2014). Relying solely on fertility control often requires considerable and ongoing management efforts to achieve population reduction (Massei & Cowan 2014). The use of fertility control in conjunction with culling for more effective management of abundance has been recommended in several species (Hobbs et al. 2000; Pepin et al. 2017), as well as kangaroos, through an initial population reduction by culling and ongoing maintenance via fertility control (Coulson & Wilson 2024; Wimpenny et al. 2021). Pepin et al. (2017) reported that the combination of fertility control and culling resulted in more effective results in closed populations of wild pigs (*Sus scrofa*) with high growth rates and low immigration. Coulson & Wilson (2024) reported that combining culling and fertility control was initially effective in reducing kangaroo density in

one population, but the population rebounded over time as the contraceptive effects diminished, highlighting the necessity of ongoing efforts or longer-lasting fertility control methods for maintaining infertility. Demographic parameters such as fecundity and mortality rates can affect the outcomes of management strategies, therefore an understanding of these parameters could help predict the effectiveness of culling intensity efforts and the proportion of animals that need to be culled to yield more effective outcomes (Pepin et al. 2017)

In terms of welfare implications, culling can be an effective and humane management strategy for controlling kangaroos, as demonstrated by the absence of significant stress-induced responses to culling and assuming that culling is conducted in accordance with the Codes of Practice (Chapter 4). Fertility control may be regarded as more harmful than lethal strategies owing to the capture and handling stress resulting from darting or anaesthetising animals (Hampton et al. 2015b; Hampton et al. 2019). However, this can be mitigated by utilising remotely delivered contraception and well-developed capture approaches.

Fertility control methods, such as Suprelorin®, discussed in Chapters 2 and 3, could be ideal for small populations facing urban threats like road construction and human-wildlife conflicts. Due to its reversibility, the population can recover and rebound in cases of decline caused by natural or anthropogenic factors. This strategy can serve as a complementary tool for lethal population management, considering the logistical challenges and delayed outcomes of fertility control. Additionally, Suprelorin® was demonstrated to be an efficient and effective method for suppressing reproduction for a period and can be safely administered remotely, minimising welfare risks associated with capture and handling, while improving economic costs and increasing the scalability of distribution (Massei 2023; Wimpenny et al. 2021).

5.5 Summary and conclusions

This thesis aimed to provide evidence-based recommendations for implementing management strategies that benefit kangaroos, humans, and the environment while considering the welfare implications of these methods.

Chapters 2 and 3 sought to address the key characteristics of effective wildlife fertility control highlighted in the review by Wimpenny et al. (2021) within the context of kangaroo management, exploring how fertility control methods can be enhanced to meet these criteria and serve as a humane and effective tool for population control. The use of Suprelorin® contraceptive meets most of the desired characteristics for a contraceptive agent for use in eastern grey kangaroos. Specifically, (1) it has demonstrated specificity to the target species, successfully inhibiting reproduction in female kangaroos; (2) a single dose of the implant was as effective as the double dose in maintaining suppression of reproduction; (3) it is safe to be used in pregnant females, with successful gestation and lactation demonstrated to continue post-treatment; (4) demonstrated humaneness in its delivery methods with neither capture-and-hand injection, nor remote delivery, inducing physiological or behavioural outcomes that could be indicative of pain or stress; and (5) can be remotely delivered with comparable suppression effectiveness and contraceptive duration to the hand-injected method, enhancing the cost-effectiveness of treatment. The long-lasting preference criterion, part of criterion number 2, could not be achieved with a single dose, due to Suprelorin®'s short-term action which requires annual additional re-treatment to maintain reproduction suppression.

Chapter 4 explored the physiological stress responses of kangaroos to culling, using faecal glucocorticoid metabolites as a proxy to measure stress at the population level before and after a cull. It demonstrated that culling was not associated with an increase in stress levels in conspecific kangaroos at culled sites. These findings demonstrate that culling can be an effective and humane management strategy for controlling kangaroo populations without

inducing acute stress. However, interpretation of results requires careful consideration of other factors influencing stress levels. This research serves as an important first step in understanding the stress response of kangaroo populations to culling as one metric of the impacts on kangaroo welfare. It provides empirical evidence on how culling operations affect stress levels in surviving kangaroos, challenging previous assumptions about stress responses to lethal control in wildlife populations. Expanding the understanding of stress physiology in kangaroos will allow for better evaluation of management interventions to minimise adverse welfare outcomes at the individual or population levels.

This research emphasises the importance of using scientific evidence to select management practices that effectively balance population control with the promotion of positive welfare outcomes. Additionally, the findings from this study provide valuable insights that can inform management strategies for kangaroos and other wildlife species subject to population management interventions.

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