Wild Horse Welfare:
Assessment and associations with population and behavioural ecology

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

Under the supervision of Daniel Ramp, Rosalie Chapple, and Fiona Hollinshead

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Certificate of Original Authorship

I, Andrea Harvey, declare that this thesis is submitted in fulfilment of the requirements of the award of Doctor of Philosophy, in the School of Life Sciences, Faculty of Science at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

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Acknowledgements & Preface

My PhD candidature and this thesis are only a small part of a long and hectic journey fully immersed in the world of 'brumby' health and welfare. I feel that it is important to say a few words about my broader story to provide a context for where this thesis fits in. It is hard to separate out my PhD from the rest of my life during my 6 year candidature, as it has all been closely intertwined, with every aspect of my immersion in this field influencing my ideas and thought processes throughout my PhD work.

I use the colloquial name here for Australian wild horses, 'brumby', because whilst my thesis focuses on the scientific assessment of the health and welfare of free-roaming wild horses, my life during this time has been dedicated to all aspects of 'brumby' welfare in many different settings from the wild to the transition into domestic lifestyles, and their training, husbandry and healthcare within domestic environments. Everything that I have learnt about, and learnt from, these horses, once in captivity, has also enhanced my understanding and knowledge of them, and inspired ideas with respect to researching them in the wild, and vice versa. An article that I wrote for the Centre of Veterinary Education's Control & Therapy series (Appendix 1) tells the story of how, as a UK small animal internal medicine specialist veterinarian, I came to start this journey with Australian wild horses, and a later article in Horse & People magazine (Appendix 2) briefly summarises much of my story working with brumbies, to date, in addition to aspects of my research that I have not included in this thesis.

My wider work in this field is also relevant to the large number of acknowledgements that I need to give. Whilst the PhD itself has often been a very lonely journey, there are a huge number of people that I have met through this work, who have inspired me, motivated me, reignited my passion when it has been waning, shaped my journey, matured my character, challenged my intellect, and enriched my life in so many ways.

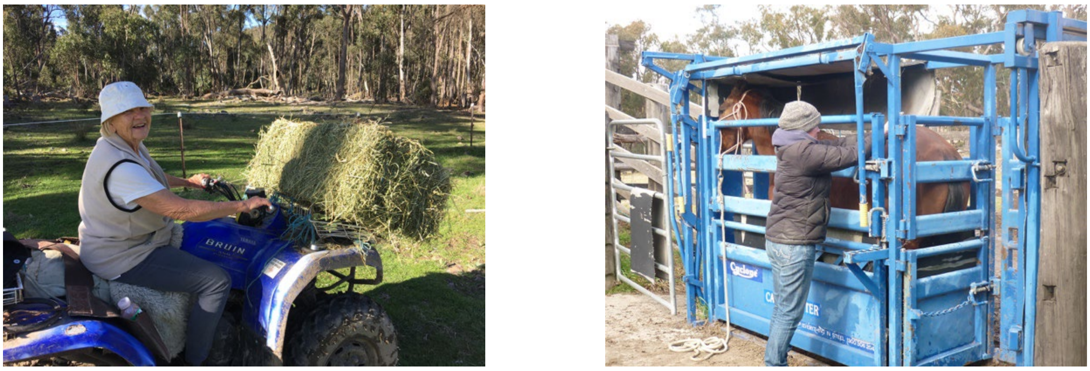
My thanks has to both start and finish with my partner, Richard, without whom I would never have had the life that inspired and enabled this journey. Nor would I have been brave enough, at a mature age, to take a break from my well-established career as a small animal internist, and embark on a journey with wild horse ecology research and animal welfare science, let alone enrol for a PhD in the area. I certainly wouldn't have completed this PhD without Richard's unfailing support in every aspect. Even during Richard's many periods of illness and throughout several stints in hospital, his priority has always been that I keep working on my PhD. During his most recent hospitalisation only a few weeks ago, he managed, from his hospital bed, to still continue nagging me to keep writing! Richard has also encouraged, facilitated, and supported the development of our own brumby sanctuary striving to demonstrate gold standard welfare practices (Figure 1), and the expansion of my skill set in aspects of equine medicine, anaesthesia and surgery to enable me to better manage the healthcare of brumbies in domesticity. Richard also supported me studying for Australian & New Zealand College of Veterinary Science Membership examinations in Animal Welfare which I did alongside field work in the first year of my candidature. I could not have done any of this without Richard's financial support. The funding available for research into the welfare of a non-native species is virtually non-existent. I also had a strong desire to undertake completely independent research where no claims could me made of research bias as a result of a funding agency. As such, all my research has been completely self-funded, other than my Australian Government Research Training Program and University of Technology Sydney Chancellor's Research scholarships.

(a) **(b)**

Figure 1. (a) Our farm in rural New South Wales that gradually became a brumby sanctuary during my PhD candidature; **(b)** Richard with 'Gertie' the goat, and 'Apache' a rescue pony.

As a boarded Specialist veterinarian, I also have an obligation to maintain credentials through ongoing clinical work, teaching, and publications in this field. Juggling these commitments was challenging, and I have to thank all my small animal medicine colleagues for their patience and understanding during these works when my mind was often on horse welfare or drifting around somewhere in the middle of the Australian Alps.

Sincere thanks to Jan Carter of Save the Brumbies Inc. (Figure 2) who first introduced me to brumbies and encouraged and facilitated me to start some immunocontraceptive trials. Stephen Page was also instrumental in assisting with planning these trials as well as later on providing practical support in field work. Whilst my immunocontraceptive work hasn't ended up being incorporated into my thesis, this was the initial inspiration for pursuing research in wild horses and was the focus of several conference presentations, also leading to an ABC news article and an article in The Conversation (https://theconversation.com/hold-your-horses-brumby-fertility-control-isnt-that-easy-97313).



(a) **(b)**

Figure 2. (a) Jan Carter first introduced me to brumbies and facilitated my initial immunocontraceptive trials; **(b)** Injecting a brumby with a GnRH immunocontraceptive vaccine at Save the Brumbies sanctuary near Armidale, northern NSW.

To my primary supervisor, Daniel Ramp, who, during a chance meeting, managed to persuade me to turn this 'hobby' into a PhD candidature. Dan insightfully recognised the need for collaboration between the fields of animal welfare science and ecology, and encouraged me to bring my existing skill set into this domain, and to expand my skills by exploring ways of scientifically assessing the welfare of free-roaming wild animals. I am grateful to Dan for the autonomy he allowed me in shaping my own journey, and his ongoing support. Thanks also to UTS colleagues, Finbarr Horgan and Caitlin Austin for the moral support and friendship, and Esty Yanco for invaluable assistance with thesis formatting and encouragement during the final stages of writing.

The socio-politically contentious nature of wild horses makes everything about wild horse research challenging. I have to thank my co-supervisor Rosalie Chapple for her wisdom early in my candidature, in advising me on negotiating the political side of wild horse research. I also value the time Rosalie spent with me in the field, and the many chats and sage advice that arose whilst hiking through the bush. Thanks also for being instrumental in all the background work that created the possibility for horse research in Kedumba Valley, which is what provided me with the opportunity of a field based PhD project in this area.

To my other co-supervisor, friend and confidante, Fiona Hollinshead, for giving me unfailing moral support, for always believing in me, enabling me to see the positives in a bad situation, and always motivating me to keep me going, even during the darkest of times. The tragic loss of Fiona's husband, Dave Hanlon, in a fatal car accident was the most devastating event during my candidature. Dave was also a valuable and inspiring mentor in the equine reproduction components of my research, always providing constructive advice regarding my immunocontraceptive studies, and welcoming me to spend time with him in his practice in New Zealand, a time that I will always cherish. Dave also advised me on using the 'Henderson method' for gelding horses, a technique that I have now performed in well over 100 brumby stallions, a significantly higher number of mature stallion geldings than is reported in the current literature to date. This has been a life saver for these stallions that are often slaughtered in knackeries and abattoirs, partly due to the practical difficulties and costs associated with gelding, and challenges of housing and handling them until this time, before they can be rehomed. Whilst this work hasn't been included in my thesis, I consider these important aspects of my broader work into improving welfare outcomes in the management and rehoming of wild horses. There is more information on this work in Appendices 3 (gelding brumbies) and 4 (welfare issues with horse slaughter), in addition to being an important part of my ongoing work, detailed in Chapter 9.

Through the gelding and other pro-bono brumby veterinary work that I have done throughout my candidature, I met my now life friends, Alison Wardman and Kim Bensch, which has been a highlight of this journey. A long road trip with Alison to an outback sheep station in far west NSW, where a large brumby training and rehoming centre had been developed, was the beginning of biannual trips with Kim to geld brumbies. We managed to pick up an exceptional assistant along the way, Cath Healy (Figure 3), who has become an instrumental member of our gelding team always happy to travel anywhere at short notice to assist us and take vigilant anaesthetic records. I am so grateful to Cath's loyalty and dedication. Enormous thanks especially to Kim for unfailing support with all aspects of brumby veterinary work, for teaching me so much, always being there as a sounding board, and always jumping in to provide practical assistance. This work inspired me to establish an organisation 'Brumby Vet', and a state of the art wild stallion rehabilitation centre on our property. With Kim's particular expertise in equine dentistry, I must also thank her for all her time examining wild horse skulls to evaluate age at death from the dentition, and record any dental pathologies.

(a) **(b)**

Figure 3. (a) Brumby stallions in Outback Australia waiting to be gelded; **(b)** Gelding brumby stallions with Alison Wardman, Kim Bensch and Cath Healy.

The broad multidisciplinary nature of my project made good collaborators critical to its success. Huge thanks to Jan Slapeta at University of Sydney, for warmly welcoming me into his parasitology lab, generously giving his time to teach me practical techniques, and his infectious enthusiasm for parasitology. Paul Canfield and Mark Westman were also treasured sounding boards at various stages.

It has been an enormous honour to have the wisdom, support and friendship of one of the greatest minds and 'godfathers' of the discipline of animal welfare science, Professor Emeritus at Massey University, David Mellor. David is truly like a rare diamond, possessing a unique combination of amazing intellect, insightful life experiences, deep wisdom, and exceptional kindness, patience and generosity. Particularly throughout the last year of writing, David frequently called me on the phone, sometimes just to check that I was OK, other times to offer positive and motivating comments, or to congratulate me on the metrics of my last paper and insist that I celebrate, and mostly to instil his wise thoughts into my writing. It was really David's faith in me that kept me writing in the final year when I often lost confidence in my work and on occasion felt like giving up. Even in his well-deserved retirement whilst having his own new projects of writing books based on his life journeys, creating Haiku-Senryu poems, and playing golf, he would always take time to discuss my work or read drafts of chapters, always with great attention to detail, positive words, and sage advice. Words cannot express the immense gratitude that I have for David's advice, support and friendship.

Sincere thanks also to Ngaio Beausoleil, at Massey University, for always taking time within her own busy research and teaching schedule to enthusiastically, thoughtfully and encouragingly discuss my work, always offering so many valuable insights. The first time that I met Ngaio during a visit to Massey early in my candidature, she didn't hesitate to warmly welcome me to stay in her home for a few days with her lovely family. The warmth and generosity, in addition to academic support is so much appreciated. Ngaio also provided me with many opportunities for sharing my work at conferences and in workshops. It was a particular honour to co-author the seminal paper on Conservation Welfare, with a prestigious group of co-authors, led by Ngaio (Appendix 5).

Heartfelt thanks to Catherine Butler and Elyssia Watts from the University of Tasmania, and their supervisory team, for warmly welcoming me to collaborate with them on work in the Australian Alps. 'Cat' and 'Talsy' made this period of work productive and enjoyable, and I will always treasure all our time together in the field (Figure 4). Through many long hours observing horses, collecting faecal samples, getting rained out of swags and recovering bogged vehicles, we were never short of laughs and I made more life friends. I was also proud to be a co-author on Elyssia's honours paper (Appendix 6). Again, although most of my work in the Australian Alps hasn't made it into my thesis, this is a large body of research that will be an important part of my future works (Chapter 9).

None of the fieldwork would have been possible without the support of Blue Mountains and Southern Ranges branches of National Parks and Wildlife Service (NPWS), WaterNSW and Parks Victoria, all of whom have been exceptional to work with. Thanks in particular to NPWS Chris Banffy and Rob Gibbs for being so generous with their time and knowledge, providing field guidance, and expanding my knowledge of the regions and the challenges in wild horse management. Thanks also for valuing my research in the context of wild horse management. This led me to be appointed by the Minister for the Environment as the veterinarian and animal welfare expert, and Deputy Chair, of the NSW Governments Scientific Advisory Panel for wild horse management in Kosciuszko National Park, for the last 2 years. Whilst this substantially slowed down my thesis writing, having the opportunity to have directly advise on wild horse management policy has been hugely rewarding and the ultimate culmination of my PhD research (Appendix 7). Huge thanks to the rest of the panel, and also NPWS Mick Pettit and Donna Sampey for being such a pleasure to work with, receptive to my ideas, and continuing to teach me so much. In this role I have also enjoyed and valued working alongside members of the Community Advisory Panel, and I thank them also for teaching me particularly about many of the historical aspects of wild horses in the Australian Alps, and sharing their valuable local knowledge.

(a) **(b)**

Figure 4. (a) Cat Butler, Kedumba River, Blue Mountains; **(b)** Talsy Watts (right) and myself, Cooleman Plain, Kosciuszko National Park.

To all my field volunteers, many of whom were colleagues from my veterinary work, for making my field work the most memorable and enjoyable aspect of my PhD. My love for the Australian bush certainly flourished, and the time spent in the bush became much more than just researching wild horses. Working dawn until dusk and then crashing out in a swag under the stars became part of normal life. Kedumba valley and The Australian Alps were my 2^{nd} home for a couple of years, and they will always hold a special place in my heart full of fond memories. Particular thanks to Vibeke Russell for literally being my right hand for over a year of field trips in Kedumba Valley (Figure 5). I will forever be so grateful to Vibeke for sharing and whole heartedly participating in this part of my journey. Some of the first wild horses we ever observed together are now at our sanctuary, and a daily physical reminder of what we achieved together, but that is another long story! (Chapter 9).

(a) **(b)**

Figure 5. (a) Co-supervisor Rosalie Chapple (left) and myself overlooking Kedumba Valley, Blue Mountains; **(b)** Vibeke Russell (right) and myself on fieldwork.

Data analysis and statistics would not have been possible without the help of John Morton. I cannot express the extent of my gratitude to John, who provided data and statistical analysis, but also so much more than that. John showed genuine interest in my work and always went above and beyond, being probably the only other person in the world to become almost as excited as me about my data! John became such a valuable sounding board and his kindness, patience and enthusiasm in each zoom meeting lifted my spirits and motivated me during periods when my morale was often low.

Most importantly, heartfelt thanks have to go to all my animals, who enthuse and inspire me daily, continue to teach me so much, and are my reason for living when life gets tough. The saying in our household is 'welfare starts at home', meaning that the welfare of the animals under our care at home and at our sanctuary always take priority. By necessity this meant that my PhD had to often take a backseat whilst I treated sick animals, grieved over animals that we had to say goodbye to, and when I had to intensively care for them during the trifecta of natural disasters that spanned over 3 years of my PhD candidature with drought, bushfires, and floods and the long recovery period that followed (Figure 6), not to mention the COVID-19 pandemic that impacted us in the final year of my candidature.

A memorable and perhaps surreal moment during the early stages of writing up, was responding to reviewers comments on my Chapter 2 paper as bushfires were ripping through our property for the 4th consecutive day. Having worked tirelessly to keep all

our animals safe and staying up all night checking for embers around the house, I had barely slept for a week by that stage. I thank the *Animals* Editorial assistant for their understanding during this time when I had to pack up my laptop ready to leave the house and tell them 'I'm not sure when I will get back to this!' It was often hard to find the time and focus for my thesis during the long and ongoing recovery period that followed, but different aspects of animal welfare were always at the forefront of my mind. I captured some of the animal welfare issues that I grappled with during the bushfires in an article for The Veterinarian (Appendix 8). I thank the many friends and colleagues that played a role in lifting my spirits and giving me the strength to continue writing my thesis during these turbulent times when it often felt meaningless.

(a) **(b)**

Figure 6. (a) Some of the brumbies at our sanctuary that I cared for during a trifecta of natural disasters spanning 3 years of my candidature; **(b)** The devastation around us following the bushfires. The red cross marks where our house is.

Finally, thanks to my parents, who as a result of my field work, followed by our trifecta of natural disasters, and then the COVID-19 pandemic, I have been unable to see since they visited here right at the beginning of my candidature in 2015. Despite having been on the other side of the world for the last 11 years, and a fairly absent workaholic veterinarian for the preceding 10 years, the philosophies they instilled in me as a child to be humble, kind, generous and work hard, have always continued to stand me in good stead to succeed in a range of endeavours. Their encouragement to always follow my heart led me back to my passion for horses, that I had since a young child. And the motto of 'having a job that you love means you never work a day in your life', has enabled me to continue to disguise long hours of working as just having fun.

The journey of my PhD candidature through many adverse times, certainly has been a labour of love, but has proved to myself that with determination and resilience, anything can be achieved.

List of Chapters and Statement of Author Contribution

This thesis has nine chapters. It is a compilation of 3 manuscripts published in peer-reviewed journals (Chapters 2, 4 and 5), and 6 chapters that are not yet published, but will also be submitted for publication. Referencing styles were standardised throughout the thesis in accordance with those of the journal *Animals*.

Chapter 1

Introduction: The wild horse controversy and the importance of incorporating animal welfare science in decision making

Contributor	Statement of contribution
Andrea M. Harvey Production Note: Signature removed prior to publication.	Conceptualisation 80% Writing original draft 100% Review and editing final draft 80%
David J. Mellor Production Note: Signature removed prior to publication.	Review and editing final draft 10%
Daniel Ramp Production Note: Signature removed prior to publication.	Conceptualisation 20% Review and editing of final draft 10%

Chapter 2 – Published Paper

Harvey, A.M.; Beausoleil, N.J.; Ramp, D.; Mellor, D.J. A Ten-Stage Protocol for Assessing the Welfare of Individual Non-Captive Wild Animals: Free-Roaming Horses (*Equus Ferus Caballus)* as an Example. *Animals* **2020**, *10*, 148.

Contributor	Statement of contribution
Andrea M. Harvey Production Note: Signature removed prior to publication.	Conceptualisation 100% Methodology 100% Literature review 100% Writing original draft 100% Review and editing final draft 70%
Ngaio J. Beausoleil Production Note: Signature removed prior to publication.	Project overview and advice 50% Review and editing original draft 60% Review and editing final draft 10%
Daniel Ramp Production Note: Signature removed prior to publication.	Review and editing final draft 10%
David J. Mellor Production Note: Signature removed prior to publication.	Project overview and advice 50% Review and editing original draft 40% Review and editing final draft 10%

Chapter 3

A review of the species-specific information required to enable assessment of wild horse welfare

Contributor	Statement of contribution
Andrea M. Harvey Production Note: Signature removed prior to publication.	Conceptualisation 100% Literature review 100% Writing original draft 100% Review and editing final draft 60%
David J. Mellor Production Note: Signature removed prior to publication.	Review and editing final draft 40%

Chapter 4 – Published paper

Addressing the knowledge gap of gastrointestinal parasitology in free-roaming wild horses in south-east Australia

Published as:
Harvey, A.M.; Meggiolaro, M.N.; Hall, E.; Watts, E.T.; Ramp, D., Šlapeta, J. Wild horse populations in south-east Australia have a high prevalence of *Strongylus vulgaris* and may act as a reservoir of infection for domestic horses. *Int. J. Parasitol. Parasites Wildl.* **2019**, *8*, 156-163.

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Andrea M. Harvey Production Note: Signature removed prior to publication.	Conceptualisation 50% Methodology 50% Data curation & analysis 70% Writing original draft 100% Review and editing original draft 50% Review and editing final draft 40%
Maira N. Meggiolaro Production Note: Signature removed prior to publication.	Data curation 20% Data analysis 10%
Evelyn Hall Production Note: Signature removed prior to publication.	Statistics 100%
Elyssia T. Watts Production Note: Signature removed prior to publication.	Data curation 10%
Daniel Ramp Production Note: Signature removed prior to publication.	Review and editing final draft 10%
Jan Šlapeta Production Note: Signature removed prior to publication.	Conceptualisation 50% Data analysis 20% Methodology 50% Review and editing original draft 50% Review and editing final draft 40%

Chapter 5 – Published Paper

Harvey, A.M.; Morton, J.M.; Mellor, D.J.; Russell, V.; Chapple, R.S.; Ramp, D. Use of Remote Camera Traps to Evaluate Animal-Based Welfare Indicators in Individual Free-Roaming Wild Horses. *Animals* **2021**, *11*, 2101.

Contributor	Statement of contribution
Andrea M. Harvey Production Note: Signature removed prior to publication.	Conceptualisation and design 80% Literature search & methodology 100% Data curation 80% Data analysis 60% Data interpretation 100% Writing original draft 100% Review and editing final draft 55%
John M. Morton Production Note: Signature removed prior to publication.	Data analysis 40% Statistics 100% Review and editing original draft 30% Review and editing final draft 5%
David J. Mellor Production Note: Signature removed prior to publication.	Review and editing original draft 70% Review and editing final draft 25%
Vibeke Russell Production Note: Signature removed prior to publication.	Data curation 15% Review and editing final draft 5%
Rosalie Chapple Production Note: Signature removed prior to publication.	Data curation 5% Review and editing final draft 5%
Daniel Ramp Production Note: Signature removed prior to publication.	Conceptualisation 20% Review and editing final draft 5%

Chapter 6

Scientific validation of welfare indicators for using the Five Domains Model to grade the welfare status of individual free-roaming wild horses

Contributor	Statement of contribution
Andrea M. Harvey Production Note: Signature removed prior to publication.	Conceptualisation 100% Methodology 100% Writing original draft 100% Review and editing final draft 70%
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Chapter 7

Dynamic changes in wild horse social organisation and habitat use revealed with remote camera-trap monitoring

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Andrea M. Harvey Production Note: Signature removed prior to publication.	Conceptualisation 70% Methodology 100% Data curation & interpretation 100% Data analysis 60% Writing original draft 100% Review and editing final draft 50%
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John M. Morton Production Note: Signature removed prior to publication.	Data analysis 40% Statistics 100% Review and editing final draft 10%
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Chapter 8

The cascading influence of resource availability on the welfare status of wild horses, and association with demography, social organisation and habitat use

Contributor	Statement of contribution
Andrea M. Harvey Production Note: Signature removed prior to publication.	Conceptualisation 70% Methodology 100% Data curation 100% Data analysis 60% Data interpretation 100% Writing original draft 100% Review and editing final draft 50%
Daniel Ramp Production Note: Signature removed prior to publication.	Conceptualisation 20%
Rosalie Chapple Production Note: Signature removed prior to publication.	Conceptualisation 10%
John M. Morton Production Note: Signature removed prior to publication.	Data analysis 40% Statistics 100% Review and editing final draft 10%
David J. Mellor Production Note: Signature removed prior to publication.	Review and editing final draft 40%

Chapter 9

Conclusions and future directions

Contributor	Statement of contribution
Andrea M. Harvey Production Note: Signature removed prior to publication.	Conceptualisation 60% Writing original draft 100% Review and editing final draft 70%
Fiona Hollinshead Production Note: Signature removed prior to publication.	Conceptualisation 20%
Daniel Ramp Production Note: Signature removed prior to publication.	Conceptualisation 20%
David Mellor Production Note: Signature removed prior to publication.	Review and editing final draft 30%

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Chapter 1

Thesis Abstract

Knowledge of the welfare of wild animals contributes vital information to ethical, legal, and political debates about our interaction with wild animals and their habitats. Despite this, there has been little insight into how to assess their welfare and factors affecting it. Wild horse management exemplifies a highly controversial wildlife issue, where to date, welfare has not been assessed or incorporated into management decision making.

My thesis aims to (i) develop methodologies for scientifically assessing the welfare of free-roaming wild animal species, (ii) apply these methods to assess the welfare of free-roaming wild horses across a range of habitats, and (iii) explore the spatial and temporal changes in welfare, and the relationship between welfare status, population dynamics, social organisation, habitat use, and resource availability.

Chapter 1 introduces the controversies in wild horse management and why welfare assessment is important. In Chapter 2 I develop a novel conceptual framework, the 'Ten-Stage Protocol', for advancing the practical capacity to assess welfare in free-roaming wild animal species. Chapters 3 to 8 then apply this framework to wild horses, demonstrating how this protocol can be practically applied to obtain meaningful, systematic, structured, transparent, and scientifically objective assessments of welfare. Whilst my thesis is based on free-roaming wild horses, it has been developed specifically to form the basis of application of these processes to other species.

In Chapter 3, I review the current state of wild horse knowledge, presented in a holistic and multidisciplinary framework. I demonstrate (Chapter 4) the type of research required to address knowledge gaps, using the example of wild horse parasitology.

Chapter 5 investigates the use of remote cameras for identifying individual wild horses across different habitats, and for acquiring data on an extensive range of welfare indicators. Chapter 6 comprises scientific validation of these welfare indicators and refinement of a Five Domains welfare grading scheme. I then (Chapter 7) evaluate population dynamics, and temporal and spatial changes in social organisation and habitat use of a wild horse population over a 15-month period, using the remote camera methodology established in Chapter 5. I further evaluate welfare status demonstrating the cascading effects of resource availability, and how this correlates with population dynamics, social organisation, and habitat use (Chapter 8).

Finally, Chapter 9 summarises how this research has advanced knowledge, assisted with management decision-making to improve the welfare of wild horse populations, and contributed to conflict resolution in wild horse management.

Thesis outline summarizing chapter titles and contents		
Chapter	**Chapter title**	**Brief description**
1	**Introduction: The wild horse controversy & the importance of incorporating animal welfare science in decision making.**	**Summary of the history of wild horses, their cultural significance, environmental impacts and controversies in wild horse management. Introduces the importance of animal welfare science in wild animal controversies.**
2 Published	A Ten-Stage Protocol for assessing the welfare of individual non-captive wild animals: free-roaming horses as an example.	Presentation of a novel conceptual framework that I designed in order to guide a systematic and scientific approach to assessing the welfare of free-roaming wild animals, using the Five Domains Model. Summarizes the principles of interpreting indicators of biological function and behaviour in terms of the mental experiences that those indicators reflect (Stage 1 of my Protocol), and how the Five Domains Model is used for assessing welfare (Stage 2 of my Protocol).
3	Literature review: A review of the species-specific information required to enable assessment of wild horse welfare.	The current status of wild horse knowledge is summarized in a novel holistic and multidisciplinary framework drawing together the relevant literature on horses across each of the four physical/functional domains of the Five Domains Model. It addresses Stage 3 of my Ten-Stage Protocol.
4 Published	Addressing the knowledge gap of gastrointestinal parasitology in free-roaming wild horses in south-east Australia.	An example of the type of detailed original research required for addressing any knowledge gaps identified in Stage 3. This chapter describes a detailed parasitological investigation of 293 faecal samples collected from 6 wild horse populations. It describes results of faecal egg counts, larval cultures and molecular diagnostics.
5 Published	Use of remote camera traps to evaluate animal-based welfare indicators in individual free-roaming wild horses.	A large body of original research investigating for the first time, both the use of remote cameras for identifying individual horses across a range of habitats, and for acquiring data on an extensive range of animal-based welfare indicators. It addresses Stages 4-6 of my Ten-Stage Protocol.
6	Scientific validation of welfare indicators for using the Five Domains Model to grade the welfare status of individual free-roaming wild horses.	Addressing Stage 7 of my Ten-Stage Protocol, this chapter evaluates the scientific evidence linking the described objective measurable/observable welfare indicators to physical/functional impacts in Domains 1-4, and the relationship between those impacts and the mental experiences that are inferred in Domain 5. This concludes with the formulation of a Five Domains Model wild horse specific welfare grading scheme.
7	Dynamic changes in wild horse social organisation and habitat use revealed with remote camera trap monitoring.	With the aim of evaluating traditional ecological metrics alongside welfare status, this chapter describes original research using the remote camera trapping methodology described in chapter 5, to evaluate population dynamics, and temporal and spatial changes in social organization and habitat use of a wild horse population over a 15 month period.
8	The cascading influence of resource availability on the welfare status of wild horses, and association with population demographics, social organization and habitat use.	This original research applied the methodology from all preceding chapters to assess welfare status and changes in welfare status in individual wild horses over a 15 month period, addressing stages 8 -10 of my Ten-Stage Protocol. It further evaluates drivers of change in welfare status and correlations between welfare status, and the population dynamics, social organization and habitat use described in chapter 7.
9	Conclusions, application, and future directions.	Summarizes overall conclusions, and contributions to the fields of wild animal welfare, wild horse ecology and welfare, wild horse management, and general horse welfare. Highlights ongoing work, some of which I have already partially completed that was not included in the main body of the thesis.

Chapter 1. Introduction: The wild horse controversy & the importance of incorporating animal welfare science in decision making

The overall aims of this doctoral research were to (i) develop an innovative conceptual framework for advancing the practical capacity to assess welfare in free-roaming wild animal species, (ii) to develop practical methods for assessing welfare, particularly in free-roaming wild horses, and to then (iii) apply these methods to assess the welfare of specific populations of free-roaming wild horses in south-east Australia.

I further sought to evaluate (i) the main drivers of change in the welfare status of these wild horses, (ii) how welfare status may vary across populations in different environments and habitats, and (iii) how changes in welfare status may correlate with changes in habitat use, social organisation, and population dynamics. Finally, I demonstrate the importance of these findings in assisting with wild horse management decision making to ensure the optimal welfare of wild horse populations, and how this may contribute to conflict resolution in wild horse management.

My thesis describes how I achieved all these aims. It focuses on Australian wild horses, specifically in south-east Australia (New South Wales and Victoria), where the most social-politically contentious populations of horses reside (Figure 1). However, the methodology and results from my thesis are also highly applicable to any wild or semi-wild equid population worldwide, whether they are feral populations with a contentious existence, endangered populations undergoing conservation, or new populations being actively introduced to habitats undergoing rewilding programs. Furthermore, the methodology I have developed for assessing welfare, may also be applied to significantly advance the field of wild animal welfare more broadly, by application to other free-roaming wild species.

Figure 1. Wild horses in Warragamba Special Area, Kedumba Valley, Blue Mountains, NSW, Australia. Images A.M.Harvey.

Most wild horse populations present throughout the world today have descended from domesticated horses and are therefore by definition 'feral' (once domesticated but now wild), with Przewalski horses being the only true wild horses remaining. However, the term 'feral' also has negative connotations, implying that the animal is 'out of place and doesn't belong'. It is widely recognised that such labels influence policy and contribute significantly to polarisation in wild horse management debates worldwide (Bhattacharyya et al. 2011; Scasta et al. 2020). Therefore, throughout my thesis I use the term 'wild' to mean both animals that are of a wild species and those that are currently non-domesticated. I use the term 'free-roaming' to distinguish between animals that roam freely in a wild habitat from those that are in captivity (e.g., in a zoo or sanctuary).

In this first chapter, to set the scene, I introduce the background on wild horses by (i) summarising the history of wild horses globally, (ii) the history of wild horses in Australia, (iii) wild horse controversies, (iv) the heritage value of Australian wild horses, (v) the environmental impacts of the horses, (vi) historical and current management of wild horses and (vii) ongoing controversies. This is an original synthesis of detailed information on these broader aspects of wild horses, derived from a wide variety of sources. The depth of this background information presented on wild horses and their relationships with people, is necessary in order to fully appreciate the controversies in wild horse management. Finally, I highlight why assessment of welfare is an important and currently missing piece in wild horse debates.

1. A brief history of wild equids

Wild equids evolved over 55 million years ago, with the earliest member of the Equidae family, the Hyracotherium living during the Eocene period (45-55 million years ago), which evolved to the Mesohippus, which lived 32-37 million years ago ("Hyracotherium", "Mesohippus" *Fossil Horses in Cyberspace,* Florida Museum of Natural History*).* In North America, the modern equine species that we recognise today (*Equus caballus*) had evolved by about 5 million years ago. Over this time, equids also evolved from being a leaf eating forest dweller, browsing on soft tropical plants, to eating drier plant material and finally adapting to eat tougher grasses inhabiting semi-arid plains. By 15,000 years ago during the late Pleistocene, wild horses were present in many parts of the world including the steppes of Eurasia, Europe, Beringia, and North America (Weinstock et al. 2005).

Wild equids became extinct in North America around 12,000 years ago (Nunez et al. 2016) and also declined elsewhere, suspected to be due to climate and habitat changes as grasses gave way to shrub tundra covered with unpalatable vegetation (Vila 2001; Luis 2006; Haile 2009). In North America, horses were reintroduced in the 15th century, with the arrival of the Spanish Conquistadors, and wild populations began to re-establish soon thereafter. A European wild horse known as the Tarpan was found in Europe and much of Asia throughout this time but became extinct in 1909 (Dohner

2001). Humans began to domesticate horses in Asia around 4,000BC and their domestication is thought to have been widespread by 3,000BC, based on palaeological and archaeological data, and DNA analysis (Matossian 1997; Outram et al. 2009).

Przewalski wild horses were described in the 19th century to be roaming the Mongolia-China border (Boyd & Houpt 1994), but they become extinct in the wild during the 20th century, due primarily to interbreeding with domesticated horses, hunting, loss of habitat and loss of water sources (Boyd & Houpt 1994). They have subsequently been reintroduced to their native habitats, with about 1,500 in existence today, but only through intensive breeding programs in captivity to preserve and expand their diminished gene pool (King et al. 2005).

2. The wild horse controversy

Wild horse populations across the world are viewed differently in different locales. Being endangered true wild horses, Przewalski horses are highly valued and subject to conservation programs in Mongolia, Hungary, and China. In Poland, a project to recreate tarpan-like horses began in the 1920's, resulting in the creation of 'Konik horses', some of which are maintained as free-roaming populations in large forest sanctuaries (Gorecka-Bruzda et al. 2020). In the UK, some free-roaming populations such as the New Forest pony are considered iconic, their presence dating back to 500,000 BC. In Wales, Carneddau Mountain ponies have been free-roaming since the Bronze Age and are considered a valuable genetic resource (Fraser et al. 2019; Harley et al. 2021). Similar situations exist in European countries, such as Spain where conservation programmes to preserve wild horse populations have been undertaken since the 1980s (Vega-Pla et al. 2006). Other populations derived from early European settlers, such as the Sable Island horses off the east coast of Canada have become legally protected (Plante et al. 2007). In many parts of Europe, horses are now being valued for their positive ecological roles with free-roaming horses being actively introduced to areas as part of rewilding projects (Linnartz & Meissner 2014).

In the United States, Australia and New Zealand, views regarding wild horses are extremely polarised and management of these horses has become a complex socio-political issue (Chapple 2015; Scasta et al. 2019). In North America, wild horse populations became particular focal points for controversy. There were power struggles over land use during the 20th century, which culminated in legal protection with the '1971 USA Wild Free-Roaming Horses and Burros Act', predicated on these horses as 'living symbols of the historic and pioneer spirit of the West' (Bhattacharyya 2012). Despite legal protection, their management is undertaken to maintain them at 'appropriate levels', and remains highly controversial (Scasta et al. 2019) with whole books devoted to the topic (*The Wild Horse Dilemma,* Gruenberg 2015; *The Wild Horse Conspiracy*, Downer 2012).

In Australia, wild horse populations have only existed since the 1800's. Some view wild horses as 'feral pests' that damage the fragile ecosystems that didn't evolve in the presence of 'hard hoofed' animals. This is reflected in legislation with the '1975 Australian National Parks and Wildlife Conservation Act' stipulating that, exotic animals are to be exterminated or controlled in order to protect the natural and native values of National Parks. Conversely, others deeply cherish the horses, regarding them as national icons for their role in Australia's survival in the early colonial days and its subsequent development. For many Australians, they hold a special place in their psyche, epitomizing the spirit of freedom and courage beloved by most Australians (Massey & Sun 2013). More recently, others view wild horses as having become entrenched in modern day Australian landscapes and consider that they may even be filling an important ecological niche (Ripple et al. 2015; Lundgren et al. 2020).

These opposing views are the core reason for controversy regarding wild horse management.

In NSW, wild horse management controversy was especially sparked in 2000 following world-wide public outrage, litigation, and significant media coverage following the aerial shooting of 606 horses in Guy Fawkes River National Park, in the NSW Northern Tablelands (Chapple 2005).

3. Australian wild horses

3.1 The history of wild horses in Australia

The wild horse population of Australia, the largest in the world, originated from domestic horse stock with the first seven horses arriving on the First Fleet in 1788. Continued importation by European settlers had brought over 3,000 horses to Australia by the 1820s, with the earliest arrivals being from the South Africa, UK, other European countries, and Timor and comprising a combination of Thoroughbred, Arab, Cape horse, Timor pony, Clydesdale, and Percherons. Breeds were mixed to produce horses suited to the needs of the early settlers, developing a strong versatile horse that could thrive in the harsh Australian landscape, with a quiet temperament and great stamina.

Horses played a vital role in the exploration and development of the new colony and became utilised widely from ploughing fields, building roads and railways, carrying mounted police and bushrangers, day to day transport, and in the growing horse racing industry. By the 19th century, domestically utilised horses were growing in number, with an estimated 14,000 working horses in 1830 having risen to 160,000 in 1850 in Eastern Australia alone, by which time distribution had extended to most states and territories (Heritage Working Party 2002) (Figure 2).

Whilst most regions where wild horses reside in NSW are now National Parks, prior to this during early white settlement, the land was largely used for farming sheep and cattle. Horses were indispensable for farm work. This was especially so in the Australian Alps in the then grazing culture of seasonally moving livestock to higher pastures in spring and summer. In the early- to mid-1800's, when pastoralists abandoned their settlements, and when horses escaped or were released to fend for themselves, wild horse populations began to establish.

Figure 2. Locals in Jindabyne (Snowy Mountains region) in 1920. Image courtesy of Leisa Caldwell, personal family collection.

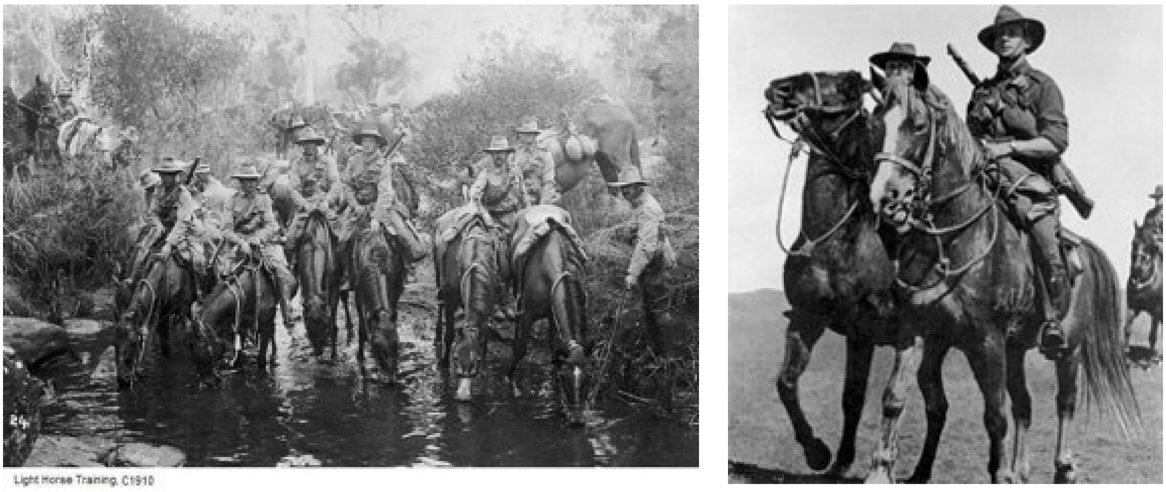
Kedumba Valley, in the Blue Mountains (described in Chapters 5 – 8) was one such region where pastoralists settled from 1824 onwards. Large portions of land were cleared for running sheep and cattle (Edds 2001). In addition, pit ponies were used to support coal mining operations in the region in the 1870's. The mining operations ceased in the early 1900's (Eardley & Stephens 1974), and as the Blue Mountains grew to be a tourist destination, these pit ponies were no longer required and are thought to have contributed to the wild horse population in the area, along with escaped or released pastoralists' horses. The Warragamba Special Area, which includes Kedumba Valley was proclaimed as a water catchment area in 1942 and consists of the stored waters of Lake Burragorang (the main water supply for Sydney) and adjacent lands and is now part of the Blue Mountains National Park, a UNESCO World Heritage site.

Similarly, the Monaro and Snowy Mountains region in the Australian Alps was first settled from the 1830s, and a wild horse population was established in the Mt Kosciuszko area by the 1840-1850s. In 1861, during a climbing expedition near Thredbo, the members sighted ‘immense herds of wild horses, which would be impossible to break in’ (The Age, 7 January 1861). There are various accounts in the late 1800s of horses accidentally escaping at Mt Kosciuszko, including the horses belonging to scientific explorers, and domesticated horses intentionally released by graziers and stockmen in order to 'improve' the wild horse population with fresh stock. By the 1890s, the wild horse population in the Alps was thought to be greater than elsewhere (Melville et al. 2015).

The Kiandra region of the Snowy Mountains was settled with the gold mining boom giving rise to a large human population by 1860, with an 1866 parish map showing ‘wild horse plain’ adjoining the township. An article in the Upper Murray District in 1885 noted the wild horses that occupied the nearby Snowy Mountains on the border between Victoria and NSW and described them to be ‘remarkably swift and agile’ (Northeastern Ensign {Benalla}, 24 Nov 1885) (Melville et al. 2015). Following many decades of rather disparaging attitudes towards the wild horses, there was a discernible shift from the 1880s in popular attitudes to the wild mountain horses.

By the mid-1800s, the quality of horses in NSW had become known to the British Army in India, and an export market in remounts (war horses) became established, a trade that flourished for over 100 years. These famous war horses from NSW became known as 'Walers' (Figure 3). Over time these horses became the new colony's first major export market with more than half a million Walers shipped overseas to destinations including South Africa, the Middle East, South-East Asia, Japan, the South Pacific, New Zealand and America (Fahey 1984). Several historical records describe Walers being bred in the Guy Fawkes River region, and a 1941 Sydney Morning Herald article describes horses for the Australian Light horse remount depots being taken from the Kiandra region of the Snowy Mountains (Caldwell 2020).

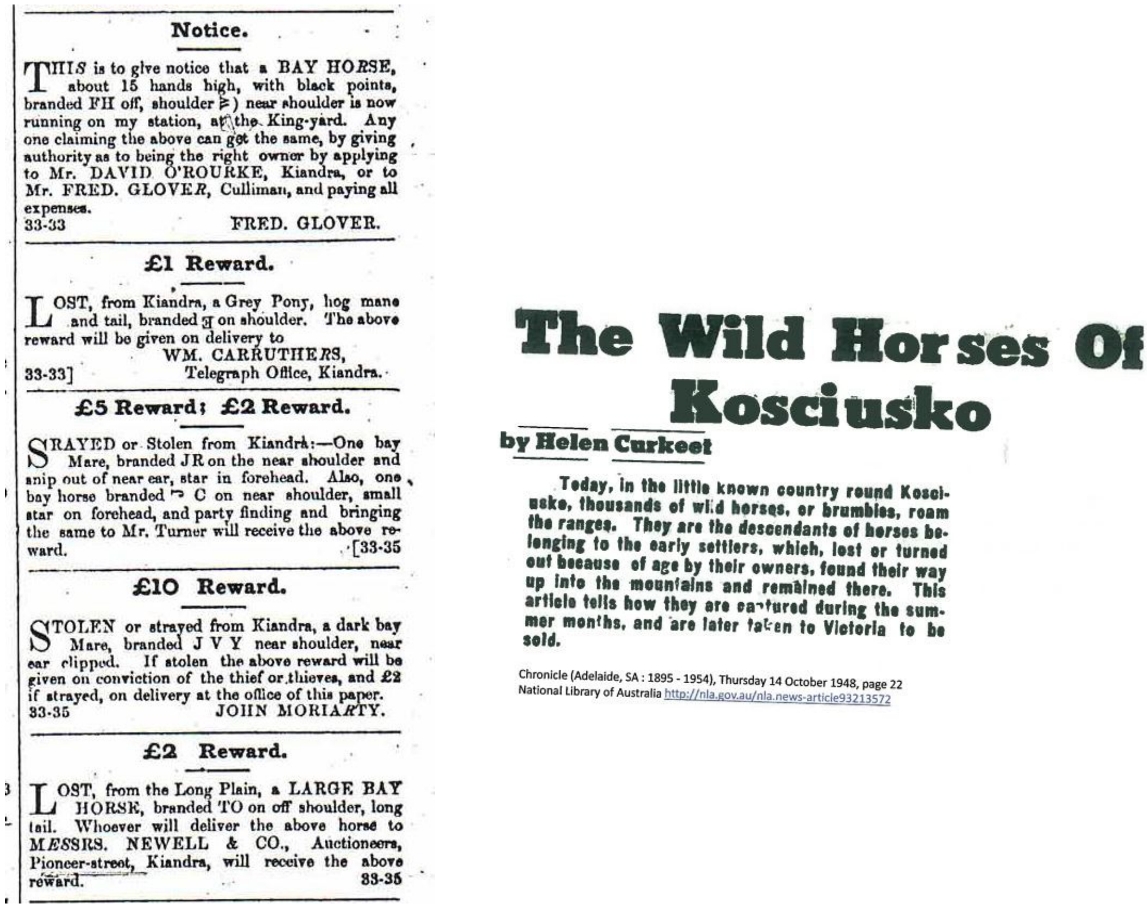
These horses became indispensable to Australians in the Light Horse mounted forces in the Second Boer War and World War 1, with 16,314 horses dispatched overseas from Australia during the Boer War, and 121,324 horses being sent overseas in World War 1. The Australian Light Horse played a key role in defeating the Turks in World War 1 and became known as 'The youngest nation in the world defeating one of the oldest on its home ground', with men of the Light Horse famously saying 'We couldn't have done this without our Walers' (Stringer, cited in Heritage Working Party 2002). Over 70,000 of these horses lost their lives in World War 1, and after the war, due to quarantine restrictions the light horses could not return to Australia and were either sold to the British Army as remounts for Egypt and India or destroyed.



(a) **(b)**

Figure 3. (a) Australian Light Horse Training, c1910; **(b)** Australian light horsemen on Walers prior to their departure from Australia. Images Wikipedia (https://en.wikipedia.org/wiki/Australian _Light_Horse).

As demand for remounts declined in the 1940s, the commercial trade ended, and together with a rise in mechanisation, many horses were no longer required and were left to remain in the bush. There are numerous accounts of working horses and remounts being bred in the Kosciuszko region (Melville et al. 2015; Caldwell 2020) and a local weekly newspaper in 1860 noted many lost horses in the district, exemplifying how many of these domestic bred stock likely joined the wild horse population (Caldwell 2020). From that time mountain cattlemen continued to manage the horses; a historical article in the *Chronicle* in 1948 describes how mountain cattlemen captured wild horses roaming Kosciuszko, during the summer months to sell (Figure 4). 'Brumby-runners', as they were known, mustered wild horses on horseback into yards or caught them via roping (Melville et al. 2015). Following creation of Kosciuszko National Park in the mid-1900s, livestock grazing leases were soon terminated, due to recognition of the environmental damage that hard-hoofed animals were causing (Robin 1998, cited in Riebow 2017).



Notice.

THIS is to give notice that a BAY HORSE, about 15 hands high, with black points, branded FH off, shoulder ⋷) near shoulder is now running on my station, at the King-yard. Any one claiming the above can get the same, by giving authority as to being the right owner by applying to Mr. DAVID O'ROURKE, Kiandra, or to Mr. FRED. GLOVER, Culliman, and paying all expenses.
33-33 FRED. GLOVER.

£1 Reward.

LOST, from Kiandra, a Grey Pony, hog mane and tail, branded ꞡ on shoulder. The above reward will be given on delivery to
WM. CARRUTHERS,
33-33] Telegraph Office, Kiandra.

£5 Reward; £2 Reward.

STRAYED or Stolen from Kiandra:—One bay Mare, branded JR on the near shoulder and snip out of near ear, star in forehead. Also, one bay horse branded ⤼ C on near shoulder, small star on forehead, and party finding and bringing the same to Mr. Turner will receive the above reward. [33-35

£10 Reward.

STOLEN or strayed from Kiandra, a dark bay Mare, branded J V Y near shoulder, near ear clipped. If stolen the above reward will be given on conviction of the thief or thieves, and £2 if strayed, on delivery at the office of this paper.
33-35 JOHN MORIARTY.

£2 Reward.

LOST, from the Long Plain, a LARGE BAY HORSE, branded TO on off shoulder, long tail. Whoever will deliver the above horse to MESSRS. NEWELL & CO., Auctioneers, Pioneer-street, Kiandra, will receive the above reward. 33-35

The Wild Horses Of Kosciusko

by Helen Curkeet

Today, in the little known country round Kosciusko, thousands of wild horses, or brumbies, roam the ranges. They are the descendants of horses belonging to the early settlers, which, lost or turned out because of age by their owners, found their way up into the mountains and remained there. This article tells how they are captured during the summer months, and are later taken to Victoria to be sold.

Chronicle (Adelaide, SA : 1895 - 1954), Thursday 14 October 1948, page 22
National Library of Australia http://nla.gov.au/nla.news-article93213572

(a) **(b)**

Figure 4. (a) Kosciuszko local newspaper cutting from 1860; **(b)** *Chronicle* article from 1948. Images Caldwell 2020.

Similarly, in the Guy Fawkes River region of northern NSW, a Heritage Working Party report (Heritage Working Party 2002) details numerous first-hand accounts describing the uncontrolled breeding of horses in large numbers, with mustering of offspring that were then sold (Fahey 1984; Newbury 1986). There were also descriptions of 'the wild horses originating from former stock mares which had outgrown their usefulness on the stations and been turned out to run free' (Newbury 1986).

Free-roaming wild horses and are now found in all states and territories of Australia, inhabiting a huge range of habitats from deserts in central and northern Australia, to the snowy mountains and alpine regions of south-east Australia. The total population of wild horses now in Australia is said to be anywhere between 500,000 to one million (Dobbie et al. 1993; Nimmo & Miller 2007), with the largest populations dispersed across desert plains in the Northern Territory (Dobbie et al. 1993), Central Australia (Berman 1991), and throughout Queensland (Mitchell et al. 1985; Dawson et al. 2006), South Australia (Axford et al. 2002) and Western Australia (Dawson et al. 2006).

In NSW, the largest wild horse population is in Kosciuszko National Park (KNP) with approximately 14,000 horses estimated to be in the park in 2020 (Cairns 2020), with others found in Guy Fawkes River National Park (estimated 1742 in 2014), Oxley Wild River National Park (estimated 207 horses in 2006), and Warragamba Special Area of the Blue Mountains National Park (estimated 30 horses in 2021). In Victoria, wild horses are most prevalent in the Alpine National Park, with an estimated 5200 horses (Walter 2002), numbers estimated to have halved following the 2013 bush fires (Walter 2003), with smaller populations of approximately 100 horses (2005) in Bogong National Park, and a similar number in Barmah State National Park (Dawson et al. 2006).

3.2 The heritage value of Australian wild horses

The Australian wild horse, colloquially known as the 'Brumby' (a name attributed to Sergeant James Brumby's horses, which were left to run loose on his land when he was transferred to Tasmania in the 1830s) are considered by many to have heritage value. In 2001, a Heritage Working Party was formed by the NSW Minister for the Environment. Its purpose, to assess the claims of heritage status of brumbies in Guy Fawkes National Park, based on the belief that the horses where part of 'The Waler' legend (Heritage Working Party 2002).

Walers were highly valued and quoted by an English cavalry officer as 'undoubtedly the finest cavalry mounts in the world' (Lieutenant Colonel RMP Preston, The Desert Mounted Corps), performing with distinctions as the Australian Light Horse, excelling in desert warfare, culminating in the charge on Beersheba, the last great cavalry charge in history (Chapple 2005). Several books have provided incredible accounts of these horses during war, for example 'The Desert Column (Ion Idriesss 1932), 'A Military History of Australia' (Grey 2008) and 'Bill the Bastard: The Story of Australia's Greatest War Horse (Perry 2012) (Figure 5). Several films include the charge at Beersheba in 1917, such as '40,000 Horsemen (1940) and 'The Light horsemen' (1987), and a song 'As if He Knows' (Eric Bogle 2001) reflects the feelings of Light Horsemen farewelling their mounts in Egypt at the end of World War I.

The Heritage Working Party concluded that the Guy Fawkes brumbies did indeed have significant heritage value, stating that 'the value of horses to this country's survival in the early colonial days and its historic development cannot be challenged. Horses have found a place in the hearts of the Australian people that goes back to the beginning of European settlement. Horses have historic, cultural and a very significant economic value in Australia. They have their beginnings with the early days of European settlement, and they have been an integral part of our nation and its history for over 200 years and continue to be important in Australian culture' (Heritage Working Party 2002).

(a) **(b)**

Figure 5. (a) The Australian Light Horse in the charge on Beersheba, 1917. Image Wikipedia (https://en.wikipedia.org/wiki/Australian_Light_Horse); **(b)** 'Bill the Bastard', a novel based on a Waler that excelled during the Charge of Beersheba.

A further study was commissioned by National Parks and Wildlife Service in 2015, to better understand the cultural heritage values associated with wild horse populations in KNP. This identified that the wild horse population in the park is an attribute associated with the cultural heritage significance of the park related to the following: historical values associated with High Country pastoralism and the 'Alps experience'; cultural values associated with alpine pastoral landscapes; aesthetic values associated with the uniquely wild and remote alpine landscapes that represent a cultural icon; social values associated with the love of the High Country cultural landscape and The Man from Snowy River legend; and the role of the High Country environment and landscape in the lives and works of significant people, in particular writer Elyne Mitchell (Silver Brumby series) and the Australian bush poet, AB 'Banjo' Paterson (who wrote the poem 'The Man from Snowy River' first published in 1890) (Melville et al. 2015) (Figure 6). Certainly, for many local people wild horses became a source of local identity and cultural pride, "When over 120 years of mountain grazing ended, the mountain people's world changed dramatically. The brumbies became sacred as they were the last link to the heritage that they treasured" (Riebow 2017).

(a) **(b)**

Figure 6. (a) The famous film 'The Man from Snowy River' based on an 1890 poem by 'Banjo' Paterson **(b)** 'The Silver Brumby' series of books by Elyne Mitchell.

Most recently, the heritage value of horses in the Snowy Mountains (KNP) has been legally recognised, with 'The Kosciuszko Wild Horse Heritage Act 2018', the object of the Act being 'to recognise the heritage value of sustainable wild horse populations within parts of the Kosciuszko National Park and to protect that heritage, whilst ensuring that other environmental values of the park are also maintained.' A report concerning the heritage value of KNP horses produced from a Community Advisory

Panel, appointed under the new legislation, details the short but rich history of the horses in the Snowy Mountains since the beginning of white settlement in the region, in the early 1800's (Caldwell 2020).

Brumbies have become known throughout the world as an Australian icon immortalized in the films the 'The Man from Snowy River'(I and II), the 'Silver Brumby' series of books and movie (starring Russel Crow), the 19th century 'bush poetry' of one of Australia's most famous poets, 'Banjo' Paterson (who was also Officer-in-Charge of the Remount Unit in Egypt during World War 1), the appearance of a Snowy mountains brumby on the $10 note (Figure 7) together with the opening lines of the poem 'The Man from Snowy River', and the popular 'Great Australian Muster' which has become the symbol of the Sydney Royal Easter Show.

Figure 7. Snowy mountains brumbies even feature on the Australian $10 note.

3.3 Growing wild horse populations and environmental impacts

Conflicting with the heritage value of these horses, there have long been concerns about the negative environmental impacts of wild horses (Costin 1954), which began to be scientifically examined in Australia in the late 1980s (Dyring 1990). Widespread concerns include soil compaction and erosion (Kauffmann & Krueger 1984; Dyring 1990; Whinam & Hope 2005; Hope et al. 2012; Robertson et al. 2019) and runoff and soil displacement to the side of trails in wet peaty areas (Marshall & Holmes 1979, cited in Dyring 1991; Lance et al. 1989).

In recent years, the wild horse population in KNP has received the most attention in terms of research on population estimates and environmental impacts, as well as socio-political attention. KNP is one of the largest conservation reserves in Australia, being an UNESCO Biosphere Reserve and is an integral component of the Australian Alps National Parks and Reserves, which were included on the National Heritage List in 2008.

The park is home to mainland Australia's highest mountain (Mount Kosciuszko), the headwaters of snow-fed major rivers that provide some of Australia's most important water catchments, and extensive sphagnum bogs (commonly called peat moss) and snow patch communities that play an important water-holding role. It is the only true alpine area in mainland Australia, has the most extensive peatlands in the Australian Alps, glacial landforms and karst (i.e. limestone) systems and 21 plant and animal species found nowhere else in the world. A total of 853 species of native plants have been recorded in the park (Montague-Drake 2005). The alpine sphagnum bogs, wetlands and heaths are endangered ecological communities which also provide habitat for several endangered species (Office of Environment & Heritage 2016a, 2016b) (Figure 8).

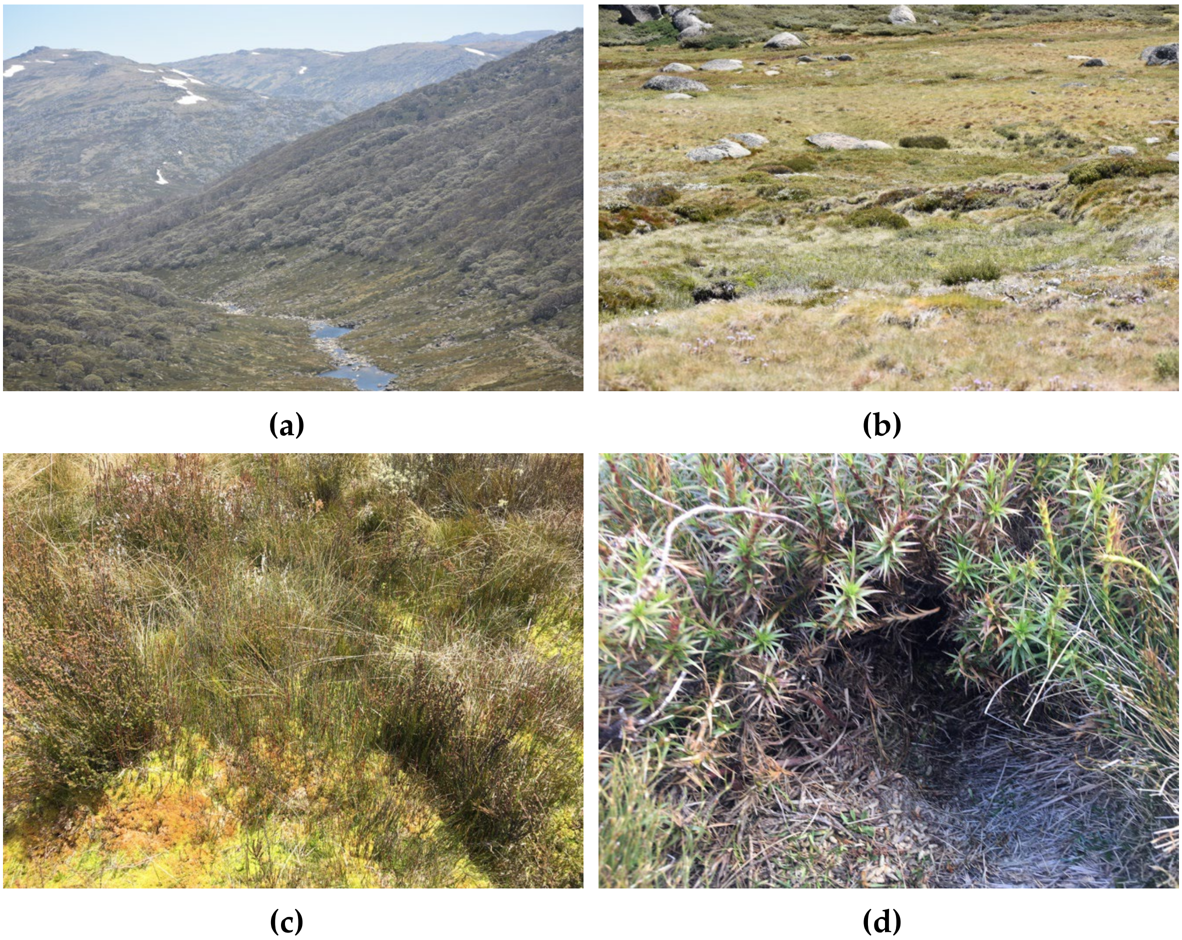


Figure 8. **(a)** Alpine region near Mount Kosciuszko; **(b)** An alpine region where horses currently do not reside; **(c)** Close up image of sphagnum moss; **(d)** A burrow of the endangered Broad-toothed rat in wetland heath. Images A.M. Harvey.

Although population estimates of horses are hotly debated, horse numbers within KNP have clearly increased substantially over the last 40 years, from several hundred horses in the late 1980's (Dyring 1990), to an estimated 14,000 horses in 2020 (Cairns 2020) (Figure 9). Regular population surveys throughout that time have supported evidence for growth in both distribution and density of horses across this region with horses now inhabiting about 50% of the park (Dyring 1990; Walter 2003; Dawson 2005; Montague-Drake 2005; Dawson 2009; Cairns & Robertson 2015; Office of Environment & Heritage 2016a, 2016b; Cairns 2019).

Figure 9. Population estimates are performed via helicopter surveys with estimates hotly debated. Image shows aerial view of horses in waterways on open plains in northern Kosciuszko. The 'counting beam' on the helicopter can be seen in the bottom right of the image. Image A.M. Harvey.

The growing populations have been associated with increased concern for the negative impacts on the environment. Peat communities are threatened by physical damage by trampling, which leads to loss of vegetation cover and altered hydrology and channelling of water (McDougall & Walsh 2002; Hope et al. 2012). This is linked to declines in sphagnum and sedges, increased erosion, and increased probability of wetland draining, which in turn will negatively affect soft plants (e.g., sphagnum moss) and fauna with specific requirements for well-aerated soils (Dyring 1990; Hope et al. 2012; Rogers 1991). This habitat loss may also threaten a range of endangered species (Robertson et al. 2019) including broad-toothed rats (*Mastacomys fuscus*) (Cherubin et al. 2019; Schulz et al. 2019), the Northern Corroboree Frog (*Pseudophryne* *pengilleyi*) (Evans 2018; Scheele & Foster 2018) and the Mountain Pygmy Possum (*Burramys parvus*) (Figure 10).

The new KNP legislation sets out to identify 'environmentally sustainable' wild horse populations, but identification of what an 'environmentally sustainable' population of horses is, is challenging. Many environmentalists argue that no horses should be present in National Parks. However, while horses at a high density clearly have a significant negative environmental impact, there is a lack of robust scientific evidence regarding the precise relationship between horse densities and negative impacts, across different habitats. Another emerging contentious theory is that there may even be positive environmental impacts of horses (Lundgren et al. 2020), at least when their densities are low. Positive impacts observed under light grazing regimes in drier areas could include recycling of nutrients and maintenance of patchy habitat and improve floristic diversity (Menard et al. 2002). For example, higher plant species diversity was maintained by wild horse grazing in the Australian Alps (Wild & Poll 2012; Williams et al. 2014) and ungulate grazing has been beneficial for an endangered daisy in Tasmania (Gilfedder & Kirkpatrick 1994). Further research is required to identify what sustainable populations are, as horse densities are reduced, in addition to further research on potential positive impacts.


(a) **(b)**


(c) **(d)**


(e) **(f)**

Figure 10. **(a)** Aerial view of horses near the head of the Murray River in the Australian Alps, on the border between NSW and Victoria; **(b)** Aerial view of the banks of the Lower Snowy River, southern Kosciuszko; **(c)** Aerial view of horses traversing wetlands in northern Kosciuszko; **(d)** Aerial view showing stream bank damage in northern Kosciuszko; **(e-f)** Close up images of stream bank damage in sub-alpine region, southern Kosciuszko. Images A.M. Harvey.

4. Wild horse management

4.1 Globally

Whilst some populations globally are subject to natural predation by pumas (Turner Jr. et al. 1992; Ransom 2012), lions (Turner Jr. et al. 1992) and wolves (Gomes & Oom 2000; Van Duyne et al 2009; Lagos 2013), the majority of free-roaming horse populations around the world today are without natural predators and are managed by human intervention. The degree of intervention is variable, with some free-roaming populations having a high level of intervention with regular monitoring, supplementary feeding when required, and annual removal of weanlings (Gorecka-Bruzda et al. 2020), whilst other populations are regularly culled through removal or shooting in situ, and some have virtually no intervention at all (Nunez et al. 2016).

In the US, although lethal control is prohibited by legislation, large numbers of horses are rounded up, usually by helicopter mustering and taken to holding areas. From there they are available for adoption by members of the public, but with demand being low, thousands are retained in holding yards long term (Nunez et al 2016). There has also been an illegal trade transporting horses to Mexico for slaughter (Bloch 2019). Immunocontraception administered by darts is used to limit reproduction in some small populations but has significant limitations (Hobbs & Hinds 2018). 'Reserve design' has also been proposed as a way to 'establish naturally self-stabilizing equine populations' (Downer 2014).

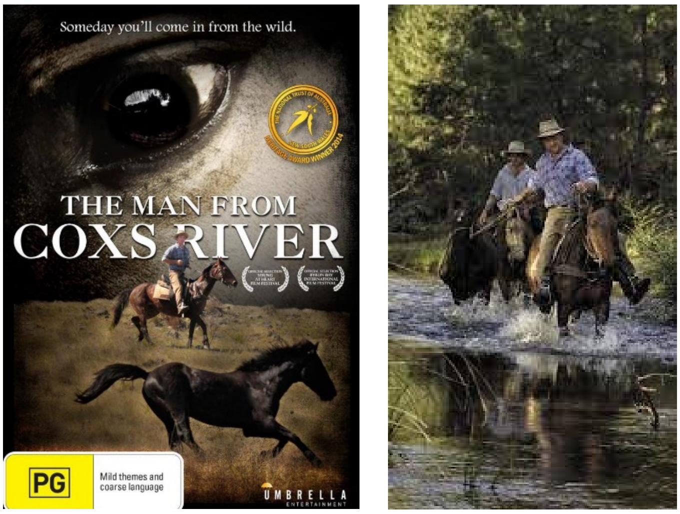
4.2 In NSW, Australia

Prior to purchase of the lands by National Parks and Wildlife Service (NPWS) in the mid-to -ate 1900's, free-roaming horses were generally managed by property owners/leasers, and mountain cattleman who would capture horses for their own use or for sale, and/or shoot them (Wright 1971, cited in Heritage Working Party 2002; Caldwell 2020). Periods with no reported management occurred after lands became National Parks, with some management in the 1980's and 1990's consisting of removal of horses from Guy Fawkes River National Park and shooting in the Blue Mountains. In Kosciuszko National Park, involvement of local mountain cattleman in wild horse management was prohibited from the late 1980s, with management responsibility then continued by NPWS.

The public condemnation following the 2000 Guy Fawkes aerial shooting of horses, led to an enquiry culminating in a ban on shooting wild horses in NSW National Parks, with the Minister of the Environment (Labour party, Bob Debus) stating 'despite the weight of scientific advice supporting aerial shooting, he had listened to the community on this issue' (Daily Telegraph, Nov 17, 2000). According to NPWS, the shooting was undertaken because of a long drought followed by bushfires, with horses becoming emaciated and congregating next to a river with no remaining food resources. However, no formal welfare assessments had been performed prior to, or during the intervention, resulting in poor transparency and loss of public trust.

As such a new wild horse management plan was developed for Guy Fawkes National Park, beginning in 2004, with passive trapping and removal of horses to be placed in private homes, with those not rehomed being taken to knackeries. The result of this enquiry also influenced new wild horse management plans throughout other NSW National Parks, including Kosciuszko National Park in 2002, Oxley River National Park in 2006, and Blue Mountains National Park (Warragamba Special Area) in 2007, highlighting the political importance of gaining public support (Chapple 2005). In the remaining state and territories of Australia, shooting from the ground and the air is still commonplace for controlling large populations in these vast landscapes, but is increasingly receiving adverse public attention.

The management of horses in the Kedumba Valley, Blue Mountains National Park (the population of horses described in Chapters 5, 7 & 8), attracted public attention in 2014 with release of an award-winning documentary, 'The Man from Coxs River', which filmed the process of horses being trapped and removed from this rugged inaccessible area (Figure 11). Despite involving non-lethal management, it generated much controversy with questions raised about the justification for removing the horses, as well as ethical and welfare issues about the handling of the horses. As such, no further management occurred until 2017 (see Chapter 9).



(a) **(b)**

Figure 11. (a) Award winning documentary following a horse removal program from Kedumba Valley, Blue Mountains; **(b)** Horse being led out of remote area following trapping and halter breaking. Image by Russell Kilbey@Empress Arts.

Kosciuszko National Park is currently the centre of controversy for wild horse management in Australia. A new draft plan of management was released in 2016, which for the first time incorporated (i) an extensive public consultation process, (ii) recognised the heritage value of the horses and proposed retaining horses in the park and (iii) incorporated trials for reproductive control. Despite this, it was met with public outcry due to the inclusion of escalated trapping, as well as ground shooting of horses with the purpose of reducing the overall population by 50% during the first 5-10 years and by 90% over 20 years (Office of Environment & Heritage 2016a, 2016b). As a result, the plan was rejected by Nationals Party Leader, and member for Monaro, John Barilaro, and ongoing lobbying by local brumby advocates resulted in him later introducing the Kosciuszko Wild Horse Heritage Bill 2018, to shape a new Wild Horse Heritage plan of management (Barilaro 2018). The 2021 Draft Plan was released for public consultation in October 2021 and having struck a balance considered acceptable to most environmentalists and brumby advocates, it was adopted by the Minister for Energy and Environment on 24th November 2021 (NSW DPI 2021).

5. Ongoing controversies in wild horse management in Australia

It is widely recognised that socio-political factors frequently compete with science in policy decision making on contentious issues, and that politics often takes precedence (Chapple 2005; Chapple et al. 2011). Wild horse management is acknowledged to be a complex socio-political issue and this unavoidably has a major influence on the development of management plans (Chapple 2005; Nimmo & Miller 2007; Riebow 2017; Scasta et al. 2020).

Wild horse management plans will only be successful if they are socially acceptable and this largely depends on education, transparency, and trust (Scasta et al. 2020). Integrating community opinion can potentially make an important contribution to the development of management plans that are both ecologically and socio-politically sound (Chapple 2005; Nimmo & Miller 2007; Bhattacharyya et al. 2011; Riebow 2017).

However, despite apparent progress in understanding the socio-political dimensions of wild horse management over the last two decades, 21 years on from the 2000 Guy Fawkes cull, the issue remains as contentious as ever, with no signs of conflict mitigation. The enormous growth of social media in this intervening period in addition to the proximity of south-east Australian national parks to metropolitan areas has led to much wider public interest in the wild horse debate, adding further complexity to it due to a wider range of disparate opinions. Shifts in the focus of debates away from just management methodology, towards, on the one hand, a growing number of horse advocates taking the view that the horses should be left 'wild and free' without human intervention at all, and on the other hand, many environmentalists advocating for complete eradication of horses from National Parks.

The 2018 KNP legislation provides an opportunity to achieve a balance between preserving wild horse populations in addition to protecting environmental assets, and for compromises to be reached between stakeholders. However, despite this, immense conflict remains with heightened vitriol and constant provocation by those on different sides of the debate, recently documented in the news and a book (*The Brumby Wars*, Sharwood 2021).

6. The importance of assessing welfare in wild free-roaming animals

Competing values and different priorities create ethical dilemmas and public discourse (Gamborg et al. 2012) but caught in the middle of the social disagreements and tensions are the horses themselves (Scasta et al. 2020).

Regardless of whether the presence of different free-roaming horse populations are valued positively or negatively, concerns regarding their health and welfare can arise. Media reports can be found across the globe describing welfare concerns for both feral horse populations, semi-feral, and those in rewilding projects. For example, in 2018 there was international media coverage when many horses in Barmah National Park, Victoria were reported to be starving following artificial flooding of the area, limiting access to food resources. In the same year, during a severe drought, horses were found dying of starvation along the Lower Snowy River in south-eastern Australia, whilst on the other side of the world there was negative media publicity about a Netherlands nature reserve undertaking a rewilding project where large numbers of horses were found to be emaciated. The following year there was a mass death of wild horses in Central Australia near a dried-up water hole during extremely hot weather. A UK study identified public concern for overbreeding, a lack of food in winter, and roundups in free-roaming ponies (Horseman et al. 2016). In wild Przewalski horses, genetic health is a particular concern (King et al. 2005). The need for more systematic evaluations of the welfare outcomes in Australian horse management has been recently identified (Scasta et al. 2020).

Despite all the controversies in wild horse management, and the obvious concern for welfare, strikingly, to date, wild horse welfare has received little or no attention, in wild horse management debates and in scientific research.

More broadly, in many wild animal controversies it has been proposed that scientific assessment of animal welfare and incorporation of animal welfare into decision making may assist in resolving controversies (Sandoe & Gamborg 2017). It is increasingly recognised that knowledge of the welfare status of individual wild animals may contribute information directly relevant to ethical, legal, and political debates about the ways in which we interact with wild animals and their associated habitats (Kirkwood et al. 1994; Beausoleil 2014; Ramp & Bekoff 2015; Dubois et al. 2017; Finn & Stephens 2017; Wallach et al. 2018; Finn 2019; Hampton & Hyndman 2019; Hampton & Teh-White 2019). Despite this, the welfare of wild animals has not received much attention from animal welfare scientists, conservationists, legislators, or the public (Hampton et al. 2015; Hampton & Hyndman 2019), and there has been very little research on the welfare of any free-roaming wild animals.

Although methods for assessing welfare have been well developed for a range of captive animals (Temple et al. 2011; Samuel et al. 2012; Andreasen 2014; Heath 2014; Hockenhull & Whay 2014; Mullan 2014; Dalla Costa et al. 2016; Blatchford 2017; Czycholl et al. 2018), including for wild species (Hill 2009; Whitham & Wielebnowski 2009; Clegg et al. 2015; Kagan et al. 2015; Sherwen et al. 2018), and a need to develop methodologies for assessing the welfare of free-roaming wildlife has been highlighted (Kirkwood 1994), to date, no protocols have been found that enable purposeful, systematic, and scientific assessments of the welfare of free-roaming wild animals engaged in their normal daily activities. Therefore, little is known about what positive and negative welfare impacts many free-roaming wild animals might be experiencing and why. Moreover, robust scientific methods for capturing reliable and informative data to enable welfare assessments of free-roaming wild animals have not been well described.

One of the reasons for this knowledge gap is that attempting to assess welfare in any free-roaming wild animal is inherently challenging (McCulloch & Reiss 2017). These challenges include: the complex logistics of such studies; some entire habitats may be difficult to access; observation of the animals may be obscured by natural geographical features such as vegetation and topography; fear of humans may lead them to remain distant from observers; and observing them for significant periods or at repeated intervals may not be possible. In some situations, it may also be difficult to observe the individuals that are experiencing severe welfare impacts, as they may hide, be less mobile, be more distant from conspecifics and in habitats/terrain that make visualising them difficult.

7. Thesis outline

In light of the above observations, my doctoral research began with the development of an innovative conceptual framework, 'The Ten-stage Protocol' (Table 1), which I designed to provide a step-by-step guide for wildlife biologists and others who wish to apply a systematic, scientifically based approach to assess the welfare of individual free-roaming wild animals (Chapter 2). The content of Chapter 2 has been published as a peer-reviewed paper (Harvey et al. 2020).

The Protocol incorporates existing conceptual frameworks, and thus incorporates decades of previous work in the science of animal welfare assessments, into a contemporary framework for novel applications to free-roaming wild animals. This innovation forms a major contribution to the field of wild animal welfare science, by advancing the practical capacity to assess welfare in free-roaming wild animals.

This novel conceptual framework, based on the 'Five Domains Model' (Mellor & Reid 1994; Mellor et al. 2009; Mellor & Beausoleil 2015; Mellor 2017; Mellor et al. 2020), also provided the basis for me to develop practical methods for capturing robust data for assessing welfare in wild free-roaming animals. I then applied these methods to assess the welfare of a population of free-roaming wild horses in south-east Australia over a 15-month period. Throughout my thesis, I demonstrate how my Ten-stage Protocol, can be practically applied to obtain meaningful, systematic, structured, transparent, and scientifically objective assessments of welfare. Also outlined are the limitations of such assessments, which are imposed by the circumstances of data collection and interpretation that occur when studying free-roaming wild animals. The primary focus of my thesis is free-roaming wild horses; however, the approaches developed and tested can also be applied to other such species.

The concept for Stage 1 of the Ten-stage Protocol arose from a workshop between conservation and animal welfare scientists (3rd International Compassionate Conservation Conference, Sydney 2017), where a dominant theme that emerged was a need for a common language and understanding in relation to wild animal welfare. This gave rise to a publication on the newly proposed field of 'Conservation Welfare' (Beausoleil et al. 2018), of which I was a co-author (Appendix 5). Stage 2, related to acquiring an understanding of the Five Domains Model required extensive reading and understanding of all the previous literature on the Model. Chapter 2 describes the Ten Stage Protocol in detail and summarises these first two stages.

Table 1. The Ten-Stage Protocol for assessing the welfare of non-captive wild animals (Harvey et al 2019).

1. Acquire an understanding of the principles of Conservation Welfare.
2. Acquire an understanding of how the Five Domains Model is used to assess welfare status.
3. Acquire species-specific knowledge relevant to each Domain of the Model.
4. Develop a comprehensive list of potential measurable/observable indicators in each physical domain, distinguishing between welfare status and welfare alerting indices.
5. Select a method or methods to reliably identify individual animals.
6. Select methods for measuring/observing the potential welfare indices and evaluate which indices can be practically measured/observed in the specific context of the study.
7. Apply the process of scientific validation for those indices that are able to be measured/observed and insert validated welfare status indices into The Five Domains model.
8. Using the adjusted version of the Model that includes only the validated and practically measurable/observable welfare status indices, apply The Five Domains grading system for grading welfare compromise and enhancement within each Domain.
9. Assign a confidence score to reflect the degree of certainty about the data on which welfare status has been graded.
10. Including only the practically measurable/observable welfare alerting indices, apply the suggested system for grading future welfare risk within each domain.

Chapter 3 relates to Stage 3 of the Protocol, i.e., acquiring species specific knowledge relevant to each Domain. This is the main literature review chapter, reviewing existing literature on wild horses in addition to relevant aspects of domestic horses that may be used to assist in advancing knowledge in wild horse health. This literature review is presented in a novel holistic and multidisciplinary framework by drawing together the relevant literature on horses across each of the four physical/functional domains of the Five Domains Model, namely nutrition, environmental conditions, health, and behavioural interactions. Importantly this review enables identification of knowledge gaps where further research may be required.

Once such knowledge gap identified in Chapter 3 was gastrointestinal parasitology of wild horses. Chapter 4 is a published paper (Harvey et al. 2019) that addresses this and is a specific example of the type and detail of multidisciplinary research that may be required to advance species specific knowledge. Specifically, this is the first report globally, demonstrating the high persisting prevalence of the pathogenic parasite *Strongylus vulgaris*, in wild horse populations. The importance of this work extends beyond the health of the wild horses themselves. This finding also demonstrates that wild horses may be an important reservoir of infection for domestic horses, where *Strongylus vulgaris* has been considered to be virtually 'extinct' in recent years. This research therefore has broad implications for any horse populations worldwide where there is overlap between wild and domestic populations.

Chapter 5 and 6 relate to the development of methodologies for obtaining information to enable scientific assessments of welfare to be made, whilst Chapter 7 describes application of that methodology. Specifically, Chapter 5 is a published paper (Harvey et al. 2021) describing a large body of novel original research that incorporates Stages 4 – 6 of the Ten Stage Protocol. This is the first published report describing the use of remote camera traps to identify wild horses in woodland habitats where they could not be directly visualised, in addition to use of camera traps to acquire data on an extensive range of welfare indicators.

Chapter 6 relates to Stage 7 of the Protocol; scientific validation of welfare indicators and refinement of a Five Domains welfare grading scheme for specific use in the wild horse populations being studied. This brings together information from chapter 3 (species specific knowledge), together with results from chapter 5 related to identifying welfare indicators that were feasible to detect with direct observations, and camera traps images and video. Bringing together that information, I then go through a process of scientific validation of those indicators, in relation to the mental experiences that those physical indicators may reflect. This also involved careful evaluation, review, and synthesis of a range of literature from several discipline including physiology, behavioural science and neuroscience.

Chapters 7 and 8 comprise a large body of novel original research resulting from 15 months of field work involving the application of stages 8-10 of the Ten-stage Protocol. This involved extensive analysis of data collected over that period from the population of free-roaming wild horses in Kedumba Valley, Blue Mountains National Park. In Chapter 7, I evaluate population dynamics, and temporal and spatial changes in social organisation and habitat use over that time. In Chapter 8, I describe the assessment of welfare status and explore drivers for the changes described in Chapter 7, demonstrating the cascading effects of resource availability on welfare status. I also investigate how welfare status is associated with population dynamics, social organisation, and habitat use. This is the first ever report of systematic and scientific assessment of the welfare of a population of wild free-roaming horses.

Finally, in Chapter 9, I summarise the overall conclusions from the work presented in Chapters 1 – 8, and the contributions that it has made to the fields of wild horse ecology and welfare, and the broader field of wild animal welfare, in advancing knowledge of (i) the practical assessment of the welfare of free-roaming wild animals, specifically of horses, and (ii) the welfare status of free-roaming wild horses across different habitats, the drivers of welfare status, and how welfare state correlates with population dynamics, habitat use and social organisation. I also highlight how the welfare assessment data can be simplified to an explicit practically achievable assessment for land managers at the population level, and finally, how this information can have direct input into management decisions and assist in addressing controversies.

During my candidature I also carried out substantial additional research including:

1) Continued assessment of the population of horses in the Kedumba Valley, Blue Mountains described in Chapters 5, 7 and 8, during and following a passive trapping and removal management program which arose as a result of my welfare assessments.
2) Further welfare assessments across larger populations of horses in different habitats throughout Kosciuszko National Park and the Victorian Alpine National Park.
3) Immunocontraceptive studies using a GnRH vaccine in captive wild horses.
4) Refinement of handling, anaesthetic, and surgical techniques for gelding wild stallions when they are brought into captivity, in order to optimise welfare outcomes during subsequent rehoming.
5) Development of frameworks for assessing the welfare impacts of different management methods
6) Development of a welfare assessment protocol and application of that protocol for assessing welfare impacts of a passive trapping and removal program in Kosciuszko National Park.

Due to time constraints, in addition to word count constraints for a PhD thesis, I have not included this additional research in my thesis but have briefly summarised these further and ongoing works in Chapter 9.

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Thesis outline summarizing chapter titles and contents		
Chapter	**Chapter title**	**Brief description**
1	Introduction: The wild horse controversy & the importance of incorporating animal welfare science in decision making.	Summary of the history of wild horses, their cultural significance, environmental impacts and controversies in wild horse management. Introduces the importance of animal welfare science in wild animal controversies.
2 Published	**A Ten-Stage Protocol for assessing the welfare of individual non-captive wild animals: free-roaming horses as an example.**	**Presentation of a novel conceptual framework that I designed in order to guide a systematic and scientific approach to assessing the welfare of free-roaming wild animals, using the Five Domains Model. Summarizes the principles of interpreting indicators of biological function and behaviour in terms of the mental experiences that those indicators reflect (Stage 1 of my Protocol), and how the Five Domains Model is used for assessing welfare (Stage 2 of my Protocol).**
3	Literature review: A review of the species-specific information required to enable assessment of wild horse welfare.	The current status of wild horse knowledge is summarized in a novel holistic and multidisciplinary framework drawing together the relevant literature on horses across each of the four physical/functional domains of the Five Domains Model. It addresses Stage 3 of my Ten-Stage Protocol.
4 Published	Addressing the knowledge gap of gastrointestinal parasitology in free-roaming wild horses in south-east Australia.	An example of the type of detailed original research required for addressing any knowledge gaps identified in Stage 3. This chapter describes a detailed parasitological investigation of 293 faecal samples collected from 6 wild horse populations. It describes results of faecal egg counts, larval cultures and molecular diagnostics.
5 Published	Use of remote camera traps to evaluate animal-based welfare indicators in individual free-roaming wild horses.	A large body of original research investigating for the first time, both the use of remote cameras for identifying individual horses across a range of habitats, and for acquiring data on an extensive range of animal-based welfare indicators. It addresses Stages 4-6 of my Ten-Stage Protocol.
6	Scientific validation of welfare indicators for using the Five Domains Model to grade the welfare status of individual free-roaming wild horses.	Addressing Stage 7 of my Ten-Stage Protocol, this chapter evaluates the scientific evidence linking the described objective measurable/observable welfare indicators to physical/functional impacts in Domains 1-4, and the relationship between those impacts and the mental experiences that are inferred in Domain 5. This concludes with the formulation of a Five Domains Model wild horse specific welfare grading scheme.
7	Dynamic changes in wild horse social organisation and habitat use revealed with remote camera trap monitoring.	With the aim of evaluating traditional ecological metrics alongside welfare status, this chapter describes original research using the remote camera trapping methodology described in chapter 5, to evaluate population dynamics, and temporal and spatial changes in social organization and habitat use of a wild horse population over a 15 month period.
8	The cascading influence of resource availability on the welfare status of wild horses, and association with population demographics, social organization and habitat use.	This original research applied the methodology from all preceding chapters to assess welfare status and changes in welfare status in individual wild horses over a 15 month period, addressing stages 8 -10 of my Ten-Stage Protocol. It further evaluates drivers of change in welfare status and correlations between welfare status, and the population dynamics, social organization and habitat use described in chapter 7.
9	Conclusions, applications and future directions.	Summarizes overall conclusions, and contributions to the fields of wild animal welfare, wild horse ecology and welfare, wild horse management, and general horse welfare. Highlights ongoing work, some of which I have already partially completed that was not included in the main body of the thesis.

Chapter 2. A ten-stage protocol for assessing the welfare of individual non-captive wild animals: Free-roaming horses (*Equus ferus caballus*) as an example

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Simple Summary

Vital for informing debates about the ways we interact with wild animals and their associated habitats is knowledge of their welfare status. To date, scientific assessments of the welfare of free-roaming wild animals during their normal day-to-day lives are not available, in part because the required methodology had not been developed. Accordingly, we have devised, and here describe, a ten-stage protocol for systematically and scientifically assessing the welfare of individual free-roaming animals, using wild horses as an example. Applying this ten-stage protocol will enable biologists to scientifically assess the welfare of wild animals and should lead to significant advances in the field of wild animal welfare.

Abstract

Knowledge of the welfare status of wild animals is vital for informing debates about the ways in which we interact with wild animals and their habitats. Currently, there is no published information about how to scientifically assess the welfare of free-roaming wild animals during their normal day-to-day lives. Using wild horses as an example, we describe a ten-stage protocol for systematically and scientifically assessing the welfare of individual free-roaming animals. The protocol starts by emphasising the importance of readers having an understanding of animal welfare in a conservation context and also of the Five Domains Model for assessing welfare. It goes on to detail what species-specific information is required to assess welfare, how to identify measurable and observable indicators of animals' physical states and how to identify which individuals are being assessed. Further, it addresses how to select appropriate methods for measuring/observing physical indicators of welfare, the scientific validation of these indicators and then the grading of animals' welfare states, along with assigning a confidence score. Finally, grading future welfare risks and how these can guide management decisions is discussed. Applying this ten-stage protocol will enable biologists to scientifically assess the welfare of wild animals and should lead to significant advances in the field of wild animal welfare.

Keywords: Five Domains; welfare assessment; wildlife; free-roaming, wild, feral, horses

1. Introduction

There is a growing awareness of how human activities, including wildlife population management and rehabilitation, land management and other conservation activities, may influence the welfare of free-roaming animals in the wild (Kirkwood et al. 1994;

Main et al. 2003; Beausoleil 2014; Ramp & Bekoff 2015; Dubois et al. 2017; Finn & Stephens 2017; Beausoleil et al. 2018; Fraser-Celin & Hovorka 2019; Hampton & Hyndman 2019). Conservation and wildlife management practices have traditionally focused on assessing animal populations, using metrics like abundance, density and diversity; and demographic parameters like sex ratios and age classes; and fitness metrics like survivorship and reproductive success. While valuable for some conservation purposes, such metrics provide little information about the welfare of individual animals within populations. However, survival does not necessarily imply good welfare since animals can survive despite persistently experiencing chronically unpleasant states (Main et al. 2003; Whay et al. 2003; Linklater et al. 2010; Walker et al. 2012a). Furthermore, the welfare of individual animals can influence the success of some conservation activities. For example, poor welfare may reduce fitness and reproductive success, and thus alter population trajectories. In addition, the public are increasingly aware of, and concerned about wild animal welfare (Temple et al. 2011; Beausoleil 2014). Therefore, having knowledge of the welfare status of individual wild animals may contribute information directly relevant to ethical, legal and political debates about the ways in which we interact with wild animals and their associated habitats (Finn 2019).

Methods for assessing welfare have been well developed for a range of captive animals (Temple et al. 2011; Samuel et al. 2012; Andreasen et al. 2014; Heath et al. 2014; Hockenhull & Whay 2014; Mullan et al. 2014; Dalla Costa et al. 2016; Blatchford 2017;

Czycholl et al. 2018), including for wild species (Hill & Broom 2009; Whitham & Wielebnowski 2009; Clegg et al. 2015; Kagan et al. 2015; Sherwen et al. 2018). Although a need to develop methodologies for assessing the welfare of free-roaming wildlife has been highlighted (Kirkwood et al. 1994), to date, such assessments have been largely restricted to impacts of non-lethal or lethal control of unwanted species, such as rodents, possums, rabbits, kangaroos, camels, badgers, and horses (Littin et al. 2004 2014; Baker et al. 2005 2016; Gray & Cameron 2010; Sharp & Saunders 2011; Beausoleil et al. 2012; Hampton et al. 2014; Beausoleil & Mellor 2015; Hampton et al. 2015a, 2015b; Sharp et al. 2015; Hampton et al. 2016a, 2016c, 2016d, Hampton & Forsyth 2016; Hampton et al. 2017; Allen et al. 2019; Hing et al. 2019). Whilst a recent study explored some aspects of welfare in the daily lives of free-roaming wild dogs (Fraser-Celin & Hovorka 2019), protocols for purposefully, systematically and scientifically assessing the welfare of free-roaming wild animals undertaking their normal daily activities, remain elusive. Therefore, little is known about what positive and negative welfare impacts they might be experiencing and why. Moreover, robust scientific methods for capturing reliable and informative data to enable assessment of free-roaming wild animal welfare have not been well described.

In light of the above observations, we describe a ten-stage protocol designed to guide wildlife biologists and others who wish to apply a systematic, scientifically based approach for assessing the welfare of individual free-roaming wild animals. We use the term 'free-roaming' to distinguish between wild species that roam freely in a wild habitat from those that are in captivity (e.g. in a zoo or sanctuary). We use the term 'wild' to mean animals that are of a wild species or those that are non-domesticated (feral). We have avoided the term 'feral' since this is associated with negative public perceptions, and whether an animal is truly wild or feral has no influence on the principles of how its welfare is assessed. This protocol, based on the 'Five Domains Model' (Mellor & Reid 1994; Mellor et al. 2009; Mellor & Beausoleil 2015; Mellor 2017), will ensure that such assessments are as scientifically objective, systematic, structured, transparent and comprehensive as possible. Applying the protocol will also enable researchers to present a clear understanding of the limitations imposed on their particular assessment by the circumstances in which data collection and interpretation necessarily occur when studying free-roaming animals. We also suggest methods that may be employed to capture robust data to support such welfare assessments.

We illustrate the application of the ten-stage protocol by using wild free-roaming horses as an example. This species was chosen since the study is part of a broader project regarding the practical assessment of the welfare of wild free-roaming horses and implications for their management.

2. The ten-stage protocol

1. Acquire an understanding of the principles of Conservation Welfare.
2. Acquire an understanding of how the Five Domains Model is used to assess welfare status.
3. Acquire species-specific knowledge relevant to each Domain of the Model.

4. Develop a comprehensive list of potential measurable/observable indicators in each physical domain, distinguishing between welfare status and welfare alerting indices.
5. Select a method or methods to reliably identify individual animals.
6. Select methods for measuring/observing the potential welfare indices and evaluate which indices can be practically measured/observed in the specific context of the study.
7. Apply the process of scientific validation for those indices that are able to be measured/observed, and insert validated welfare status indices into The Five Domains model.
8. Using the adjusted version of the Model that includes only the validated and practically measurable/observable welfare status indices, apply The Five Domains grading system for grading welfare compromise and enhancement within each Domain.
9. Assign a confidence score to reflect the degree of certainty about the data on which welfare status has been graded
10. Including only the practically measurable/observable welfare alerting indices, apply the suggested system for grading future welfare risk within each Domain.

2.1 Stage 1: Acquire an understanding of the principles of Conservation Welfare.

A new discipline of Conservation Welfare has recently been proposed to align traditional conservation approaches that historically focused on measures of 'fitness' (physical states), with more contemporary animal welfare science concepts which emphasise 'feelings' (mental experiences or affective states), that result from physical states. This enables a more holistic understanding of animals' welfare states (Beausoleil et al. 2018). A common language and understanding relating to wild animal welfare are important starting points, since the way in which welfare is conceived influences the way it is evaluated and the emphases put on its different features (Beausoleil et al. 2018). The reader is referred to Beausoleil et al. (2018) for a more detailed consideration of the value of seeking a shared welfare-related understanding between conservation scientists and animal welfare scientists under the heading of Conservation Welfare.

Animal welfare is characterised mainly in terms of an animal's mental experiences, in other words, how the animal may be experiencing its own life (Green & Mellor 2011; Stafford 2013; Mellor et al. 2015; Mellor 2016; Beausoleil et al. 2018). In animal welfare science, welfare is conceptualised as a property of individuals, belonging to species considered have the capacity for both pleasant (positive) and unpleasant (negative) mental experiences, a capacity known as sentience (Duncan 2006; Fraser 2008; Broom 2016a 2016b; Beausoleil et al. 2018; Ledger & Mellor 2018; Mellor 2019). Contemporary animal welfare science aims to interpret indicators of biological function and behaviour in terms of the mental experiences that those indicators are likely to reflect (Beausoleil et al. 2018). Mental experiences, or affective states, are subjective and cannot be measured directly, but indirect indices can be used to cautiously infer affective experiences (Mellor & Reid 1994; Mellor et al. 2009; Mellor & Beausoleil 2015; Beausoleil & Mellor 2017; Mellor 2017; Beausoleil et al. 2018).

Negative affective states

There is a growing body of neurophysiological and behavioural evidence in non-human animals regarding the basis of negative affective states such as breathlessness, thirst, hunger, pain, fear, nausea/sickness, dizziness and weakness, and there are also validated links between measurable indicators of physical/functional states and some of these mental experiences (McMillan 2003; Gregory 2004; Panksepp 2005; Murrell & Johnson 2006; Boissy et al. 2007; Fraser 2008; Denton et al. 2009; Beausoleil & Mellor 2015; Kenward et al. 2015; Mellor 2015; Beausoleil & Mellor 2017; Mellor 2019). For example, body condition is a measurable physical state that can be used as an indicator of hunger in some situations (Verbeek et al. 2011, 2012a, 2012b, 2014). Likewise, certain behaviors can be used as indices of pain. For example, in horses, the combination of rolling, gazing and/or kicking at the abdomen along with inappetence may be interpreted as reflecting abdominal pain (Ashley et al. 2005).

Some affective experiences are generated by the animal's brain processing sensory inputs that register specific features of the internal physical/functional state. For example, water deprivation causes dehydration which leads to osmoreceptorstimulated neural impulses passing to the brain generating the affective experience of thirst (Denton et al. 2009). Thirst elicits the behaviours of seeking water and drinking, in order to correct dehydration, after which the mental experience of thirst ceases.

Other affective experiences may arise from externally stimulated sensory inputs that contribute to the animals perception of its external circumstances. For example, threatening situations such as the presence of predators or humans, separation from conspecifics, or environmental hazards such as fire, are registered via cognitive processing of sensory inputs from visual, auditory and/or olfactory receptors giving rise to anxiety and fear (Gregory 2004; Panksepp 2005; Boissy et al. 2007; Denton et al. 2009; Mellor 2015; Beausoleil et al. 2018).

Whilst some negative experiences such as thirst and hunger motivate the animal to be behaviourally active in order to achieve resolution of the experience, others motivate the animal to reduce its activity. For example weakness, sickness and pain often induce inactivity and seeking to be isolated from other animals (Mellor & Beausoleil 2015). These and other types of behaviour are referred to as 'sickness' behaviours and may facilitate recovery from disease and injury thereby enhancing survival (Gregory 1998; Mellor et al. 2009). Experiencing negative emotions to some degree is therefore essential in order to motivate life-sustaining behaviours, but it is the incidence, intensity and duration of these experiences that are important in determining the overall impacts on an animal's welfare state. It is when negative experiences become extreme, prolonged, or unavoidable, that an animal experiences the most severe compromises to its welfare (Mellor et al. 2009; Beausoleil 2014; Mellor & Beausoleil 2015).

Positive affective states

Animals can also experience a range of positive affective states, and when experienced, these may enhance the animal's welfare state (Fraser & Duncan 1998; Spinka et al. 2001; Boissy et al. 2007; Yeates & Main 2008; Held & Spinka 2011; Mellor & Beausoleil 2015). Some positive mental experiences may occur as a result of behaviours that are directed at minimising negative affects (Mellor & Beausoleil 2015). For example, the smell, taste, textural and masticatory pleasures of eating a range of foods and the comfort of post-prandial satiety may occur with eating that is directed at relieving hunger (Deag 1996; Gregory 1998; Balcombe 2009; Mellor & Beausoleil 2015). Alternatively, other positive experiences may replace negative experiences when an animal is able to express more of its behavioural repertoire (Mellor et al. 2015; Mellor & Beausoleil 2015; Mellor 2017; Fraser & Duncan 1998; Spinka et al. 2001; Held & Spinka 2011). For example, foraging, affiliative social interactions, adolescent play behaviour, maternal behaviour and sexual activity are behaviours that infer positive mental experiences (Panksepp 2005; Balcombe 2009; Spinka & Wemelsfelder 2011; Mellor 2015; Mellor et al. 2015; Mellor & Beausoleil 2015). Despite living in stimulus-rich environments, expression of rewarding behaviours can be hindered in wild free-roaming animals. For example, in malnourished horses, more time and energy is spent searching for food. Hunger is also likely to dominate awareness and this, in turn, may reduce motivation to undertake rewarding behaviours (Mellor & Beausoleil 2015; Mellor 2017). Conversely, when food is plentiful, relief from the negative experience of intense hunger may re-motivate animals to utilise existing opportunities to engage in a range of rewarding behaviours (Mellor 2017). Therefore, it is important to consider indicators of positive, as well as negative welfare states in wild free-roaming animals and to understand particular features of their 'natural' circumstances may compromise or enhance theirl welfare (Yeates 2018).

2.2 Stage 2: Acquire an understanding of how the Five Domains Model is used to assess welfare status.

The Five Domains Model (Mellor & Reid 1994; Mellor et al. 2009; Mellor & Beausoleil 2015; Mellor 2017) is consistent with, and structurally represents, the understanding that physical and mental states are linked (Figure 1). It is a device that facilitates systematic and structured welfare assessment of individual sentient animals, based on current understanding of the functional bases of negative and positive subjective experiences that animals may have (Mellor & Reid 1994; Mellor et al. 2009; Mellor & Beausoleil 2015; Mellor 2017). Originally developed to assess welfare compromise in animals used in research, teaching and testing (Mellor & Reid 1994), it has since been broadened for use in companion animals, livestock, captive wild animals, and animals designated as 'pests' (Mellor et al. 2009; Portas 2013; Beausoleil & Mellor 2015; Kagan et al. 2015; Mellor et al. 2015; Mellor & Beausoleil 2015; Beausoleil et al. 2016; Littlewood & Mellor 2016; Mellor 2017; Clegg & Delfour 2018; McGreevy et al. 2018).

The Five Domains Model comprises four interacting physical/functional domains of welfare; 'nutrition', 'environment', 'health' and 'behaviour', and a fifth domain of mental state (affective/mental experience) (Figure 1). The physical/functional domains focus on internal physiological and pathophysiological states (Domains 1-3) and external physical, biotic and social conditions that may alter the animals' behavioural expressions (Domain 4) (Mellor et al. 2009; Mellor & Beausoleil 2015; Mellor 2017). Following measurement of animal-based indices within each physical domain, the anticipated negative or positive affective consequences are cautiously assigned to Domain 5. It is these experiences that contribute to descriptions of the animal's welfare state (Mellor et al. 2009; Mellor & Beausoleil 2015; Mellor 2017).

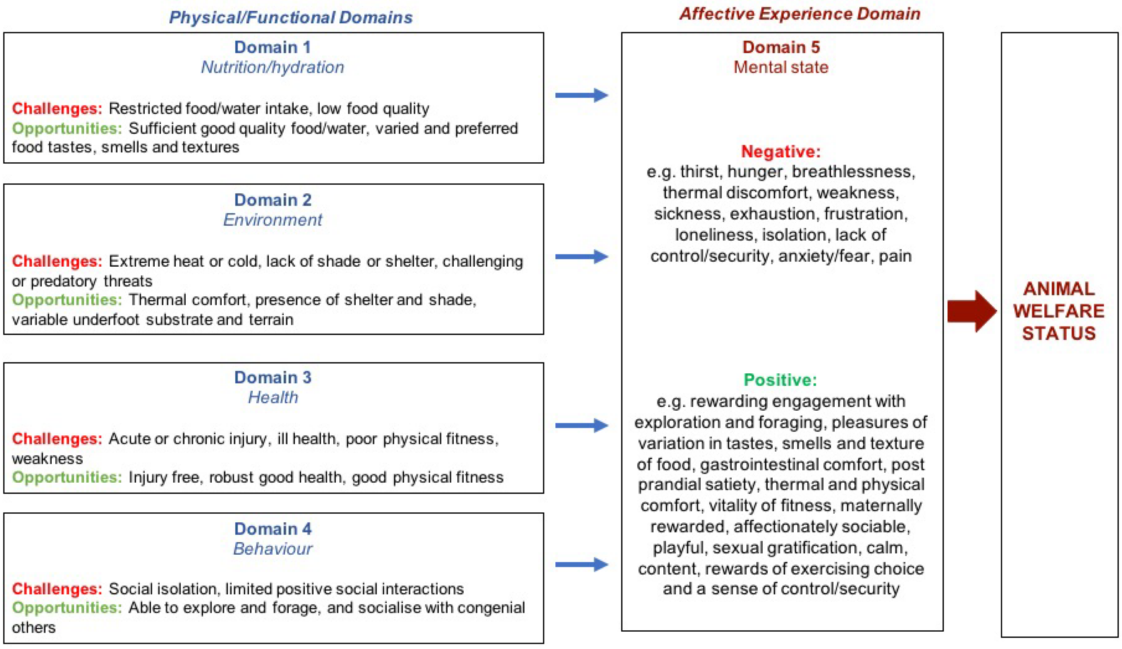


Figure 1. An abbreviated schema of the Five Domains Model (adapted from Littlewood & Mellor 2016), showing negative and positive physical/functional states or situations (Domains 1-4) and examples of their associated negative and positive mental experiences or affects (Domain 5), relevant to free-roaming wild horses. Taken together, these mental experiences represent the overall welfare state of the animal. A more detailed schema is available elsewhere (Mellor & Beausoleil 2015).

It is imperative that a sound understanding of the principles of Conservation Welfare (Stage 1) and the Five Domains Model (Stage 2) is gained prior to progressing to the next stages of the protocol.

2.3. Stage 3: Acquire species-specific knowledge relevant to each Domain of the Model.

In order to appropriately apply the Five Domains Model to assess animal welfare, detailed species-specific knowledge is required. Table 1 illustrates the species-specific information within each of the four physical/functional domains that is required to enable assessment of the welfare of free-roaming horses. Without a thorough understanding of what is normal for a species under optimal conditions, it is not possible to identify or interpret abnormalities. Acquiring species-specific knowledge will likely require extensive reading and advice from others having species-relevant practical experience, in addition to species-relevant nutritional, environmental, health and behavioural expertise. Accordingly, such holistic welfare assessments require multidisciplinary input (Mellor et al. 2009; Mellor & Beausoleil 2015; Mellor 2017).

All of the information required to make an informed assessment of the animal's welfare status may not be available for the wild species of interest. However, systematically undertaking Stage 3 will help to identify knowledge gaps and related limitations in welfare assessments, thus guiding further research.

Table 1. Illustration of the species-specific information required to assess welfare of free-roaming horses.

Domain	Species-specific information required
1: Nutrition	Water requirements: volume, frequency, preferred water sources, factors influencing water requirements, adaptations to and impacts of water restriction
	Nutritional requirements and preferences
	Common nutritional deficiencies and excesses and their causes, plant toxicities
	Assessing body condition, body condition scoring systems, optimal body condition score, factors affecting body condition
2: Environment	Habitat preferences, and factors affecting habitat selection and use
	Preferred underfoot substrate and terrain
	Thermoneutral zone, impacts of extreme climate events, signs of thermal stress
3: Health	Common non-infectious diseases and their clinical signs, risk factors, aetiology, diagnosis and prognosis
	Common infectious diseases and their clinical signs, epidemiology, mode of infection, characteristics of infectious agent (e.g., life cycle, survival in environment, involvement of other species)
	Common injuries and their clinical signs, risk factors, aetiology, diagnosis and prognosis
	Sickness and pain behaviours
4: Behaviour	Social organisation and factors affecting it
	Population dynamics
	Reproductive physiology and behaviours; oestrous, courtship, mating, gestation, parturition, lactation, maternal and newborn behaviour
	Normal range of behaviours and time budgets
	Social behaviour (including 'rewarding behaviours' e.g., play, allogrooming and other positive affiliative behaviours) and communication

2.4. Stage 4: Develop a comprehensive list of potential measurable/observable indicators in each physical domain, distinguishing between welfare status and welfare alerting indices.

Based on knowledge of the theory of animal welfare and its importance in a conservation context (Stage 1 and 2), and on species-specific knowledge (Stage 3), the next stage is to develop a list of potential indicators of various physical and thus affective states (both positive and negative) that the animals might experience. Measurable or observable indicators can be animal-based, such as body condition score and behaviour, or resource-based, such as forage quality and weather conditions (Table 2). Some indices (specifically animal-based indices) will be direct indicators of physical states, and therefore reflect aspects of welfare status. Others will be indicators of the risk of particular states occurring, or welfare alerting indices (all resource-based indicators and some animal-based indicators). Welfare alerting indices do not directly reflect the animal's current welfare state, but they can direct attention in future assessment towards specific animal-based indices (e.g. Figure 2). All assessments are made on individuals, but some resource-based indicators may apply to a number of individuals and therefore have group applications.

Search for previously described indices

Literature searches should be performed to develop a list of potential indices that may have already been described for use in welfare assessments of the species of interest, either in a free-roaming context or in a domesticated/captive context, and to evaluate their suitability. For example, various horse welfare assessments have been described and some of the indices used may be practical to apply to wild horses (Samuel et al. 2012; Dalla Costa et al. 2014b; Hockenhull & Whay 2014; Mullan et al. 2014; Dalla Costa et al. 2016; Czycholl et al. 2018; Somerville et al. 2018). Published information may also exist with regard to methods for measuring or observing some of these indices. For example, in horses there are well described protocols for assessing body condition score (Henneke et al. 1983; Carroll & Huntington 1988) and behavioural (Ashley et al. 2005) and facial (Gleerup et al. 2015) signs of pain have been described, with development of a horse grimace scale for assessing some types of pain (Dalla Costa et al. 2014a).

Table 2. Examples of animal-based and resource-based indices that may be measured or observed in free-roaming horses and which measures directly reflect mental experiences, i.e., welfare status, compared to welfare alerting indices that reflect welfare risk.

Domain	Animal-based indices		Resource-based indices
	Index	Status/Alerting	All alerting
1: Nutrition	Body condition score	Welfare status	Water availability/sources (e.g., number, accessibility, reliability, proximity to core home range)
	Reproductive status (e.g., mature lactating female)	Welfare alerting	Predominant vegetation type in home range Mineral analysis of vegetation Grass quality and length Competition for resources
2: Environment	Spatial and temporal use of habitat	Welfare alerting	Weather (e.g., temperature, humidity, direct sun exposure, wind, rainfall, extreme weather conditions such as snow, hail, fire)
	Shivering Profuse sweating	Welfare status	Habitat (e.g., presence of and nature of protection from wind/sun/rain (i.e., shelter and shade), terrain, substrate, ability to disperse to other habitats Anthropogenic activities (e.g., presence of roads, fencing, habitat destruction, use of habitat for other activities, noise
3. Health	Coat, skin, hoof condition Lameness Visible injuries, other visible physical abnormalities General demeanour, mobility, gait, posture Sickness behaviours Faecal quality	Welfare status	Environmental conditions that may predispose to certain health conditions (e.g., heavy rain, moist substrates) Hazards that may predispose to injury (e.g., fencing, roads, terrain) Presence and abundance of toxic plants
	Dentition of any skulls found (e.g., dental pathology and age at death)	Welfare alerting	
	Faecal egg counts, *Strongylus vulgaris* molecular diagnostics (PCR)	Welfare alerting	
4. Behaviour	Quantitative (e.g. time-budget behaviours, frequency/duration of positive affiliative interactions) and qualitative (e.g. alert, relaxed, weak) assessment of behaviours	Welfare status	Opportunities to express complete range of normal behaviours; affected by environment and conspecifics
	Population dynamics and social organisation	Welfare alerting	

Figure 2. Ingestion of plants such as Fireweed (*Senecio madagascariensis*), can cause pyrrolizidine alkalosis in horses resulting in chronic liver failure and eventual clinical signs of diarrhoea, weight loss, subcutaneous oedema, neurological disease and ultimately death (Clegg & Delfour 2018). Observing an abundance of these plants within a wild horse's habitat should act as a welfare alerting factor to prompt further monitoring of horses for presence of these clinical signs, and/or to consider this as a potential cause of any unexplained mortalities. Image A.M. Harvey.

Some animal-based indices provide welfare status information

Only animal-based indices can contribute information to the assessment of overall welfare status, since they provide the most direct evidence of what the animal may be experiencing (Mellor & Reid 1994; Mellor et al. 2009; Mellor & Beausoleil 2015; Hampton et al. 2016b; Mellor 2017). Animal-based indices may be externally observable, or internally measurable, as illustrated in Table 3. Externally observable indices can provide easily observable evidence of welfare compromises in each domain, and are the most practical indices to use in free-roaming animals (Figure 3, Table 3). Quantitative measures of behaviour, such as time budgets, have been most commonly applied by wildlife biologists (Linklater et al. 1999; Cameron et al. 2008; Ransom & Cade 2009; Ransom et al. 2010). However, since behaviour reflects a complex level of functioning, qualitative assessment can also inform assessment of the animals' affective state and whether positive or negative mental experiences are occurring (Wemelsfelder et al. 2001; Wemelsfelder 2007; Hintze et al. 2017; Minero et al. 2018) (Figure 4). To date, qualitative behavioural assessments do not appear to have been scientifically studied in free-roaming animals, and it is important that the context of the behaviour is considered carefully when making such assessments (Wemelsfelder et al. 2000).

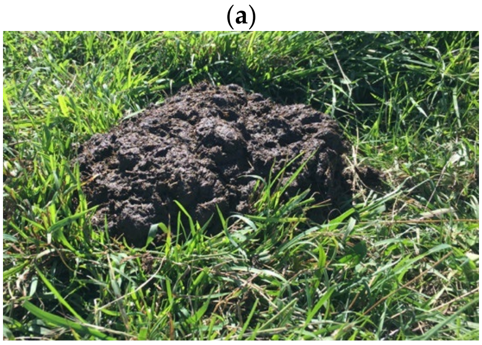
Internally measurable indices relate to physiological, pathological or clinical conditions (Table 3). These indices are not routinely used for day-to-day welfare assessments, and are problematic to measure in free-roaming wild animals. Some indices such as cortisol and reproductive hormones can be measured in faeces, which makes this more feasible for use in wild animals. However, interpretation of many of these indices is not straightforward. For example, while faecal (Keay et al. 2006; Linklater et al. 2010; Sherwen & Fanson 2015) and hair (Rakotoniaina et al. 2017; Kalliokoski et al. 2019) cortisol concentrations have been employed as a physiological index of stress (Keay et al. 2006; Linklater et al. 2010; Sherwen & Fanson 2015; Rakotoniaina et al. 2017; Kalliokoski et al. 2019), the significance of non-specific stress for an animal's mental experience is unclear (Barnard & Hurst 1996; Beausoleil et al. 2018). Cortisol and many other physiological parameters are non-specific and do not indicate if the experience was positive (e.g., excitement, arousal) or negative (e.g., pain, fear, hunger). Further, lack of elevated cortisol concentrations does not mean that the animal is not experiencing something unpleasant. Cortisol concentrations are also affected by many other variables (e.g., species, sex, reproductive status, circadian rhythms), further hindering interpretation (Beausoleil et al. 2018). Accordingly, an absence of detailed contextual information limits how informative cortisol measurements are in free-roaming animals.

Table 3. Examples of animal-based indices that may provide information about welfare status.

Externally observable indices	Internally measurable indices
Growth rates and achievement of developmental milestones in young animals	Measurement of heart rate and core body temperature
Reproductive success	Measurement of various blood parameters such as complete blood count and serum biochemistry
Body weight and/or body condition score	Measurement of cortisol and reproductive hormones in urine, faeces and hair
Presence of injuries, wounds, lameness, diarrhoea, nasal discharge, food pouching, quidding	Faecal egg counts
Coat condition and presence of skin lesions	
Social behaviours, sickness or pain behaviours	

(**a**)

(**b**)



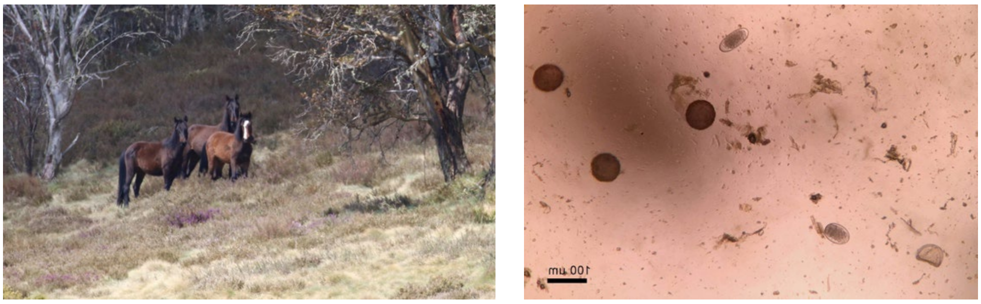
(**c**)

Figure 3. Examples of externally observable animal-based indices that provide information about welfare status **(a)** An emaciated mare with a body condition score of 1/9 (Somerville et al. 2018) with a stunted yearling; **(b)** A horse with a left hind lower limb injury; **(c)** Diarrhoeic faeces, indicative of gastrointestinal disease such as parasitism. Images A.M. Harvey

Figure 4. Examples of qualitative assessment of behaviour as an externally observable animal-based indicator of positive mental experiences associated with: **(a)** Play and **(b)** Affectionate sociability. Images A.M. Harvey

Some animal-based indices provide welfare alerting information

Animal-based indices traditionally collected by wildlife biologists (e.g., population dynamics, home range features and size, reproductive rates and survival rates), may not directly reflect the mental experiences of individuals, however, they may provide relevant contextual information. For example, low reproductive success, smaller herd sizes and/or larger home ranges, may reflect physiological states (e.g., chronic malnutrition) that would generate negative affective states of relevance to welfare (Klingel 1975; Henneke et al. 1983; Garrott & Taylor 1990; Linklater et al. 2004; Grange et al. 2009) (Figure 5a). Consequently, such indices may provide information about future welfare risks, and thus become important welfare alerting indices. Some other animal-based indices, such as faecal egg counts (FECs), may also only provide welfare alerting rather than welfare status information (Figure 5b, Table 2), because, when FEC is elevated, free-roaming horses frequently do not exhibit overt clinical signs of disease (Slivinska et al. 2006; Harvey et al. 2019). Hence, interpreted in isolation they do not necessarily indicate presence of intestinal pathology and any related negative experience.

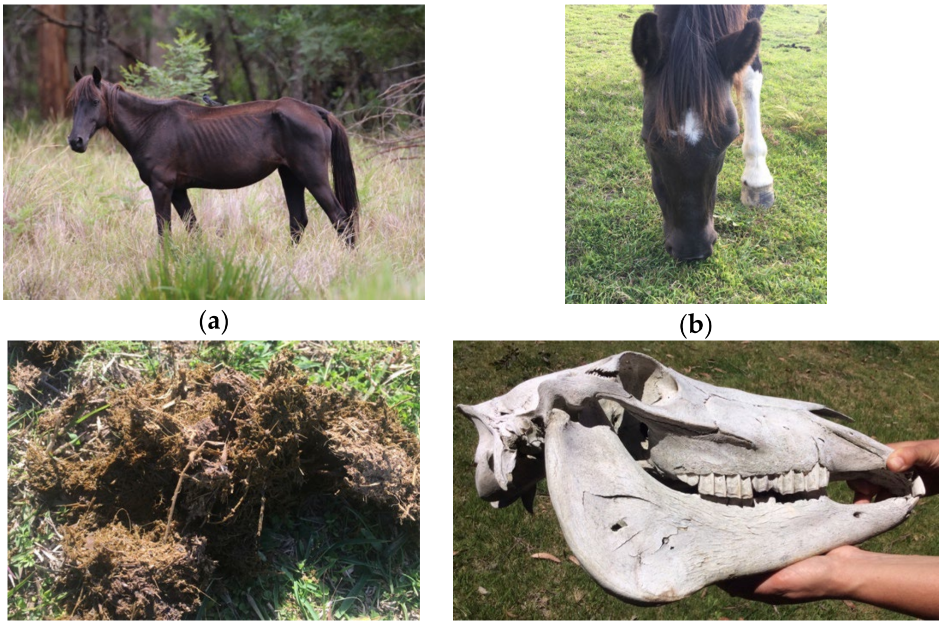


(**a**) (**b**)

Figure 5. (a) Low reproductive success, i.e., an absence of foals, particularly when combined with observing poor forage availability, may be an alerting indicator for chronic malnutrition, since poor nutrition in horses is known to have negative impacts on their fertility (Henneke et. al 1983); **(b)** Presence of parasite eggs in the faeces can be used as an indicator of the presence of certain gastrointestinal parasites (Harvey et al. 2019). However, faecal egg counts (FEC) give no indication of the severity of any associated pathology and cannot be used directly to make inferences about the animals' mental experience. FECs therefore are welfare alerting indices, with a high FEC raising awareness that gastrointestinal pathology and subsequent clinical signs (e.g., diarrhoea, abdominal pain) may be more likely to arise in the future. Images A.M. Harvey

Some animal-based indices can be interpreted in combination with resource-based indices

In some situations a combination of resource-based and animal-based indices may provide indirect relevant information about current welfare status and future risk. For example, dental disease can be an important cause of both morbidity (e.g., pain, malnutrition) and eventual mortality (malnutrition) in horses (Klugh 2010; Dixon et al. 2011), and several externally observable indices can be suggestive of clinically significant dental disease (Figure 6).



(**a**) (**b**) (c) (d)

Figure 6. (a) Dental disease may be suspected as one possible cause of low body condition. For example, if an individual horse is in poor body condition when feed is plentiful, con-specifics are in good body condition, and there is no obvious alternative reason for the individual to be in poor condition (e.g., not lactating or injured); **(b)** Quidding (dropping food from the mouth whilst chewing) and/or food pouching in the mouth lateral to the cheek teeth (as shown on the horse's right cheek in the photograph) are associated with pain from dental disease; **(c)** Long unchewed grass fibres in the faeces are suggestive of reduced chewing ability with dental disease (Klugh 2010; Dixon et al. 2011); **(d)** Information on the incidence of dental disease in a population as a whole (i.e., alerting information) may be provided by examination of the dentition of skulls found in the horses' habitat. Images A.M. Harvey.

2.5. Stage 5: Select a method or methods to reliably identify individual animals.

In order to assess animal welfare at an individual level, individuals need to be identifiable. Non-interventional identification methods may be suitable for some species. For example, in horses a combination of coat colour and natural markings may be used (Linklater et al. 2000a; Cameron et al. 2003; Scorolli & Cazorla 2010). Where such approaches are not possible, alternative methods may be required, such as marking with paints or dyes, or applying tags (Beausoleil et al. 2004). Factors such as distance from the animal during observations and visibility are important considerations in choice of identification method. Animal welfare impacts associated with capture/handling/restraint, application of any marks/tags, wearing of the mark, and impacts of observations should be assessed. The welfare impacts of different methods of marking have been previously reviewed, and should be considered along with other advantages and disadvantages of the marking method, before deciding upon those most appropriate for identification (Calvo & Furness 1992; Beausoleil et al. 2004; Hawkins 2004; Mellor et al. 2004; Casper 2009; Walker et al. 2012b).

2.6. Stage 6: Select methods for measuring/observing the potential welfare indices and evaluate which indices can be practically measured/observed in the specific context of the study

Having decided, based on species-specific knowledge (Stage 3), what resource-based and animal-based indices are important for assessing welfare in the species of concern (Stage 4), and how individual animals are going to be identified (Stage 5), the methods of practically measuring/observing the required indices then need to be considered.

Collecting information on the welfare of free-ranging individual animals is logistically challenging: their habitats may be difficult to access; the animals may be difficult to observe because of natural features such as vegetation and topography, in addition to fear of humans, and they may be unobservable for significant periods or at repeated intervals. In some situations it may also be challenging to locate the individuals that may be experiencing the worst welfare impacts, as they may hide, be less mobile, more distant from con-specifics and in habitats/terrain that make visualising them difficult.

Historically, data on free-roaming animals have been obtained using methods such as direct observations (e.g., herd size, behaviour, body condition score), trapping (e.g., sex, weight, size) and GPS collaring (e.g., home range, distance travelled) (Calvo & Furness 1992; Linklater et al. 1999 2000a; Cameron et al. 2003; Beausoleil et al. 2004; Hawkins 2004; Mellor et al. 2004; Cameron et al. 2008; Casper 2009; Ransom & Cade 2009; Hampson et al. 2010; Ransom et al. 2010; Scorolli & Cazorla 2010; Walker et al. 2012b). Although these methods can yield useful information, they themselves often have significant welfare implications (Calvo & Furness 1992; Beausoleil et al. 2004; Hawkins 2004; Mellor et al. 2004; Casper 2009; Walker et al. 2012b), provide a very narrow range of data, and there may be bias of the individuals sampled (e.g., direct observation is likely biased to those individuals within habitats where direct visualisation is possible). With more recent advances in technologies, it is now possible to obtain a wider range of information about free-roaming animals, and for longer periods of time, using techniques such as camera traps and drones (Silver et al. 2004; Karanth et al. 2010; Si et al. 2014; van Gemert et al. 2014; Ivošević et al. 2015; Vas et al. 2015) (Table 4). Advantages and limitations of each potential method need to be considered for the species and context of the research, and the highest yielding methods may vary. For example, for free-roaming horses residing on open grassland or desert habitat, direct observations or drones may be the most effective way to obtain animal-based data. In contrast, in a woodland habitat, where trees may interfere with direct visualisation of animals, camera traps may be more appropriate. Combined, these methods can provide complementary information (Figure 7).

Table 4. Summary of methods that may provide information relevant for the welfare assessment of free-roaming wild horses.

Method	Relevant information
Assessment of maps	Identification of cleared vs forested areas and size of different habitats, geographical limitations to dispersal and recruitment, steepness of terrain, location of roads, rivers/creeks
Geographical and meteorological data	Temperatures, rainfall, snow, wind, known environmental information, vegetation types, etc.
Ground surveys	Essential for verifying information from maps, identifying presence of water in creeks, access to water sources, type of vegetation and abundance of food, direct visualization of the species of interest, and other species sharing the same habitat, presence/distribution of faeces, identify good camera trap sites
Direct observations with or without photographs and/or videos	Best for evaluating behaviour and identifying herd composition but the variable distance between observer and horse can be more limiting in accurate body condition scoring, assessing hooves, skin lesions etc. Importantly many horses cannot be directly observed
Camera trap individual still images	Identifying individuals, sex, coat condition, skin lesions, hoof condition, body condition, limited behavioural information such as social interactions but not possible to identify sickness behaviours and gait abnormalities
Camera trap group still images	Herd compositions and sizes, foaling rate, approximate home ranges, mortality rate
Camera trap video images	Gait, demeanor, presence of lameness, weakness, occurrence of quidding/food pouching, play behaviour, positive affiliative interactions
Drones	Herd compositions, foaling rate, body condition, behaviour
Collection of faecal samples	Faecal consistency and colour, faecal egg counts, specific parasite molecular diagnostics, faecal cortisol and other hormone assays (Linklater et al. 2000b 2010; Keay et al. 2006; Sherwen & Fanson 2015)

In some situations, direct animal-based indices may be impractical, but there could be alternative indices that indirectly provide relevant information. For example, it is not practical to assess the dentition of free-roaming horses, but some indices can be observed that are indirectly suggestive of clinically significant dental disease (Figure 5).

(**a**)

(**b**)

Figure 7. Where possible, combining methods can provide the most comprehensive information. For example: **(a)** is a photograph taken from direct distance observation showing herd size, structure and social interactions. Direct observations, where possible, also provide a wealth of behavioural information, whereas **(b)** is a close-up camera trap image. This can enable easier measurement/observation of animal-based indices such as body condition score, coat condition, hoof condition, presence of injuries and presence of food pouching. Images AM Harvey.

Methods should be evaluated by undertaking pilot studies to identify which of the potential indices are practically feasible to measure/observe in the context of the study. Indices that are not practically able to be measured/observed with currently available methods should be archived. This enables them to be considered at a later stage when evaluating the limitations of the welfare assessments (Stage 9), and to be revisited when future technological advances may make them more feasible to measure or observe.

2.7. Stage 7: Apply the process of scientific validation for those indices that are able to be measured/observed, and insert validated welfare status indices into The Five Domains model

Once it has been established which indices can be practically measured/observed in the species and context of interest (Stage 6), these indices then need to be scientifically validated. Ideally, validation of welfare indices requires prior demonstration of the relationship between an observed indicator and the physical/functional impact (Domains 1-4), and of the relationship between the physical/functional impact (Domains 1-4) and the inferred mental experience (Domain 5). These steps of scientific validation have been described in detail elsewhere (Beausoleil & Mellor 2017). For example, detection of raised plasma osmolarity by osmoreceptors increases water-seeking and drinking behaviour, and drinking eliminates water-seeking behaviour (Denton et al. 2009), validating the link between the externally observable indicator of water-seeking behaviour/drinking, and the internally measurable indicator of dehydration, plasma osmolarity. Affective neuroscience provides evidence of the link between the physical state of dehydration (increased plasma osmolarity) and the mental experience of thirst, via neurohormonal pathways transmitting afferent inputs from osmoreceptors to higher brain centres associated with emotions (Denton et al. 2009).

Ideally, evidence of these relationships should relate to the species and context of interest, but where this is not available, evidence from the same species in a different context (e.g., in captivity), or a similar species, can be cautiously extrapolated. In many situations, the complete body of evidence to achieve such validation is not available and the level of confidence in the validation of indices should be indicated (Beausoleil & Mellor 2017).

Thus, this process will also highlight further knowledge gaps, and what further evidence may be required to strengthen the confidence between the suggested animal-based indices and inferred mental experiences.

In some cases, a direct animal-based indicator may not be practical to measure/observe in free-roaming animals, but there may be scientific evidence to support the use of an indirect indicator, which may be resource-based. For example, in free-roaming animals, water seeking or drinking behaviours can be difficult to observe. Therefore, thirst may be indirectly judged based on the resource-based indices of how available water sources are in relation to required frequency of drinking, based on the best available data for the species of interest. In the absence of direct measures, strength of motivation to drink could also be assessed by the distance the animal is willing to travel to reach a water source. Factors other than location of water sources would also need to be considered since impaired water access may occur for other reasons, such as illness or injury.

Indices that cannot be scientifically validated as indicators of the animals' mental experience (e.g., poor hoof condition in the absence of an abnormal gait), should be archived for consideration in future validation studies. Some of these archived indices may still provide valuable alerting information. All welfare alerting indices (Table 3) should be evaluated and graded separately from welfare status indices, as described in Stage 10.

2.8. Stage 8: Using the adjusted version of the Model that includes only the validated and practically measurable/observable welfare status indices, apply The Five Domains grading system for grading welfare compromise and enhancement within each Domain.

Once the indices that can be practically measured/observed (Stage 5), which are deemed to be sufficiently validated (Stage 7), have been inserted into The Five Domains Model, the next stage is to apply the grading system.

In order to standardise the assessment of animal welfare across different individuals and/or different assessors, and to monitor animal welfare over time, a reliable, repeatable and practical method of grading is required. Grading welfare compromise and welfare enhancement, and the operational details of the Five Domains Model have been previously described (Mellor & Beausoleil 2015; Beausoleil et al. 2016; Littlewood & Mellor 2016; Mellor 2017). It should be noted that such grading does not necessarily provide a comprehensive assessment of welfare status; rather it provides an assessment of those indices of welfare that can be assessed and interpreted in terms of the mental experience they are associated with, in the particular species and context of interest. In the case of free-roaming animals the range of welfare-relevant indices that can be assessed will usually be more limited than that for animals in captivity.

Grading the impact of mental experiences on welfare status involves a different approach depending on whether the experiences are negative (welfare compromise) or positive (welfare enhancement) (Mellor & Beausoleil 2015; Littlewood & Mellor 2016; Mellor 2017) (Table 5).

Table 5. A conceptual matrix of combined grading of welfare compromise and welfare enhancement (adapted from Mellor & Beausoleil 2015).

Welfare compromise grade	**Welfare enhancement grade**			
	None (0)	Low level (+)	Med level (++)	High level (+++)
A (None)	-	A/+	A/++	A/+++
B (Low)	B/0	B/+	B/++	-
C (Mild to moderate)	C/0	C/+	-	-
D (Marked to severe)	D/0	-	-	-
E (Very severe)	E/0	-	-	-

Grading welfare compromise (negative mental experiences)

The grading system applies a five-tier scale (A to E) to each of the Five Domains, representing increasingly severe impacts, ranging from none to very severe (Table 6) (Mellor & Beausoleil 2015; Littlewood & Mellor 2016; Mellor 2017). Information from the scientifically validated measurable/observable indices decided upon in Stage 7 is used to assign the grade of physical impact (A to E) in the first 4 domains. Knowledge of the association between those physical impacts and the associated mental experiences is used to infer the type of unpleasant experiences in Domain 5. The grades assigned in Domains 1-4 are used to infer the severity and duration of those experiences in Domain 5. The grade assigned in Domain 5 is usually the same as the highest of the grades in Domains 1-4, to reflect the most severe negative mental experience. This grade is the overall welfare compromise grade (Table 6).

Table 6. An example of grading welfare compromise in a horse with a lower limb injury resulting in severe lameness.[1]

Domain of potential welfare compromise					
1 Nutrition	**2 Environment**	**3 Health**	**4 Behaviour**	**5 Mental status**	**Overall welfare compromise grade**
C	B	D	C	D	D

[1] Based on the severity of the injury and associated debility, a D grade has been assigned in Domain 3 (Health). The lameness has been observed to moderately impact on behaviour (inability to keep up and interact with the rest of the herd), leading to C grade in Domain 4 (Behaviour). Observations of reduced ability to forage and graze and a body condition score of 3/9, led to a C grade in Domain 1 (Nutrition). The horse's environment is unchanged and the horse has easy access to shade and shelter, however the steep terrain is more challenging for the injured horse to negotiate, leading to a B grade in Domain 2 (Environment). The inferred mental experiences from these physical states include pain, hunger, and likely exhaustion, and possibly frustration and isolation. These are integrated to assign a grade in Domain 5 (Mental status). As the pain associated with the degree of lameness is considered to be severe, and of chronic duration, grade D has been assigned to Domain 5. This is the overall welfare compromise grade.

There may, however, be insufficient information to define impacts with the degree of precision implied by a five-tier scale, and in this case the grading matrix can also be adapted to a simpler three-tier scale to represent 'no to low', 'moderate', and 'severe' compromise (Mellor 2017) (Table 7).

Table 7. An example of a modified three-tier grading system for assessing physical impacts in free-roaming horses within Domain 1 and associated negative experiences in Domain 5.

Measurable/ observable indices	Compromise grade		
	No to low	Moderate	Severe
Access to water	Able to access water at least every 6-12 hours. May be up to 12 hours interruption in water supply in cool weather	Able to access water every 12-24 hours. May be up to 12 hours interruption in water supply in hot weather and up to 24 hours interruption in water supply in cool weather. Interruption to water supply may be due to distance to water, or difficulty accessing water due to reduced mobility or competition with conspecifics for limited water supply	Unable to access water within 48 hours in cool weather or 24 hours in hot weather. Water not available, water sources blocked/dried out in drought, or injury/illness preventing ability to access water
Domain 5 Negative affective experience inferred: Thirst	*No to very low-level thirst*	*Moderate thirst*	*Severe thirst*
Body condition score (BCS) and food availability	Optimal body condition (5-6/9) with good grass coverage within home range	Moderately thin (4/9) to thin (3/9) body condition with poor grass coverage within home range	Very thin (2-3/9) to emaciated body condition (1/9 or less) with very poor grass coverage within home range
Domain 5 Negative affective experience inferred: Hunger	*No to very low-level hunger*	*Moderate hunger*	*Severe hunger, weakness*

Grading welfare enhancement (positive mental experiences)

The described grading system applies a four-tier scale (0, +, ++, +++), representing 'no', 'low-level', 'medium-level', and 'high-level' enhancement (Mellor & Beausoleil 2015; Littlewood & Mellor 2016; Mellor 2017), but as above could also be simplified to a two- or three-tier scale when information relating to positive mental experiences is sparse.

Grading of welfare enhancement has three elements; i) the availability of *opportunities* for the animal to engage in self-motivated rewarding behaviours, ii) the animals actual *utilisation* of those opportunities, iii) making a cautious judgement of the degree of '*positive affective engagement*'. For example, in free-roaming horses, when grading positive mental experiences (Domain 5) associated with impacts in Domain 4 (behaviour), opportunities for horses to engage in free movement, exploration, foraging a range of vegetation of varying tastes and textures, to have affectionate social interactions with bonded conspecifics and engage in maternal, sexual or play behaviour, would be expected. However, for a variety of reasons, a horse may not be able to utilise these opportunities, and consequently will not exhibit behaviours that would provide evidence of positive mental experiences. This may occur where there is welfare compromise. For example, malnutrition, dehydration, hypothermia, injury and illness may all impair an animal's ability to engage in activities that may otherwise be pleasurable (McMillan 2003; Gregory 2004; Beausoleil & Mellor 2015; Mellor & Beausoleil 2015). The ability to engage in positive social interactions may also be impacted by aspects of social organisation and group composition (Sigurjónsdóttir & Haraldsson 2019) (Figure 8). Table 5 illustrates one way in which the interaction between compromise and enhancement has been conceptualised, i.e., severe compromise hinders enhancement.

(**a**)

(b)

Figure 8. The images of the two groups of horses in a) and b) were taken from a large population of horses, but due to the social organisation of herds, illustrate the difference in the ability of some horses to engage in affectionate social interactions, maternal, sexual and play behaviour much more than others: **(a)** These horses, 2 bachelor stallions, would be graded as '+' for welfare enhancement associated with opportunities in Domain 4, whereas **(b)** These horses, being in a large mixed age/sex herd with multiple foals, would be graded as '+++' for welfare enhancement associated with such opportunities in Domain 4. Images A.M. Harvey.

The use of numerical scores in the grading system is explicitly rejected in order to avoid scientifically unjustified aggregation of scores and to avoid implying a degree of precision that is not achievable when qualitatively assessing subjective affective states (Mellor & Reid 1994; Mellor et al. 2009; Mellor & Beausoleil 2015; Mellor 2017). Scientifically informed best judgement is an important aspect of grading with the Five Domains Model, and so the grading scheme should act as a guide only, but be utilised alongside informed interpretation (Mellor & Beausoleil 2015; Mellor 2017). Detailed examples of species and situational specific grading matrixes and application of this grading system can be found elsewhere (Mellor & Beausoleil 2015; Beausoleil et al. 2016; Littlewood & Mellor 2016; Mellor 2017).

2.9. Stage 9: Assign a confidence score to reflect the degree of certainty about the data on which welfare status has been graded.

When the grading system is applied to assess individual animal welfare (Stage 8), a confidence score should then be assigned to the overall welfare status grade, to reflect the degree of certainty about the data upon which the grade was based (Beausoleil et al. 2016). We recommend a three-tier scoring system where L = low confidence, M = moderate confidence, and H = high confidence.

The confidence score should reflect the knowledge gaps and limitations of the assessment, including gaps in species-specific knowledge (Stage 3), any challenges with individual animal identification (Stage 5), and the archived indices that could either not be practically measured/observed with currently available methods (Stages 6), or which could not be sufficiently validated (Stage 7). These are critical actions both for directing further research to improve future welfare assessments, and for informing the level of confidence with which individual welfare can currently be assessed in the species and context of interest.

In addition, a range of other factors should be considered including: whether all indices in the grading scheme could be measured/observed in the individual being assessed; the number of and/or duration of observations of the animal; whether indices were measured/observed from several methods combined or a single method; the implications if all methods could not be applied (e.g., still images only vs video recordings vs direct observations); and the distance of the assessor from the animal/image/video recordings when measurements/observations were made. The importance of some of these factors may also vary depending on the degree of welfare compromise. For example, if a welfare compromise status grade of E is assigned to a horse with a body condition score of 1/9, or a horse with a broken leg, the confidence in that score may be high despite the possibility that the grade was based on data from a single still image of the horse. In contrast, if a welfare status grade of A was assigned to a horse based on a single still image, the confidence in that score would likely be low.

2.10. Stage 10: Including only the practically measurable/observable welfare alerting indices, apply the suggested system for grading future welfare risk within each Domain.

From the comprehensive list of potential welfare alerting indices (Stage 4), select only those that can be measured/observed and interpreted (Stage 6). Some of these may be animal-based measures that were not able to be scientifically validated as indicators of mental experiences (Stage 7). Assessing such alerting indices separately from assessment of welfare status (Stage 8), can draw attention to risks of future welfare compromises and to what, if any, actions may be taken to mitigate these risks (e.g. Figure 9). This is particularly relevant to the situation of free-roaming animals, as unlike animals in captivity, immediate action based on a single welfare assessment or routine frequent monitoring of welfare may be impractical.

(**a**) (**b**)

Figure 9. These images illustrate the value in grading welfare alerting indices. Both of these mares have the same grading for physical impacts in Domain 1 based on the animal-based measure of a body condition score of 3/9. However alerting indices suggest that: **(a)** this mare has a low risk of further welfare compromise (and high likelihood of future improvement). This is because forage availability is good, it is the end of winter so forage quality and availability are likely to improve, and her yearling foal will soon be weaned, reducing nutritional demands on the mare. Accordingly, immediate intervention is not required, but body condition and forage availability should preferably be reassessed after another 6-12 weeks as intervention may be required if there was no improvement in body condition; **(b)** Conversely, this non-lactating mare has a high risk of further welfare compromise of increasing severity. This is because forage availability is poor and unlikely to improve because it is the end of spring, and the mare is already in poor body condition despite the absence of additional nutritional demands from nursing a foal. In this case, therefore, the recommendation may be for immediate intervention or closer monitoring with intervention if her body condition were to decrease below 3/9 within the following month. Images A.M. Harvey.

We therefore propose the use of an additional three-tiered scale for the overall grading of welfare alerting indices, representing 'no to low', 'moderate' and 'high' risk of further welfare compromise of increasing severity (Figure 9). Welfare alerting indices interpreted in combination with welfare status (Stage 8), should enable recommendations to be made relating to: i) whether any immediate intervention is required, or ii) whether further assessment or ongoing monitoring should be implemented, and what form that should take, and iii) the point at which intervention would be required to ameliorate increasing welfare compromise, where the risk of further compromise occurring is high.

3. Concluding remarks

The ten-stage protocol described here illustrates how the well-established Five Domains Model can be systematically applied to assess the welfare of individual free-roaming wild animals. This paper therefore forms a template for making such welfare assessments in free-roaming wild terrestrial species by applying the principles outlined here.

Applying the Model to such animals will help to identify previously unrecognised features of poor and good welfare by more precisely characterising scientifically validated negative and positive mental experiences, and their evaluation, as opposed to the commonly used imprecise and non-specific descriptors such as 'suffering' and 'stress' (Beausoleil et al. 2018). Utilising qualitative grading allows the monitoring of the welfare status of animals in different circumstances and at different times, thus providing scientifically informed and evidence-based guidance for decisions to intervene or not, in addition to enabling assessment of responses to any interventions that are implemented.

Nevertheless, it is important to recognise the limitations of the Model and its use in the assessment of wild animal welfare. Only specific indices and mental experiences that can be identified and interpreted can be assessed; there will be variable levels of confidence with which particular experiences may be inferred to be present in different circumstances, and differing precision with which each mental experience may be graded, as well an inability to determine relative impacts of those different experiences on welfare status (Mellor 2017). For some species, in some contexts, it may become evident that very few welfare indices can be assessed and interpreted, significantly hindering welfare assessments. However, this then highlights and identifies the knowledge gaps that need to be filled. As such, it provides a sound foundation for further research into the welfare of wild free-roaming animals.

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Thesis outline summarizing chapter titles and contents		
Chapter	**Chapter title**	**Brief description**
1	Introduction: The wild horse controversy & the importance of incorporating animal welfare science in decision making.	Summary of the history of wild horses, their cultural significance, environmental impacts and controversies in wild horse management. Introduces the importance of animal welfare science in wild animal controversies.
2 Published	A Ten-Stage Protocol for assessing the welfare of individual non-captive wild animals: free-roaming horses as an example.	Presentation of a novel conceptual framework that I designed in order to guide a systematic and scientific approach to assessing the welfare of free-roaming wild animals, using the Five Domains Model. Summarizes the principles of interpreting indicators of biological function and behaviour in terms of the mental experiences that those indicators reflect (Stage 1 of my Protocol), and how the Five Domains Model is used for assessing welfare (Stage 2 of my Protocol).
3	**Literature review: A review of the species-specific information required to enable assessment of wild horse welfare.**	**The current status of wild horse knowledge is summarized in a novel holistic and multidisciplinary framework drawing together the relevant literature on horses across each of the four physical/functional domains of the Five Domains Model. It addresses Stage 3 of my Ten-Stage Protocol.**
4 Published	Addressing the knowledge gap of gastrointestinal parasitology in free-roaming wild horses in south-east Australia.	An example of the type of detailed original research required for addressing any knowledge gaps identified in Stage 3. This chapter describes a detailed parasitological investigation of 293 faecal samples collected from 6 wild horse populations. It describes results of faecal egg counts, larval cultures and molecular diagnostics.
5 Published	Use of remote camera traps to evaluate animal-based welfare indicators in individual free-roaming wild horses.	A large body of original research investigating for the first time, both the use of remote cameras for identifying individual horses across a range of habitats, and for acquiring data on an extensive range of animal-based welfare indicators. It addresses Stages 4-6 of my Ten-Stage Protocol.
6	Scientific validation of welfare indicators for using the Five Domains Model to grade the welfare status of individual free-roaming wild horses.	Addressing Stage 7 of my Ten-Stage Protocol, this chapter evaluates the scientific evidence linking the described objective measurable/observable welfare indicators to physical/functional impacts in Domains 1-4, and the relationship between those impacts and the mental experiences that are inferred in Domain 5. This concludes with the formulation of a Five Domains Model wild horse specific welfare grading scheme.
7	Dynamic changes in wild horse social organisation and habitat use revealed with remote camera trap monitoring.	With the aim of evaluating traditional ecological metrics alongside welfare status, this chapter describes original research using the remote camera trapping methodology described in chapter 5, to evaluate population dynamics, and temporal and spatial changes in social organization and habitat use of a wild horse population over a 15 month period.
8	The cascading influence of resource availability on the welfare status of wild horses, and association with population demographics, social organization and habitat use.	This original research applied the methodology from all preceding chapters to assess welfare status and changes in welfare status in individual wild horses over a 15 month period, addressing stages 8 -10 of my Ten-Stage Protocol. It further evaluates drivers of change in welfare status and correlations between welfare status, and the population dynamics, social organization and habitat use described in chapter 7.
9	Conclusions, applications and future directions.	Summarizes overall conclusions, and contributions to the fields of wild animal welfare, wild horse ecology and welfare, wild horse management, and general horse welfare. Highlights ongoing work, some of which I have already partially completed that was not included in the main body of the thesis.

Chapter 3. Literature review: A review of the species-specific information required to enable assessment of wild horse welfare

Abstract

The Ten Stage Protocol for applying the Five Domains model to assess the welfare of individual non-captive wild animals (Harvey et al. 2020) emphasises the importance of first acquiring extensive species specific knowledge relevant to each of the four physical/functional Domains of the model (Stage 3 of the Protocol). The physical/functional Domains are the areas in which objective information is evaluated and collated. It is on the basis of this detailed knowledge that cautious interferences can then be made about the animals affective/mental experiences (in Domain 5).

This original review presented in a novel holistic framework provides a detailed resource of the multi-disciplinary knowledge of horses across all of the four physical/functional Domains (1. Nutrition; 2. Physical environment; 3.Health; 4. Behavioural interactions) of the Five Domains Model. A detailed understanding of what is normal for a species under optimal conditions is critical in being able to identify and interpret abnormalities and is the body of knowledge that underpins the assessment of welfare. Further, knowledge about conditions that may impact on welfare, and how these conditions may present or be identified, is also vital for interpreting welfare indicators.

The consolidation of the key information for horses in each physical/functional domain of the Five Domains model, provides the reference material required whenever assessment of equine welfare is being undertaken. This review is therefore an important resource for anyone wishing to assess the welfare of horses in free-roaming or domestic situations. The most likely welfare challenges to be encountered in wild horses, within each domain are also summarised.

1. Introduction

Chapter 2 detailed the Ten Stage Protocol for applying the Five Domains model to assess the welfare of individual non-captive wild animals, using free-roaming horses as an example (Harvey et al. 2020). Stage 3 of the Protocol emphasises the importance of acquiring species specific knowledge relevant to each of the four physical/functional Domains of the model, prior to considering which specific welfare indicators may be able to be assessed. The physical/functional Domains are the areas in which objective information is evaluated and collated. In Chapter 2 I highlighted what species-specific information was required to assess welfare of free-roaming horses (Table 1). These headings are the basis for this review.

A detailed understanding of what is normal for a species under optimal conditions is critical in being able to identify and interpret abnormalities and is the body of knowledge that underpins the assessment of welfare. Further, knowledge about conditions that may impact on welfare, and how these conditions may present or be identified, is also vital for interpreting welfare indicators. It is on the basis of this detailed information summarised in this review, that enables cautious interferences to be made about the affective/mental experiences (in Domain 5), which may be generated in or by the different internal states and external circumstances within each of domains 1 to 4.

Table 1. Illustration of the species-specific information required to assess welfare of free-roaming horses (from Harvey et al. 2020).

Domain	Species-specific information required
1: Nutrition	Water requirements: volume, frequency, preferred water sources, factors influencing water requirements, adaptations to and impacts of water restriction
	Nutritional requirements and preferences
	Common nutritional deficiencies and excesses and their causes, plant toxicities
	Assessing body condition, body condition scoring systems, optimal body condition score, factors affecting body condition
2: Physical environment	Habitat preferences, and factors affecting habitat selection and use
	Preferred underfoot substrate and terrain
	Thermoneutral zone, impacts of extreme climate events, signs of thermal stress
3: Health	Common non-infectious diseases and their clinical signs, risk factors, aetiologies, diagnosis and prognosis
	Common infectious diseases and their clinical signs, epidemiology, mode of infection, characteristics of infectious agent (e.g., life cycle, survival in environment, involvement of other species)
	Common injuries and their clinical signs, risk factors, aetiologies, diagnosis and prognosis
	Sickness and pain behaviours
4: Behavioural interactions	Social organisation and factors affecting it
	Population dynamics
	Reproductive physiology and behaviours; oestrous, courtship, mating, gestation, parturition, lactation, maternal and newborn behaviour
	Normal range of behaviours and time budgets
	Social behaviour (including ‘rewarding behaviours’ e.g., play) and communication

Whilst there is a vast literature on different aspects of equine nutrition, physiology, ecology, health and behaviour, there are no existing reviews that bring this multi-disciplinary information together in a holistic way as is required for assessing horse welfare. Consolidating the key species-specific information for each physical/functional domain of the Five Domains model, provides the reference material required whenever assessment of equine welfare is being undertaken. This review is therefore an important resource for anyone wishing to assess the welfare of horses in free-roaming or domestic situations.

Further, a review of this multi-disciplinary and holistic nature does not exist for any species. Thus, this review also serves as a guide for researchers of the welfare of other species, in demonstrating the breadth and depth of information required about a species in order to begin assessing welfare.

This extensive foundational understanding informs the completion of Stage 4 (i.e., Developing a comprehensive list of potential measurable/observable welfare status and alerting indicators in each physical domain), Stages 5 and 6 (i.e., Selecting methods for identifying individual animals, and for measuring/observing the potential welfare indicators), Stage 7 (i.e., Applying the process of scientific validation to selected welfare indicators and inserting validated indicators into the Five Domains Model), and design of the grading system for Stage 8 (i.e., Applying the Five Domains grading system) of the Ten Stage Protocol). Furthermore, systematically gathering this information enables identification of critical knowledge gaps. This highlights limitations in assessments, and this helps to inform Stage 9 (i.e., **a**ssigning a confidence score for the welfare grading). It may also help to direct further research (as illustrated in Chapter 4), enabling new information to be continuously added to the assessment framework as knowledge advances.

2. Sources of information

Given the broad multidisciplinary knowledge required for Stage 3, it warrants highlighting the breadth of sources of information initially used, which then helped to direct more specific literature searches on individual topics. They included a wide range of textbooks: Equine Welfare (McIlwraith & Rollin 2011), Wild Equids ecology, management and conservation (Ransom & Kaczensky 2016), Equine Applied & Clinical Nutrition (Goer et al. 2013), A Practical field guide to horse behaviour (McDonnell 2013), Equine Internal Medicine (Reed et al. 2017), Equine Medicine, Surgery, and Reproduction (Mair et al. 2013), Handbook of Equine Parasite Control 2nd Ed (Nielsen & Reinemeyer 2018), Australia's poisonous plants, fungi and cyanobacteria (McKenzie 2012), The Wild Horse Dilemma (Gruenberg 2015), Using Science to Improve the BLM Wild Horse and Burro Program (National Research Council 2013), and Care and Rehabilitation of the Equine Foot (Ramey 2011).

3. Domain 1: Nutrition

3.1 Water requirements

Daily maintenance water requirements for an adult non-lactating, non-pregnant horse at rest, and at thermoneutral temperature (see Domain 2) is approximately 50ml/kg/day, i.e., for a 300kg horse this would be 15 litres per day (Cymbaluk 2013).

Domestic horses are observed to drink peri-prandially approximately 80% of the time, or 4 times daily when fed ad lib (Sufit et al. 1985; McDonnell et al. 1999; Nyman & Dahlborn 2001). Normal drinking patterns for housed mature horses are episodic occurring 2-8 times per day (McDonnell et al. 1999). Most wild horse populations are reported to drink at least once per day (Pellegrini 1971; Feist & McCullough 1976; Scheibe et al. 1998), with free-ranging Przewalski's horses and some free-roaming horses in the US observed to drink twice daily (Ganskopp & Vavra 1986; Wang et al. 2012). In arid Australia, due to the large distance between good forage and water sources, drinking as little as once every 48 hours has been described (Berman 1991; Hampson et al. 2010a, 2010b) with some horses recorded to walk up to 55 km to watering points, with mean watering frequency every 2.67 days (range 1–4 days) (Hampson et al. 2010a, 2010b). Free-roaming Przewalski's drank less frequently post-release compared to in captivity when they drank about 5 times daily (Chen 2008; Zhang 2010), suggesting that drinking frequency is affected by availability (Schoenecker et al. 2006), and that water availability is not always optimal for free-roaming horses.

Factors influencing water requirements

The frequency of drinking and volume of water required varies with age, sex, weight, activity, reproductive status, health status, climate and diet (Cymbaluk 2013). Lactation is an important factor increasing water requirements. To offset lactational losses, water intake needs to be increased by 30-60% above maintenance. Lactating mares in the wild have been observed to drink more frequently and therefore tend to remain close to water sources (Kreuger 2008).

In wild horses, water requirements have also been shown to increase during dry conditions (Scheibe et al. 1998). In domestic horses high ambient temperatures alone (> 33°C) increase evaporative fluid loss by 4-5 times in resting horses (Morgan et al. 1997) and by 45-60% during exercise (McCutcheon et al. 1995; Jansson 1999). Part of the acclimatisation to hot ambient temperatures involves changes in water intake and does not confer resistance to dehydration (Cymbaluk & Christison 1990). In one study water intake increased from approximately 23 litres per day at thermoneutrality (20 °C, 45-50% relative humidity) to about 40 litres per day after short term exposure to a hot humid environment (33-35 °C, 80-85% relative humidity) (Geor et al. 1996).

Adaptations to and impacts of water restriction

Given horses' dependence on water, especially in warm seasons, water distribution strongly influences the distribution, space use and movement patterns of free-roaming horses (Schoenecker et al. 2006). When water sources are more abundant, horses spend less time moving (Figure 1). In regions where it snows during winter, horses can eat snow for hydration which potentially enables foraging further away from water compared to summer months (Mejdell et al. 2005; Kaczensky et al. 2010).

Water reserves in horses are more labile than energy reserves which means that horses tolerate water deprivation for much shorter periods than they can tolerate food deprivation. Water restricted horses spend less time eating with feeding activity gradually declining as water is restricted (Houpt et al. 2000). Reduced access to water is also one factor that has been shown to increase the risk of gastric ulceration in domestic horses (Luthersson et al. 2009). However, no evidence of gastric ulceration was found in any wild horses studied in different populations in outback Australia (Hampson et al. 2011b), which is consistent with other risk factors also being important.

Figure 1. Wild horses grazing on the edge of the Lower Snowy River, NSW, Australia. Image A.M. Harvey.

Dehydration greater than 15% can be fatal for a horse (Carlson 1979). On average this would occur after approximately 7 days of water deprivation depending on environmental conditions (Cymbaluk 2013).

3.2 Nutritional requirements and preferences

Horses are predominantly grazers and typically select graminoids over other species, with one study finding over 35 different grass species in the diet of free-roaming horse (Berman 1991). Forbs and shrubs are less frequently consumed, more commonly in winter, and usually only comprising a small proportion of the diet (Hansen 1976; Krysl et al. 1984; Crane et al. 1997). One potential reason for this is the high concentration of phenolics in forbs and shrubs, as equids do not have the capacity to detoxify plant phenolic compounds (Janis 1976; Duncan 1992).

Several studies report grasses making up at least 83% of the diet of free-roaming horses in all seasons (Salter & Hudson 1979a; McInnis & Vavra 1987; Smith et al. 1998), although in other populations only 56% of the diet comprised grass species with the remainder of the diet consisting of sedges, rushes and browse (Hubbard & Hansen 1976; Salter & Hudson 1979a) (Figure 2). Some authors report consistent diets in free-roaming horses throughout the year (Smith et al. 1998) whilst others report seasonal differences due to changes in forage availability (Salter & Hudson 1979a; Hanley & Hanley 1982, McInnis & Vavra 1987; Crane et al. 1997). Horses in the Australian desert were found to have up to 28% of their diet as dicotyledonous species and observations of horses eating the tops of shrubs and trees were common when grass growth ceased (Berman 1991).

Grazing studies have suggested that horses will select different qualities of forage when given opportunity, and the extent of the grazing resource and its diversity will affect the qualitative intake (Gordon 1989; Naujeck et al. 2005; Fleurance et al. 2009). When horses were offered a choice test, they appeared to select a higher energy diet (Cairns et al. 2002). Free-ranging Konik horses grazing natural vegetation selected about 50% of their diet based on the intake of digestible organic matter, a measure that is the product of bite size, bite rate, and bite digestibility from plants with the highest content of digestible organic matter (van Wieren 1996). So, when given the opportunity horses will select dietary components that will maximise their energy intake over time. Further studies have suggested that diet selection can be influenced by grass quality as horses remained grazing short patches of quality pasture rather than utilising longer swards of inferior grasses (Gordon 1989); others also report preferences for short grasses of lower bulk density rather than taller grass on perennial ryegrass swards (Naujeck et al. 2005). Data collected from stomach contents at post-mortem suggested that food resource quality cannot be determined only by the physical characteristics of the apparent forage (Hampson et al. 2011b).

(a) **(b)**

Figure 2. **(a)** Horses are predominantly grazers, however **(b)** shrubs and trees form a variable proportion of their diet. Images A.M. Harvey.

3.3 Common nutritional deficiencies and excesses and their causes

Lack of rainfall and overgrazing can result in significant dietary protein deficiency (Hampson et al. 2011b), which when prolonged may result in weight loss, abortion and reduced milk production in lactating mares (National Research Council 2007).

Starvation, when health is impaired because of inadequate nutrition (Kronfeld 1993), is discussed more under Domain 3.

In a post-mortem study of free-roaming horses in semi-arid Australia, dietary copper deficiency was present in all horses sampled at different sites, although no associated pathology was observed (Hampson et al. 2011b). Copper is essential for normal collagen biosynthesis and skeletal development (Kohnke et al. 1999; Floyd 2007). Foals born to mares with copper deficient diets have increased incidence of developmental orthopaedic disease (Kohnke et al. 1999; Douglas 2003), and in adult horses copper deficiency has been linked to osteoporosis, joint effusion and limb deformity, and secondary iron deficiency anaemia (Kohnke et al. 1999). Iron and copper deficiency have also been identified as potential causes of geophagia (Figure 3) and pica in horses (McGreevy et al. 2001; Aytekin et al. 2011).

Figure 3. Wild horses exhibiting geophagia. Image A.M. Harvey.

Secondary hyperparathyroidism ('big head disease') is recognised in horses arising from excessive phosphorus intake coupled with inadequate calcium intake, resulting in calcium mobilisation from bone (Coenen 2013). Whilst I am not aware of the condition being reported in free-roaming wild horses to date, it would be most likely associated with plant calcium oxalate toxicosis (see section 1.4 below). In the semi-arid Australia post-mortem study, evidence of wide variation in dietary phosphorus, magnesium and sodium, was found, with some levels being below recommended daily intakes (Hampson et al. 2011b). With phosphate deficient diets, phosphorus is mobilised from bone to maintain blood and tissues concentrations (Coenen 2013). Inadequate magnesium intake can be associated with poor growth, increased disease risk due to immune-incompetence, and muscle weakness (Coenen 2013). Most feeds of plant origin are low in sodium and inadequate sodium intake in horses is common, with signs being increased licking and searching for salty substances (Coenen 2013).

Dietary zinc concentrations were also very low in the Australian study (Hampson et al. 2011b) and low soil zinc concentrations are known to be prevalent in some regions (Coenen 2013). Zinc deficiency has been linked to developmental orthopaedic disease (Pagan 2003) and reduced hoof horn strength and hardness (Coenen 2013). High dietary ash content was also found, suggesting that the horses may have consumed soil (Hampson et al. 2011b), a common occurrence in horses grazing sandy soils (Granot et al. 2008) and at natural mineral licks (Salter & Hudson 1979a). Ingestion of soil or soil contaminated vegetation may also increase iron intake (Coenen 2013) and may explain both the high dietary iron and cobalt that was identified (Hampson et al. 2011b).

3.4 Plant toxicities

Plant toxicities most commonly occur in conjunction with undernutrition, since horses will most likely only ingest toxic plants when there is an inadequate alternative source of food. Diagnosis usually involves post-mortem, even then some are difficult to definitively diagnose. Presumptive diagnosis may be made when a horse presents with consistent clinical signs, in an environment with a high density of the toxic plant. There are a very wide range of plants that can cause toxicity in horses, and this can vary geographically and between different environments and habitats. Familiarisation with common and unique plants in the environments in question is therefore critical.

The most common toxicities are related to pyrrolizidine alkalosis (e.g., Patterson's curse (*Echium plantagineum*) and Fireweed (*Senecio madagascariensis*)) (Figure 4a), photosensitisation (e.g., St John's Wort (*Hypericum perforatum)*) (Figure 4b), calcium oxalate toxicosis (e.g., Purslane (*Portulaca oleracea)*, Lambsquarter (*Chenopodium album*), Kikuyu grass (*Pennisetum clandestinum*), Setaria grass (*Setaroa sphacelate*)), selenium toxicosis (accumulated in a range of plants (e.g., *Astragalus, Stanleya, Machaeranthera, Oonopsis*, and *Xylorhiza*) when growth is in alkaline soils with low rainfall), and thiaminase toxicosis (e.g., Bracken fern (*Pteridium esculentum*)). Pyrrolizidine alkalosis causes cumulative toxicity eventually resulting in chronic liver failure with diarrhoea, weight loss, subcutaneous oedema, neurological signs and ultimately death. Photosensitisation results in skin lesions, typically on sensitive areas or unpigmented skin. Selenium toxicosis results in hair loss and severe hoof pathology leading to lameness. Thiaminase toxicosis results in neurological signs and ultimately death. Calcium oxalate toxicosis results in nutritional secondary hyperparathyroidism with a relative calcium deficiency and subsequent fibrous osteodystrophy and osteoporosis, frequently presenting as 'big head' (McKenzie 2012). Plant toxicity is also therefore relevant to Domains 3 in terms of associated health problems and potential sickness behaviours.

(a) **(b)**

Figure 4. **(a)** Patterson's curse (*Echium plantagineum)* is a cause of pyrrolizidine alkalosis; **(b)** St John's Wort (*Hypericum perforatum)* is a cause of photosensitisation. Images A.M. Harvey.

Some disorders secondary to plant toxicities specifically reported in Australia include 'Australian Stringhalt', a peripheral nerve disorder, that has been associated with ingestion of flatweed (*Hypochaeris radicata)* (Huntington et al. 1989), and neurological disease associated with ingestion of *Indigofera enneaphylla* in subtropical and arid regions of Australia (Bell & Everist 1951). The study of free-roaming horses in central Australia also identified high concentrations of cobalt in the stomach of horses in five different environments, with one hypothesis being consumption of native plants that accumulate cobalt (Hampson et al. 2011b). Identified in central Australia, is a small shrub violet (*Hybanthus floribundus*) that accumulates high concentrations of nickel and cobalt (Severne & Brooks 1972), and different species of this shrub have also been identified more widely in Australia (Severne 1974). The requirements and toxicity of cobalt have not been studied in horses, but these findings illustrate the importance of having local knowledge of vegetation and an awareness of knowledge gaps.

3.5 Body condition scoring

Body condition scoring (BCS) is a standardized semi-quantitative method to visually evaluate the amount of fat and muscle on a horse's body; it takes into account the deposition of body fat in different areas by examination of the neck, back, ribs, pelvis and rump (Carrol & Huntington 1988). Scoring systems have been well described using either a 9-point scale, where 1 is emaciated and 9 is extremely fat (Henneke et al. 1983), as illustrated in Table 2, or a 6-point scale where 0 is emaciated and 5 is very fat (Carroll and Huntingdon 1988), as illustrated in Figure 5. Body condition scoring has been widely used in horses (e.g. Henneke et al. 1984; Carrol & Huntington 1988; Huntington 1988; Pritchard et al. 2005; Burn et al. 2009, 2010; Dawson & Hone 2012; Samuel et al. 2012, Hockenhull 2014; Mullan et al. 2014; Czycholl et al. 2018; Somerville et al. 2018) and, in experienced hands, is a consistent and reliable method of estimating body condition (Gentry et al. 2004; Cameron et al. 2008; Dugdale et al. 2012; Jensen et al. 2016).

Table 2. The Henneke 9-point body condition scoring (BCS) scale (adapted from Henneke et al. 1983, images A.M. Harvey).

BCS	Description	Example photo
1 (Poor)	Extremely emaciated. Spinous processes, ribs, tailhead, tuber coxae and ischii projecting prominently. Bone structure of withers, shoulders and neck easily visible. No fatty tissue.	
2 (Very thin)	Emaciated. Slight tissue covering over base of spinous processes. Spinous processes, ribs, tailhead, tuber coxae and ischii prominent. Withers, shoulders and neck structures visible.	
3 (Thin)	Slight fat coverage of spinous processes and ribs but still visible. Tailhead prominent, but individual vertebrae cannot be visually identified. Tuber coxae rounded but easily discernible. Withers, shoulders and neck structures visible but with some tissue coverage.	

4 (Moderately thin)	Ridge of spine and outline of ribs just visible, tailhead not prominent. Tuber coxae not discernible. Withers, shoulders and neck not obviously thin.	
5 (Moderate)	Spine and ribs cannot be seen but ribs can be felt. Fat around tailhead. Withers rounded over spinous processes. Shoulders and neck rounded and blend smoothly into body.	
6 (Moderately fleshy)	Slight crease down spine. Fat over ribs and tailhead. Fat deposits along sides of withers, behind shoulders and along the sides of the neck.	

7 (Fleshy)	Crease down spin. Fat filling between ribs and around tailhead. Fat deposited along withers, behind shoulders and along the neck.	
8 (Fat)	Crease down spine. Difficult to feel ribs. Fat around tailhead. Area along withers filled with fat. Fat deposits along withers and behind shoulders. Noticeable thickening of neck. Fat deposited along inner thighs.	
9 Extremely fat	Obvious crease down spine. Patchy fat appearing over ribs. Bulging fat around tailhead, along withers, behind shoulders and along neck. Fat fills in flank and is distributed along inner thighs.	

Optimal body condition score

On the 9-point scale, optimal body condition is 5-6, with BCS ≤ 3 being underweight (Carter & Dugdale 2013). Mares with body condition of less than 5 have lower reproductive performance, with delayed onset of oestrous and ovulation, reduced conception rates, higher numbers of cycles to conception, and reduced maintenance of pregnancies (Henneke et al. 1984). Mares of BCS 3-3.5 also had extended seasonal anoestrous compared to mares of BCS 7.5-8.5 (Gentry et al. 2002). Similarly, stallions also have the best reproductive success at body condition scores of 5-6.

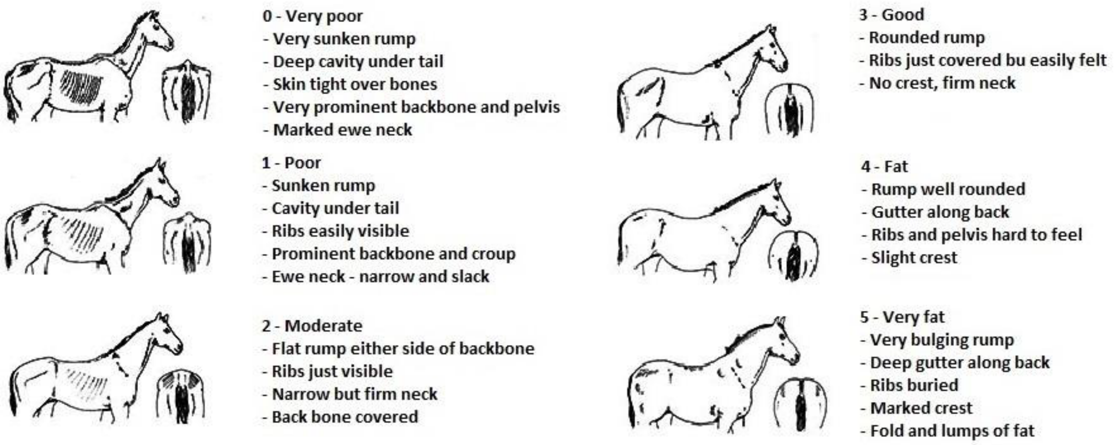

Figure 5. 6 point body condition scoring system based on visual body fat distribution. Source: Carroll & Huntington (1988), reproduced in a variety of sources.

In addition to influencing conception and birth success, body condition and resource availability may also influence the sex of offspring, with mares in poor condition at the time of conception producing more female foals (Cameron 1999), with a ratio of 0.47 male foals to 1 female observed in a resource stressed population of wild horses in another study (Stanley & Shultz 2012).

Maternal body condition has also been shown to influence the amount of time mothers spent vigilant, with mares in good condition spending more time vigilant than mares in poor condition (Watts et al. 2020). Mares in good condition can afford to spend more time alert at the expense of feeding time, whereas mares in poor condition must spend more time foraging (Lima & Dill 1990), impacting on ability to invest in the protection of their offspring.

Impacts of low body condition scores on health and reproduction are discussed in Domain 3 and 4.

Factors affecting body condition

Increased energy demands will negatively impact body condition if they are not met by adequate increases in food intake. Common causes of increased energy demand include lactation, with mares commonly having lower BCS than stallions (Grange et al. 2009; Hampson et al. 2011b; Dawson & Hone 2012), increased activity which may occur with stallions during the breeding season, horses that must travel long distances between food and water sources (Hampson et al. 2011b), and cold weather (Cymbaluk 1990). Disease may also interfere with digestion or increase nutrient utilization, such as in the case of endoparasitism, which has been associated with low body condition scores in one wild horse study (Debaffe et al. 2016), but not in another study (Cain et al. 2018).

Reduced food intake may occur with reduced food availability, related to low rainfall (Hampson et al. 2011b) or populations reaching carrying capacity (Scorolli 2021). In individual horses, injury or disease may also reduce foraging activity.

Forage quality may also impact body condition. In a study in semi-arid Australia all horses were judged to be moderately thin (mean BCS 3.9) across 5 populations (Hampson et al. 2011b). The dietary protein concentration in stomach contents of these horses varied from 4.3% to 14.9% and was found to be significantly correlated with body condition score, with thinner horses having lower protein concentrations in stomach contents. Conversely, concentrations of water soluble and ethanol soluble carbohydrates in horse stomach contents were well above those expected even for high quality forage. Combined this suggests that the diet of these horses was suboptimal (Hampson et al. 2011b).

Foraging behaviour is also relevant to this domain and is discussed under Domain 4. Habitat and weather are intrinsically related to forage availability, as discussed under Domain 2. Health problems may affect body condition, and conversely mal/under-nutrition can cause health problems, and these are discussed under Domain 3.

3.6 Most likely welfare challenges in Domain 1

For wild horses, the most likely welfare challenges within Domain 1 are mal/under-nutrition and dehydration, depending on the specific geographical location and environmental conditions. In unmanaged populations without horse predators, the predominant factor limiting population growth would be food availability and so it can be expected that at least some of the population will be undernourished.

4. Domain 2: Physical Environment

4.1 Habitat preferences, and factors affecting habitat use

Habitat selection and use is primarily influenced by forage availability and quality (King & Gurnell 2005; Henley et al. 2007), with distance to water also being important (Kaczensky et al. 2008). The primary determinant of habitat use in free-roaming horses in all seasons has been shown to be availability of preferred forage (Salter 1978; Salter

& Hudson 1979; Berger 1986; Crane et al. 1997; Girard et al. 2013). Intermittent drought and changes in rainfall can create a great deal of seasonal variation in quantity and quality of available forage which may impact habitat use.

In New Zealand changes in habitat preference coincide with the beginning of foaling and mating in spring and formation of frost inversion layers in winter (Linklater et al. 2000), with free-roaming horses occupying lower altitudes on gentler slopes, river basins and stream valley floors in spring and summer, compared to steeper slopes at higher altitudes in winter (Linklater et al. 2000). Despite the ability of horses to obtain forage beneath the snow, the presence of snow limits habitat use (Salter & Hudson 1979). Horses in the Australian Alps are limited in distribution in winter by deep snow at higher elevations (Walter 2002).

Home range is highly dependent on resource availability and varies considerably within and among populations (McCort 1984), with reported home range sizes varying from 6 – 303 km^2 (Girard et al. 2013, Salter & Hudson 1982, Berger 1986, Miller 1983). Bands generally have high fidelity to home ranges (Berger 1986). Band sizes have been correlated to home range size indicating that it is likely related to resource demand (Linklater et al. 2000).

Horses travel between feeding and resting spots to seek dry ground to lie down or resting places that provide refuge relief from insects (Zervanos & Keiper 1979; Keiper et al. 1980; Rutberg 1987; Powell et al. 2006; King & Gurnell 2010). They also travel between feed and water sources, sometimes for long distances (Hampson et al. 2010a).

In one study in Central Australia and Queensland, the average distance travelled was 15.9 ± 1.9 km/day (range 8.1–28.3 km/day). Central Australian horses watered less frequently and walked for long distances in direct lines to patchy food sources, whereas central Queensland horses were able to graze close to water sources and moved in a more-or-less circular pattern around the central water source (Hampson et al. 2010a).

4.2 Preferred underfoot substrate and terrain

As described above, habitat selection appears to be primarily driven by resource availability rather than underfoot substrate and terrain. Free-roaming horses reside in both flat and steep mountainous terrains, and a range of surfaces from rocky to wetlands. The effects of the environment on the foot of free-roaming horses have been well studied in Australia (Hampson 2011), with the hardness of underfoot substrate and travel distance affecting foot morphology (Hampson et al. 2013a) (discussed more in Domain 3).

4.3 Thermoneutral zone

Wild horses exist across many different environments, and are very adaptable to temperature variation, thriving in climates varying from tropical to subarctic (Cymbaluk 1994). In Australia alone their habitats vary from semi-arid Central Australia, to the snow-covered mountains of the Australian Alps (Berman 1991; Dawson 2005; Hampson et al. 2011b; Watts 2019). The potential environmental challenges encountered by horses will thus vary greatly between these different habitats.

The thermoneutral zone (TNZ) is defined as the range of effective ambient temperature where internal thermostability is maintained without changes in metabolic heat production (Cymbaluk & Christison 1990; Cymbaluk 1994). It comprises three zones: cool, optimal and warm (Cymbaluk & Christison 1990). The lower end of the TNZ is known as the lower critical temperature (LCT) and is the ambient temperature below which metabolic heat production increases, and thus without extra feed, feed energy is diverted to heat production rather than tissue growth. The upper end of the TNZ is known as the upper critical temperature (UCT) at which evaporative heat loss increases, and thus sweating starts, to reduce core body temperature (Cymbaluk & Christison 1990).

The thermoneutral zone for horses has been defined as 5 – 25 °C (Morgan 1998), however horses can adapt to different climates and modify their thermoneutral zone (Cymbaluk & Christison 1990). For example, acclimatised adult horses have been documented to have a LCT as low as – 15 °C (Cymbaluk 1994). The LCT also varies with seasons, breed, age, body condition and intake of dietary digestible energy (Cymbaluk 1994). For example, the LCT for newborn foals is much higher, reported to be around 14 - 22 °C, reducing gradually during the first week after birth (Cymbaluk & Christison 1990; Cymbaluk 1994) (Figure 6).

4.4 Thermoregulation and factors affecting it

Thermoregulation in cold weather is achieved through insulation, and changes in behaviour, heart, respiratory and metabolic rates, digestibility and hormones (Cymbaluk 1994). Shelter seeking and huddling occur with acute cold. Foraging and movement also decreases and may conserve 17% of daily energy expenditure (Cymbaluk & Christison 1990).

(a) (b)

Figure 6. (a) Horses can adapt to different climates and modify their thermoneutral zone. **(b)** New born foals have much higher lower critical temperatures.

Other factors such as body condition, food intake, having a wet coat, access to shade/shelter and access to water will also influence how the animal may tolerate thermal extremes. Microclimatic variables are ambient temperature, relative humidity, precipitation, wind velocity and solar radiation, with ambient temperature being the most important (Cymbaluk & Christison 1990). Being wet increases evaporative heat loss directly by reducing thermal insulation of the hair coat and by increasing convective losses (Cymbaluk & Christison 1990). Snowfall is less cooling than rain since the hair coat then retains a significant capacity to insulate against heat loss (Cymbaluk & Christison 1990).

At LCT's horses rely on insulation and food intake (Cymbaluk & Christison 1990) to maintain body temperature. Insulation is derived from muscle, fat, skin and hair with subcutaneous fat being particularly important. Thus, thermoregulatory ability is negatively impacted if horses are in poor body condition and/or if food availability is restricted (Cymbaluk & Christison 1990). For example, acclimatised ad lib fed yearlings had a LCT of -11 °C (Cymbaluk 1994) but those fed for normal growth had a LCT of 0 °C (Cymbaluk 1994).

As temperature approaches UCT, when able to, horses will seek shade where radiant heat loads are lower, limit their movement to reduce internal heat production, and may enter water holes or rivers where immersion in water has direct cooling effects and increases evaporative heat loss when they exit the water. The circulatory system and sweat glands act as major thermoregulatory mechanisms. Peripheral vasodilation increases blood flow to the skin enabling convective heat loss and sweating leads to evaporative heat loss (Guthrie & Lund 1998). Sweating rate will continue proportionate to increases in core body temperature, but at very high temperatures evaporation may not keep pace with the rate of sweating, making evaporative heat loss less effective, and also leading to large fluid losses. However, this is usually only an issue during protracted moderate-high intensity exercise (Guthrie & Lund 1998).

Impacts of ambient cold

Cold and/or wet weather increases energy requirements and can be associated with weight loss. Metabolic rates increase by 70% above resting levels with severe cold exposure (Cymbaluk 1994), which results in weight loss, or reduced weight gain in growing animals, if energy intakes are restricted. Young horses kept at -5 °C had 29% lower average daily weight gain than those kept at 10° C (Cymbaluk & Christison 1990). Energy intake therefore needs to be increased during cold exposure to avoid weight loss in mature horses, and reductions in weight gain in growing young. At temperatures below LCT, maintenance energy intakes should be increased by 2.5% per °C decrease in effective ambient temperature for adult horses, and by 1.3% for growing horses (Cymbaluk 1994).

Horses in poor body condition need significant additional feed to gain weight in colder temperatures. For example, at -10 °C a 500kg horse needs 16.4 MCal/day digestible energy (equates to approx. 9kg of hay) to maintain body weight, whereas a thin horse with a body condition score < 4 (9 point scale) needs 26.4 MCal/day digestible energy (14kg + hay) to gain 0.5 kg in body weight (Cymbaluk & Christison 1990). Acute cold exposure also alters some serum biochemical parameters reflective of an enhanced mobilisation of fat and protein to fuel cold thermogenesis (Cymbaluk & Christison 1990).

Impacts of ambient heat

With protracted intense exercise during hot and/or humid conditions, thermoregulatory failure may occur. Once core body temperature reaches 42.5°C without effective cooling, hyperthermia-induced pathological changes develop (Guthrie & Lund 1998). Severe fluid and electrolyte losses occur, and horses become fatigued, tachypnoeic and tachycardic. Fluid and sodium loss associated with excessive sweating results in hypotonic dehydration and thus affected horses can lose the desire to drink. Potentially fatal metabolic disturbances can follow, including exertional rhabdomyolysis, synchronous diaphragmatic flutter, gastrointestinal stasis, colic and renal failure. This can progress to ataxia, collapse, convulsions, coma and death (Guthrie & Lund 1998).

Impacts of extreme climatic events

The effects of catastrophic environmental factors on mortality are described under 'population dynamics' in Domain 4, but climatic events such as drought, storms, heavy snow, extreme cold, thunderstorms, lightning strikes and fire have all been reported to cause mass mortalities in wild horse populations (Berger 1983; Garrot & Taylor 1990; Berman 1991; Scorolli et al. 2006; Kaczensky et al. 2011).

4.5 Most likely welfare challenges in Domain 2

In temperate climates, significant environmental challenges may not be common. However, in colder climates, cold exposure, particularly when combined with wet conditions, undernutrition, and in young animals, it is likely to present significant threats. In more arid climates, deleterious effects of heat exposure are likely to be more common, particularly when access to water is limited. This illustrates the interaction between Domains 1 and 2. Other extreme climatic events may cause infrequent but severe welfare challenges.

5. Domain 3: Health

It is beyond the remit of this review to discuss health problems of horses in any depth, so the aims here are to: (i) briefly summarise the predominant health problems reported in the literature; (ii) emphasise those that are likely to be common in wild free-roaming horses; (iii) highlight areas where there may be significant knowledge gaps; and (iv) indicate important sources of further information.

The most common health problems in wild horses may be anticipated to vary geographically between different countries and different environments, as well as between different age groups. Anyone involved in assessing equine welfare should familiarise themselves with clinical signs, risk factors, aetiologies, and the diagnosis of and prognosis for key diseases mentioned below, and especially for those specific to their region.

5.1 Common non-infectious diseases

The most common non-infectious health problems, aside from injuries, to which US wild horses are said to be susceptible, are those secondary to limitations in food and water, followed by age related disease, club feet, and developmental orthopaedic disease with angular and flexural deformities being most common, albeit rare overall (Kane 2011, 2014; Nunez et al. 2016). Age related development of osteoarthritis in the metacarpophalangeal joints of wild horses in New Zealand has also been identified (Cantley et al. 1999).

In free-roaming horses in a Polish sanctuary, the most common health problems noted over a 70-year period of monitoring, were hoof pathology (Figure 7) and parasitic diseases. However resultant lameness or clinical signs associated with parasitism were rare. Undernutrition was also rare since the small population was maintained at a stable number, and supplementary feeding was carried out if needed (Górecka-Bruzda et al. 2020). Reported causes of mortality are described under Population Dynamics in Domain 4.

Figure 7. Hoof pathology such as cracks may be common in some populations, but relationship to lameness is not clear. Image A.M. Harvey.

However, evaluation of the peer reviewed literature suggests that health problems are a key knowledge gap in wild horses. Common health problems likely to be encountered in wild horses are difficult to extrapolate from the extensive knowledge of health and disease in domestic horses, since domestic horse health is most often related to husbandry, particularly diet and environmental factors. Poor forage availability can predispose to conditions such as colic, gastric ulceration, and abnormal dental wear, whilst over-nutrition is common resulting in obesity, equine metabolic syndrome and related disorders such as laminitis. Restrictions on movement can have negative impacts on hoof health and musculoskeletal development in addition to predisposing horses to obesity and related diseases. An indoor dusty environment is a major contributor to lower airway disease. The most common health problems in older domestic horses are cardiovascular disorders, locomotor system injuries, hoof abnormalities, dental disease, weight loss and colic (Leblond et al. 2002; McGowan et al. 2010).

Conversely, the only health related area that has been extensively researched in wild horses is hoof pathology in Australian desert horses (Hampson 2011; Hampson et al. 2011a, 2013a, 2013b) and free-roaming horses in New Zealand (Hampson et al. 2010c, 2012). The most striking finding in these studies was a high prevalence of chronic laminitis in these populations. This may be surprising since laminitis is more typically associated with diet and husbandry issues in domestic horses (Eades 2010). This pathological finding in wild horses could suggest diets high in non-structural carbohydrates, or presence of insulin resistance (Hampson et al. 2012). However other authors have suggested that this pathology in horses walking long distances on hard surfaces is likely a result of foals having to travel long distances early in life, impacting the early development of the hoof (Ramey 2011). As these studies were conducted at post-mortem, the clinical relevance for the prevalence of lameness associated with these findings, is unknown.

Food limitation may give rise to other health problems. Specific nutritional deficiencies and related disorders were discussed under Domain 1. When normal food sources are limited, horses are more likely to ingest toxic plants, also discussed under Domain 1. Soil ingestion may be more likely to occur in environments where forage is sparse (Hampson et al. 2011b), or with some nutritional deficiencies (See Domain 1). In some circumstances this may give rise to sand enteropathy (Niinistö & Sykes 2021), although this has not been specifically–documented in wild horses. Low body condition is also associated with decreased reproductive performance, with widespread effects on reproductive physiology of mares (Morley & Murray 2014).

There is limited information in horses indicating their susceptibility to different diseases when they are malnourished, but it is well established in humans that malnutrition increases risk of disease and decreases survival rate (Schroeder & Brown 1984). Acute starvation occurs when food intake ceases or is exceptionally low, leading to a rapid onset of emaciation, and related impairments to health (Kronfield 1993). In horses, systemic immunity may be severely compromised within 5 days of the onset of starvation (Naylor & Kenyon 1981). Chronic starvation gives rise to a range of metabolic changes that reduce basal energy requirements, with eventual sequalae of reduced body mass, reduced lower critical temperature, reduced integrity of the gut mucosa, gastric ulceration, and compromised immunity resulting in increased risk of infectious diseases, and endoparasitism (McGregor-Argo 2013). Chronic starvation is associated with decreased activity levels to only essential movements (Shetty 1999), with death typically occurring after a body mass loss of about 50% (Cymbaluk 2013). Death by starvation has been reported in wild horses in large numbers in semi-arid habitats during droughts (Berman 1991; Dobbie et al. 1993).

(a) **(b)**

Figure 8. **(a)** Ventral abdominal hernia in a young wild horse in the Victorian Alps, Australia. **(b)** 'Parrot mouth' in a horse removed from Guy Fawkes National Park, NSW, Australia. Images A.M. Harvey.

Sufficient genetic diversity is important for the prevention of inbreeding and subsequent development of genetic diseases. The population size necessary to prevent genetic problems from arising in horses is not known, but will depend on the genetic diversity of the population. Crude estimates of 50 breeding horses or a total of 150 horses in a population have been suggested to minimise the risk of genetic disorders

(Singer et al. 2000). Genetic disorders including club foot deformity, dwarfism or blindness associated with cataract formation, ventral abdominal hernias and cryptorchidism have occurred in the US. Also, I have observed ventral abdominal hernias, 'parrot mouth' (Figure 8), pelvic limb deformities and cryptorchidism in Australian wild horses, which may be genetic in origin (personal observations).

5.2 Common infectious diseases

Specific infectious disease risks will vary considerably between different geographical regions. In Australia, the main infectious diseases of potential concern would be gastrointestinal parasites, external parasites such pediculosis (lice) caused by *Damalinia equi* (Figure 9a) and *Haematipinus asini* (Da Silva et al. 2013), bacterial infections such as *Clostridium tetani* (Kay & Knottenbelt 2007), *Streptococcus zooepidemicus and S.equi* (Boyle et al. 2018), and viral infections such as Papillomavirus (Figure 9b) (Torres & Koch 2013), Equine Herpes Virus (Lunn et al. 2009), Ross River Virus (El-Hage et al. 2020), Hendra virus (Middleton 2014) and Lyssavirus (Warrilow 2005). However, no investigation of these infectious diseases has been performed in Australian wild horse populations. They will not be discussed in detail here, but readers can consult the texts cited.

Infectious upper respiratory tract diseases caused by *S. zooepidemicus* and *S. equi* have been documented in free-roaming wild horses in the US, however they appear to rarely cause a problem in free-roaming horse; they are mainly an issue when large numbers of horses are brought into a smaller area, or in situations where larger numbers of horses congregate in one area, for example during times of water or feed shortage (Kane 2011; Nunez et al. 2016). In the US, clinical problems have been observed in wild horses with Eastern equine encephalitis virus, West Nile Virus, and equine infectious anaemia, but they also appear to be rare (Kane 2011). Equine protozoal myeloencephalitis caused by *Sarcocystis neurona* occurs in some countries including the US, and seroprevalence has been determined in wild horses in Wyoming (Dubey et al. 2003), although the incidence of associated clinical disease has not been reported. In Venezuelan wild horses, muscular disease associated with *Trypanosoma evansi* has been identified (Mateu et al. 1994). Otherwise, very little is known about the prevalence or significance of infectious diseases in wild horse populations throughout the world.

(a) **(b)**

Figure 9. (a) Skin lesions (upper image) caused by the louse *Damalinia equi* (lower image). **(b)** Warts on the nose caused by Papillomavirus. Images A.M. Harvey.

Gastrointestinal parasitic diseases are the most studied to date, but the reports have been predominantly anecdotal descriptions of high parasite burdens at post-mortem (Hampson et al. 2011b), and in horses removed from the ranges in the US (Kane 2014). Nevertheless, there are few studies of gastrointestinal parasites in wild horses worldwide (Rubenstein & Hohmann 1989; Slivinska et al. 2006, Slivinska et al. 2009; Debaffe et al. 2016; Cain et al. 2018; Slivinska et al. 2020). A particular deficiency was the absence of information on evaluating the most pathogenic large strongyle, *S. vulgaris*. This knowledge gap was the impetus for the further work in this area, described in Chapter 4.

(a) **(b)**

Figure 10. (a) Approximately 6 month old filly removed from KNP, with poor body condition and a rough hair coat. **(b)** Following administration of anthelmintics she passed several large *Parascaris* spp. Images A.M. Harvey.

Gastrointestinal parasites described are most commonly ascarids, and small and large strongyles. *Parascaris* spp. most commonly cause pathology in horses aged < 18 months due to development of very strong acquired immunity in older horses, although anecdotally significant burdens have also been reported in the US in adult horses removed from the range (Kane 2014; Nunez et al. 2016). Migration through pulmonary tissues can result in coughing and a purulent nasal discharge (Clayton & Duncan 1978; Srihakim & Swerczek 1978), whilst parasites in the gastrointestinal tract can result in reduced food intake, diarrhea, poor growth, poor weight gain, and a rough haircoat (Figure 10).

Larval cyathostominosis, when large numbers of small strongyle (cyathostomins) larvae emerge from cysts in the intestinal wall, is associated with severe haemorrhagic oedema and inflammation in the large intestine (Love et al. 1999; Peregrine et al. 2006; Corning 2009), often associated with profuse diarrhea, weight loss, anaemia, hypoproteinaemia and accompanying ventral oedema and dehydration. This is most common in young horses, with reported mortalities of up to 50% in domestic horses (Love et al. 1999). Chronic cyathostominosis can be related to the presence of various stages of parasite's life cycle (Love et al. 1999) and can cause weight loss, dull haircoat, pot-bellied appearance, colic, and mild diarrhoea. The most pathogenic large strongyle is *S. vulgaris,* a cause of verminous arteritis in the cranial mesenteric artery. *S. vulgaris* was reported to be the cause of death in 10-33% of domestic horses presenting with abdominal crises, prior to the availability of macrocyclic lactone anthelmintics (McCraw & Slocombe 1976; Ogbourne & Duncan 1985). Other clinical signs associated with adult strongyles include caecal ulcers associated with anaemia, weight loss, and poor coat condition. Acute signs following infection with large numbers of larvae can include pyrexia, inappetence, malaise, weight loss, constipation or diarrhea, and can progress to abdominal pain, recumbency and death, being commonly fatal in young foals (McCraw & Slocombe 1976). Chronic infection from repeated smaller doses of larvae, may result in pyrexia, poor weight gain and intermittent episodes of abdominal pain (McCraw & Slocombe 1976).

5.3 Common injuries

Injuries are common in domestic horses. These are often sport related injuries, paddock injuries associated with fencing, excessive activity after periods of confinement, closer confinement with other horses that may not be in stable social groups, and kick injuries, heightened if a kick is from a shod horse (Kane et al. 2000). Injuries may therefore be less common in wild horses, however the incidence of injuries in free-roaming wild horses under different conditions is unknown. Steep uneven terrain may be more likely to increase the risk of ligament and tendon injuries. Lower limb fractures (Kane 2011) and poorly healed old injuries (Nunez et al. 2016) are most observed in US wild horses.

5.4 Sickness and pain behaviours

Sickness and pain behaviours can be quite subtle, so detection relies upon a really good understanding of normal behaviours. Sickness behaviours can be any change in ‘normal’ behaviour, emphasising the importance of species-specific knowledge under optimal circumstances. Subtle changes in time budget behaviours may indicate presence of pain with reduced mobility, grazing, alertness and social interactions

(Ashley et al. 2005; Hausberger et al. 2016) and have generally been associated with welfare compromise in domestic horses (Lesimple 2020). Although behaviour and time budgets have been well studied in wild horses (reviewed in King et al. 2016), time budgets under different conditions have not.

Wild horses usually regularly scan the environment and remain vigilant, responsive to environmental stimuli and ready to react to unusual stimuli (Waring 2003). Sick horses are often described as apathetic or depressed (Burn et al. 2010; Pritchard et al. 2005; Fureix et al. 2012; Popsecu & Duigan 2013), characterised by prolonged periods of reduced mobility, head-lower-than-withers body posture, reduced responsiveness to environmental stimuli and self-isolation. A fixed stare, dilated nostrils and clenched jaw have also been associated with apathy (Ashley et al. 2005). In addition, a dull demeanour, reduced alertness often accompanied by self-isolation and lowered head carriage have been described with non-specific and abdominal pain (Whitehead et al. 1991; Duffield et al. 2002), features often observed in combination with a 'pain face' grimace (Taylor et al. 2002; Dalla Costa et al. 2014).

Further, some behaviours that could be regarded as sickness behaviours may not be widely recognised as such. For example, coprophagia has been described as a normal behaviour in wild horses (Schoenecker et al. 2016), but is not observed in domestic horses that have adequate nutrition. Interestingly, coprophagia seen in captive plains zebra and Przewalski horses housed in a grassless paddock with no other vegetation, resolved when vegetation was provided, and when they were housed indoors with ad lib hay (Boyd 1986). This illustrates how some behaviours or clinical findings that may appear to be 'natural' are not necessarily signs of optimal welfare. In this situation, it is apparent that the knowledge gained from domestic horses and wild equids in captivity provides the helpful insight that coprophagia is suggestive of inadequate nutrition. Interestingly, it has also been found that horses that developed equine motor neuron disease were more likely to be coprophagic than unaffected horses (Divers et al. 1994). This association is due to both conditions being related to limited grass intake, the link being that equine motor neuron disease is associated with vitamin E deficiency (De la Rua-Domenech et al. 1997) and that a major source of vitamin E is fresh forage. This condition usefully illustrates how features in all four physical domains can interact: Domain 1, inadequate nutrition linked with Domain 2, environmental impairment of forage growth, Domain 3, health, vitamin E deficiency causing equine motor neuron disease, and Domain 4, the sickness behaviour of coprophagia being exhibited.

5.5 Most likely welfare challenges in Domain 3

On the basis of current knowledge, the most common health challenges in wild horses are likely to be secondary to limitations in food and water intakes and to gastrointestinal parasites. However, there is a distinct knowledge gap regarding other health problems encountered in such wild horses.

6. Domain 4: Behavioural interactions

6.1 Social organisation and factors affecting it

Horses are highly social animals exhibiting a harem social structure and usually, long term stability of social groups (Klingel 1975, 1982; Rubenstein 1986; Linklater 2000). The social unit, known as a band, usually consists of one of more stallions, one or more mares, 1–2-year-old horses of both sexes that haven't yet dispersed, and foals (Linklater 2000) (Figure 11a). Mean reported band size is 4-12.3 horses, with maximum band sizes of 8-24, whilst number of adult mares in a band has been reported to be a mean of 2-4 and maximum of 8 (Berger 1986; Keiper 1986; Linklater et al. 2000; Grange et al. 2009; Roelle et al. 2010). Wild horse populations with a female biased sex ratio have been described to live in larger groups with a mean band size of 14, maximum of 18-35, with a mean number of adult mares being 5.6 and maximum 8-22 (Keiper 1986). The largest reported band sizes in feral horse have been in populations with strongly female-biased adult sex ratios, with 28 on Assateague Island, USA (Keiper et al. 1986) and 35 in Hato El Frio, Venezuela (Pacheco & Herrara 1997).

Stallions not in a band, are usually young or displaced older stallions that form all-male bachelor groups (Klingel 1975; Keiper 1986; Rubenstein 1986) (Figure 11b). All-female and mixed-sex groups have been described in free-roaming horses on Assateague Island, USA (Keiper 1976), and in Kaimanawa mountains, New Zealand (Linklater et al. 2000), but these are uncommon and usually temporary groupings.

Solitary horses would typically be band stallions who have lost their females, and are often old or sick (Klingel 1975; Rubenstein 1986; Boyd & Keiper 2005).

An alternative social structure is observed in some other equid species, where the social group is dynamic with no long-lasting bonds other than between mares and their current foal (Klingel 1975, 1982; Rubenstein 1986, 1994; Kaczensky et al. 2008). In this social structure stallions are territorial and sometimes alone, or at other times they have temporary associations with mares; although mares are rarely alone, their social associations are only temporary.

(a) **(b)**

Figure 11. (a) A band and **(b)** a group of bachelor stallions in Kosciuszko National Park, NSW, Australia. Images A.M. Harvey.

Wild horses do not typically exhibit this social structure, but a rare scenario has been described in free-roaming horses in Shackleford Banks Island, USA where different social systems were present in the same population, under different environmental conditions. Here a female-biased sex ratio reduced the number of potential rivals whilst an open habitat made detection of other stallions easy, so the stallion could defend both mares and his territory without being with a band all the time. When the vegetation later changed to more uniform cover, social structures of all horses reverted to harems with overlapping home ranges (Rubenstein 1986, 1994).

The size of bands is influenced by a range of factors such as food and water availability and predation risk (Klingel 1975; Rubenstein 1986). Bands tend to be larger in more open environments especially when resources are abundant (Miller 1979). Severe weather conditions in winter and food limitation during dry seasons causes bands to split into smaller groups (Miller 1979) where they then roam in much larger and overlapping areas (Salter & Hudson 1979). Adult sex ratio was important in one study showing a decrease in band size as the adult male proportion increased (Kaseda & Khalil 1996). However, in an Argentinian study, changes in population density, rainfall and adult sex ratio did not influence group size (Scorolli & Lopez-Cazorla 2010). Endoparasite burden appeared to influence group size in Shackleford Banks Island, USA (Rubenstein & Hohmann 1989), but not in other studies (Harvey et al. 2019).

Within a population, bands may form a sub-population known as a herd, with similar movement patterns, a common home range, and interband dominance hierarchies that affect access to scarce resources (Miller 1983). Bands often aggregate into these larger herds around water sources, or preferred grazing sites (Miller 1979). Herd formation may reduce predation risk (Rubenstein 1986), afford shelter against harsh climatic conditions and help avoid bachelor harassment of females (Rubenstein 1994).

In unmanaged wild horse populations the overall sex ratio is expected to be 1:1, with surplus males forming bachelor groups typically comprising males younger than 5yrs, although older males can also join bachelor groups and occasionally, they will be solitary (Klingel 1975; Keiper 1986; Linklater 2000; Boyd & Keiper 2005). The mean size of bachelor groups is 3, with a range of 2-17, but these are generally not stable groups (Berger 1986; Linklater 2000; Boyd & Keiper 2005).

Band composition has been shown to be stable all year-round in many different populations studies across the world (Klingel 1982; Keiper 1986; Linklater 2000; Boyd & Keiper 2005), with several longer term studies of > 3 yrs. showing ongoing stability for many years (Rubenstein 1981; Berger 1986; Keiper 1986; Duncan 1992; Feh 1999; Goodloe et al. 2000; Linklater 2000; Scorolli & Lopez-Cazorla 2010), and less than one-third of bands disbursing within study periods (Berger 1986; Goodloe 2000; Cameron et al. 2001; Scorolli & Lopez-Cazorla 2010). The percentage of adult mares changing bands annually has varied from 2-30% (Rubenstein 1981; Berger 1986; Franke-Stevens 1990; Duncan 1992; Feh 1999; Linklater et al. 2000; Goodloe et al. 2001; Scorolli & Lopez-Cazorla 2010). Band stallion tenure may be one indicator of band stability. Mean stallion tenure is reported to be 2.8 – 4.4 years (Berger 1986; Goodloe et al. 2000; Scorolli & Lopez-Cazorla 2010), with as long as 18 years reported (Feh 1999), albeit in a managed population with a strong female-biased sex ratio.

Social stability may be influenced by water and forage availability (Franke-Stevens 1990), predation and adult male harassment of mares (Linklater et al. 1999). Where there is less competition for food horses tend to live in larger relatively stable family groups, whereas those living in more arid environments where food is spares are more often found to be in smaller unstable groups (King et al. 2016). This is also observable among other equid species; when food biomass drops below 40g/m^2 normally stable groups of plains zebras become unstable (Ginsberg 1989) and the scattered food supply in dry environments such as Ethiopian desert does not allow females to forage close enough to each other to form stable groups (Kreuger 2008). Dominance hierarchies exist both within and between bands. Larger groups generally get priority access to resources (Berger 1977) although groups already in possession of the resource may have an advantage (Franke-Stevens 1988).

Stallions obtain mares in a variety of ways; acquiring unguarded mares that have dispersed or separated from their band or whose stallion has died (Salter 1978; Keiper 1986), defeating another band stallion to take over the entire band, abducting part of a band (Miller 1979), attaching themselves as a satellite to a band and eventually departing with all or some of the mares (Welsh 1973; Salter 1978; Miller 1979; Rubenstein 1981; Khalil & Murakami 1999), or inheriting the band following death of the band stallion (Miller 1979; Rutberg & Keiper 1993; Roelle et al. 2010). In most studies stallions are around 5-6 years old before they obtain their first mares (Klingel 1982; Berger 1986; Khalil & Murakami 1999).

Multi-stallion bands are also commonly reported (Roelle et al. 2010), being more likely to form with lower ranking stallions of low-ranking mothers (Feh 1999). The most dominant stallion in the band is involved in most of the breeding (Berger 1986; Franke-Stevens 1990; Linklater & Cameron 2000). Multi-stallion bands provided added protection, and an increased potential number of mares that the band can hold (Franke-Stevens 1990; Pacheco & Herrera 1997). When faced with potential danger or rival stallions, the dominant stallion usually leads the mares or drives them away from danger, whilst the subordinate stallions place themselves between the females and the danger, and confront approaching rivals (Berger 1986; Franke-Stevens 1990; Pacheco & Herrera 1997; Feh 1999; Linklater & Cameron 2000). Multi-stallion bands may be more stable, and mares in stable bands produce more foals than those in unstable bands (Berger 1986; Kaseda et al. 1995). Natality also appears to be less affected by severe winters in multi-stallion bands (Boyd 1980) and larger bands (Welsh 1973), and one study showed higher foal survival in multi-stallion bands (Feh 1999). Other studies have found no difference between single and multi-stallion bands and the number of adult mares within the band (Linklater & Cameron 2000; Roelle et al. 2010). A New Zealand study showed mares from single-stallion bands were in better body condition than mares in multi-stallion and had higher foaling rates and lower foal mortality rates (Linklater et al. 1999; Linklater & Cameron 2000).

6.2 Population dynamics

Food is one of the most important factors limiting wild horse population densities (Fowler 1987; Gaillard et al. 1998; Gaidet & Gaillard 2008; Scorolli 2010; Dawson & Hone 2012; Scorolli 2021) with many populations exhibiting density dependence and being limited by their own impact on food resources. As forage becomes scarce, body condition scores of horses reduce, and disease and parasitism may increase, with subsequent reductions in fecundity and survival, thereby limiting population growth (Eberhardt 1987).

Primiparous births typically occur from females aged 2- 5 years with peak birth rates occurring for mares ages 6-17 yrs. When forage is abundant mares are more likely to give birth at an earlier age (Berger 1986; Tatin et al. 2009), whereas when food and water become scarce, age of primiparous birth can increase and birth rates for all females can decline quickly (Ginsberg 1989). In a range of studies across the world, mean adult birth rate was 0.56 ± 0.001 (0.23-0.92), mean foal survival rate 0.84 ± 0.002 (0.09-0.97), mean adult survival rate 0.9 ± 0.001 (0.79-0.98) and mean population growth rate was 1.18 ± 0.001 (0.84 – 1.39) (Reviewed by Ransom et al. 2016). Environmental conditions during early pregnancy are regarded as the most influential factor in determining whether a pregnancy will reach term (Roberts 1986). Oestrous and the timing and rates of births have been correlated with rainfall in populations of free-roaming horses (Siniff et al. 1986; Berman 1991; Scorolli & Lopez-Cazorla 2010; Ransom et al. 2013), synchronising births with forage availability. This may increase survival of lactating mares and their foals (Ginsberg 1989, National Research Council 2007). Each centimetre of rain in spring increased foal survival by 1.5% in one study, and significantly increased survival rates of horses > 20 years old but did not impact survival of adult horses up to 19 years old (Ransom 2012).

Populations can increase by up to 20% per year when resources are unlimited (Eberhardt et al. 1982; Garrott et al. 1991), but may frequently be much lower. In free-roaming horses in New Zealand, a population growth rate of 9.6% per year was identified, and in three different wild horse populations in the Australian Alps only one of these populations was found to be increasing whilst the other two were stable, with food limitation being the major limiting factor in population growth (Dawson & Hone 2012). In France, demographics and density dependence was investigated over an eight-year period and identified resource limited population growth and decreased survival with increasing density (Grange et al. 2009). Similarly, a seven-year study in Argentina identified a density dependant response with population growth limited by low fecundity when resources were limited (Scorelli et al. 2010).

Most wild horse populations studied exhibit relatively high survival rates with similar age structures across populations with foals comprising 8-15% of a population, juveniles 13-28% and adults 71-78% of a population (Berman 1991; Tatin et al. 2003; Ransom et al. 2016). Free-roaming horses in the US have been known to live as long as 29 years (Ransom 2012), but longevity is extremely variable. On Assateague Island the mean age at death for stallions was 10.3±0.84 years, and for mares 6.4±0.85 years. However, when mares were treated with immunocontraception their longevity increased significantly with mean age at death rising to 10.2±0.56 years after 1-2 years treatment and 19.9±1.66 years after > 3 years treatment (Kirkpatrick & Turner 2007).

Causes of adult mortality have been studied in Przewalski horses in Mongolia, with the most common causes of death being starvation (31 %) and predation (22%), with other causes including injury and parasitic disease (Dorj & Namkhai 2013). In managed free-roaming horses in a large wild horse sanctuary in Poland, reported causes of adult mortality were drowning in marshlands where horses entered to graze the green shoots of reeds, intestinal perforation associated with ascarids, dystocia, suspected wolf predation, fractured shoulder, fractured limb and internal haemorrhage due to a kick injury (Górecka-Bruzda et al. 2020).

Infanticide has been reported to be the cause of 9% of foal deaths in Przewalski horses (Dorj & Namkhai 2013). Maternal responses to infanticide risk have been studied through comparison of maternal protectiveness of foals between single and multistallion bands, showing that mares were more vigilant and spent more time close to foals in multistallion bands (Watts et al. 2020). Much of the difference between survival rates in different populations across different regions have been attributed to local differences in predation. Large bands sizes in open environments may reduce risk of predation (Kie 1999). Many predators have been documented killing both young and adult equids throughout the world (Ransom et al. 2016). In Australia faecal analysis shows that dingoes (*Canis lupus dingo*) sometimes consume horse parts (Davis et al. 2005), but it is unclear whether this is through hunting or scavenging. There is some evidence that dingoes kill foals (Dawson 2005) but there are no scientific reports evaluating the prevalence and extent of such predation in different populations in

Australia. In one study dingo call playbacks did not alter maternal protectiveness suggesting that mares did not perceive dingo calls to be an immediate threat to foals (Watts et al. 2020), and foal survival has been shown to be high in studied populations in Australia (Dawson & Hone 2012). In managed free-roaming horses in Poland, congenital defects were the most common cause of foal deaths, with gastric rupture and car accident also reported (Górecka-Bruzda et al. 2020).

Other factors also influence population growth rates including dispersal and recruitment, and catastrophic stochastic events. Reduction in fecundity may also arise from social stress (Linklater 2000; Tatin et al. 2009). Due to their longevity, high survival rates, low recruitment rates, and low susceptibility to moderate environmental stressors, most wild horse populations can persist near their carrying capacity, barring catastrophic mortality events (Ransom et al. 2016). When mass mortality events do occur, populations can be relatively slow to recover because of low recruitment rates, however, sudden reductions in populations also result in a compensatory increase in reproductive rate (National Research Council 2013; Ransom et al. 2016). Catastrophic climate events can have the greatest effect on wild horse populations with population declines of 12–61 % reported after extreme cold winters in some parts of the US (Berger 1983; Garrot & Taylor 1990; Kaczensky et al. 2011), mass mortalities during drought, with a 51% reduction reported during drought in an Australian population (Berman 1991), 28% of a population dying during a thunderstorm in Argentina (Scorolli et al. 2006) and 4% dying with a lightning strike in Pryor Mountain, US (Ransom et al. 2016).

6.3 Reproductive physiology and behaviours

Knowledge of reproductive physiology is particularly important for understanding impacts of nutritional, environmental and health factors on reproduction. The effects of body condition on equine reproductive physiology were recently reviewed in detail (Morley & Murray 2014). This is also–important knowledge when seeking to manipulate physiology to reduce reproduction (Hobbs & Hinds 2018).

Horses are seasonally polyoestrous, with oestrous determined predominantly by increased day length. Also, nutrition, physiological condition and environmental temperature can affect seasonal reproductive activity, with conception rates impacted by age, parity, hormone metabolism, disease, trauma and genetics (Morley & Murray 2014).

Females usually reach sexual maturity between 12 to 24 months. During the breeding season they typically complete about 12 oestrous cycles each lasting 21 days, with sexual receptiveness (oestrous) lasting 4-5 days (Feist 1975). When mares are in oestrous, they adopt a posture whereby the hind legs are abducted and tail held raised and to the side of the perineum, serving as a visual signal to attract males (Boyd & Houpt 1994; Ransom & Cade 2009). Mares exhibit an increased frequency of urination and 'clitoral winking', incorporating labial secretions into the urine as part of olfactory signalling (Asa 1986). Mares also exhibit a distinctive facial expression with ears back, lips pulled back, and head and neck stretched low (Klingel 1975). Only sexually receptive mares will stand for mounting, whilst unreceptive mares may kick the stallion on attempts to mount (Klingel 1975).

Following a gestation period of 327-367 days (Feist 1975; Berman 1991; Monfort et al. 1994; Ransom et al. 2016), mares normally foal over spring and summer with a single foal. Mature females are capable of foaling every year, but most commonly they raise one foal every two years (Feist 1975).

The first successful suckling of the foal should occur within 2 hours of parturition (Klingel 1975) (Figure 12). Suckling bout duration and frequency are reflective of the amount of maternal care (Mendl & Paul 1989). Suckling frequency decreases with increasing age of the foal. The main factor affecting lactation length is subsequent pregnancy with non-pregnant mares nursing their foals for longer than pregnant mares (Duncan et al. 1984), and primiparous mares lactating loner than multiparous mares (Duncan et al. 1984; Cameron & Linklater 2000). Age of weaning does not appear to be affected by the condition of the mare (Cameron & Linklater 2000), or number of stallions in the group (Cameron et al. 2003).

Figure 12. A mare suckling her newborn foal within an hour of parturition. Image A.M. Harvey.

6.4 Normal range of behaviours and time budgets

Time budgets of wild horses have been well researched. Based on several studies in Australia, US, France and Japan, the mean and standard error of the proportions of predominant behaviours are as follows; grazing (62.9 ± 4.79), stand resting (19.6 ± 2.76), standing (16.4 ± 1.77), recumbent (5 ± 1.95) and moving (9.5 ± 2.45) (reviewed King et al. 2016). The daily rhythm of behaviours is linked to sunrise and sunset (Berger et al. 1999), ambient temperature (Souris et al. 2007), season (Duncan 1985; Mayes & Duncan 1986), arising from differences in forage availability and quantity (Arnold 1984) and presence of insect pests (King & Gurnell 2010). Most grazing and social behaviours occurs when horses are most active during the crepuscular period. Horses continue to be active during the night, with one study showing no difference in their behaviours during day and night (Berman 1991), but with others reporting more resting and less feeding behaviour at night (e.g., Mayes & Duncan 1986).

Typically, grazing occurs predominantly in 2 pulses early morning and late afternoon, and ongoing during the night. Longer periods of time are spent feeding during winter than summer (Kaseda 1983; Berman 1991; Berger 1999; King 2002). Mares in poor body condition spend more time grazing than mares in good condition (Ransom et al. 2010) and lactating mares spend more time feeding that nonlactating mares (Duncan 1985; Boyd 1988; Madosky 2011). Most resting occurs during the middle of the day, with recumbent rest most common between 2am and dawn (Keiper & Keenan 1980; Boyd et al. 1988; King 2002,). Movement makes up a relatively small amount of the time budget, with movement time related to the distance between prime feeding areas and water sources. Stallions spend more time moving and less time feeding than mares (Boyd 1988; King 2002; Ransom & Cade 2009).

During the first month after birth foals stay close to their mother, being entirely dependent on her for nutrition (Tyler 1972; Boy & Duncan 1979). They begin to forage and interact with other horses by about 2 months of age, and by 5 months of age their time budget becomes more like that of adult horses (Boyd 1988), although they spend more time in recumbent rest than adults (Boy & Duncan 1979; Duncan 1979; Boyd 1988; King 2002).

6.5 Social behaviour and communication

Good communication is a requirement for living in large social groups. Ethograms have been well defined for wild horses, characterising the range of behaviours, facial expressions and ear, tail and general postures involved in their communication (McDonnell 2003; Ransom & Cade 2009).

Affiliative behaviours include allogrooming (Feh & de Mazieres 1993; Houpt & Boyd 1994; King 2002), very close proximity to each other (Wolter et. al. 2018) (Figure 13), or putting their heads on another's body whilst resting (King 2002; Ransom & Cade 2009). Mutual grooming enhances social bonds, which aids stability of the band, consequently enhancing reproductive success (Linklater et al. 1999; Cameron et al. 2009) and may increase effectiveness at protecting foals from predators. Allogrooming may also reduce heart rate (Feh & de Mazieres 1993) suggestive of a more relaxed state. Individual horses usually have preferred close companions, quantified by the proportion of time that those horses are their 'nearest neighbour'(Kimura 1998; Wolter et al. 2018); and companions usually mutually groom each other (Sigurjonsdottir et al. 2003; van Dierendonck et al. 2004). The nearest neighbour to foals is their mother until they are about 2 years old (Crowell-Davis et al. 1986; Boyd 1988; van Dierendonck et al. 2004), and outside the foal-dam relationship, they tend to be those similar in age and rank (van Dierendonck et al. 1995; Kimura 1998; Sigurjonsdottir et al. 2003).

(a) **(b)**

Figure 13. Affiliative behaviours **(a)** allogrooming between two older foals and **(b)** close spatial proximity between a mare and her 2 year old filly. Images A.M. Harvey.

Play behaviour is well described, occurring most commonly in young horses and bachelor stallions (McDonnell & Poulin 2002; Ransom & Cade 2009) and least commonly in adult mares (Sigurjonsdottir et al. 2003). Play often occurs between individuals that engage in mutual grooming (Sigurjonsdottir et al. 2003; van Dierendonck et al. 2009); and evidently helps to reinforce social relationships (Feh 1988). Play is energetically costly, which may be why it is more rarely observed in equids in arid environments (Moehlman 1998). Individuals that played more had better survival and body condition as a yearling despite weaning earlier (Cameron et al. 2008).

Agonistic behaviours such as chasing, bites, kicks and ears pinned back (McDonnell & Haviland 1995; Ransom & Cade 2009) are defensive or aggressive behaviours that result in an increased distance between the individuals involved (Feh 1988). Rates of agonistic behaviour tend to be low, and are affected by available space and band size, where populations that have larger available areas and larger bands, exhibit lower rates of agonistic behaviour (Ransom et al. 2010). Competition for resources is apparently the most common reason for agonistic behaviour between mares (Rutberg & Greenberg 1990; Rho et al. 2004), with dominance hierarchies observed (Linklater 2000).

Older and larger horses in the band are usually more dominant (Houpt & Wolski 1980; Rutberg & Greenberg 1990; Keiper & Receveur 1992; van Dierendonck et al. 1995; King 2002; Rho et al. 2004). The band stallion is usually dominant over mares, exhibited through herding behaviour (Feist & McCullough 1976; Ransom et al. 2010), whereas dominance among mares and juveniles is most expressed through agonistic behaviours (Sigurjonsdottir et al. 2003). More dominant individuals are reported to have greater reproductive success (Feh 1990; Powell 2008). Despite typically being more dominant, stallions do not necessarily lead the band. This role can alternate between different mares, usually an individual of higher social rank or with increased resource needs (e.g., pregnant or lactating mare), but any band member may initiate movement of the band (Kreuger et al. 2014).

Serious aggression is rare amongst mares but can be seen between stallions (Tilson et al. 1988) and although severe injury is reportedly rare, fatal injuries can occur. In one study of Przewalski horses, 18.8% of mortalities in males were attributed to injuries sustained in fights (Dorj & Namkhai 2013). Other stallions are however usually prevented from incursion into a band by signals provided by band stallions about relative fighting ability (Rubenstein & Hack 1992; King & Gurnell 2006). Stable relationships between stallions and mares, and band stability can lead to increased reproductive success and lower levels of agonistic behaviour (Linklater et al. 1999; Cameron et al. 2003, 2009).

Horses also communicate through visual, auditory and olfactory systems (Proops et al. 2009; Krueger & Flauger 2011; Rorvang et al. 2020), with recognition of individuals enabling expression of social behaviour to conspecifics, encouraging social stability (Linklater 2000). The sensory abilities of horses in relation to vision, hearing, olfaction, taste and tactile perception has recently been reviewed in detail (Rorvang et al. 2020).

Horses have a visual acuity of 20-23 cycles/degree (Timney & Macuda 2001) with a 60-70° anterior binocular field of view, with up to a 215° field of view out of each eye (Waring 2003), using stereopsis to determine depth (Timney & Macuda 2001). They are dichromate, being able to see colour especially blue and red, but not always being able to distinguish between some colours (Timney & Macuda 2001).

Vocal communications include neighing, nickering, snorting and squealing. Neighing is a contact call between horses, occurring when the horse hears another horse, usually from its social group, or when it is separated from its group (Policht et al. 2011). Horses may process sounds from familiar individuals differently to unfamiliar sounds (Basile et al. 2009), being able to distinguish between neighs from different individuals (Proops et al. 2009). Horses hear higher frequency (55Hz – 34 kHz) sounds than humans can, but are poor at sound localisation, only detecting differences more than 20° (Timney & Macuda 2001). Nickering is a low decibel, low frequency call to encourage close contact, usually being used by dams to attract their foals. Snorting is typically due to frustration, fear, or to clear the nostrils (Houpt & Boyd 1994), although a recent study has suggested that snorting may also be an indicator of positive emotions (Stomp et al. 2018). Squealing occurs during agonistic encounters (Houpt & Boyd 1994).

Smell is also crucial for communication, with the ability to move large volumes of air through their nose in one breath, trapping large numbers of molecules containing scent information (Saslow 2002). Stallions sniff mare's urine, contact with which draws pheromones as well as volatile odours into the vomeronasal organ, communicating with brain centres coordinating the sexual response (Asa 1986; Houpt & Boyd 1994). Curling up the upper lip, known as the flehmen response assists these odours to make contact with the vomeronasal organ (Stahlbaum & Houpt 1989). Stallions urine mark and faecal mark on the faeces of other males, and on mare excretions, particularly during the breeding season (King & Gurnell 2006). Horses can differentiate between conspecifics based on their urine odour (Hothersall et al. 2010), and differentiate their own faeces from others, as well as recognise the relative dominance of the source of the faeces (Krueger & Flauger 2011).

6.6 Most likely welfare impacts in Domain 4

Potentially, any change in social organisation, affiliative social behaviours, reproduction, and time budget behaviours may represent a negative impact on welfare. Horses being such strongly gregarious animals, social isolation may represent a very severe welfare challenge in this Domain. It is not currently well understood how welfare-relevant factors in the other Domains influence behavioural interactions.

7. Concluding comments

This is the first review to combine relevant information about horses, with a focus on free-roaming wild horses, from all 4 physical domains of the Five Domains Model for assessing welfare. It therefore serves as a useful holistic and multidisciplinary summary and point of reference for anyone wishing to assess equine welfare. It further provides a guide for researchers of the welfare of other species, regarding how to collate this information, and the breadth and depth of information required.

This novel way of synthesising existing literature through the lens of animal welfare, may also offer new insights and challenge some aspects of current wild horse ecological knowledge. Problematically, since wild horse welfare has not been scientifically assessed to date, aspects of social organisation, habitat use, behaviour and population dynamics previously reported may not always have been representative of those animals under *optimal* conditions. Welfare assessments may be misleading if data underpinning those assessments includes information collected on the species in suboptimal conditions. This may also have broader implications regarding the assessment of domestic horse welfare. The key starting point for domestic horse welfare assessment relates to knowledge of what is normal for the species in its wild state, under optimal conditions. Since the welfare of wild horses has never been scientifically evaluated to date, it is possible that there may be irregularities in what is considered to be 'normal'.

Any changes in optimal food and water availability, environmental extremes outside of the horse's thermoneutral zone, health and natural behaviours and social organisation under optimal condition may have negative impacts on welfare. From the current knowledge base, the most common welfare impacts in free-roaming horses are likely to be related to inadequate nutrition and/or water, environmental extremes often combined with inadequate food and/or water, health problems related to under nutrition, developmental abnormalities and gastrointestinal parasitism, and the subsequent impacts of any of these on the horse's behaviour and social organisation.

As highlighted in Chapter 2, features in each of the four physical/functional Domains can serve as indicators of positive or negative welfare states. The information presented here was used to formulate a list of welfare indicators in Chapter 5, which addresses methodology for evaluating welfare indicators in free-roaming wild horses. It is on the basis of this detailed information summarised in this review, that enables cautious interferences to be made about the affective/mental experiences (in Domain 5), The mental experiences that different physical indicators may represent, and the scientific evidence for the link between them is discussed in Chapter 6.

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Thesis outline summarizing chapter titles and contents		
Chapter	**Chapter title**	**Brief description**
1	Introduction: The wild horse controversy & the importance of incorporating animal welfare science in decision making.	Summary of the history of wild horses, their cultural significance, environmental impacts and controversies in wild horse management. Introduces the importance of animal welfare science in wild animal controversies.
2 Published	A Ten-Stage Protocol for assessing the welfare of individual non-captive wild animals: free-roaming horses as an example.	Presentation of a novel conceptual framework that I designed in order to guide a systematic and scientific approach to assessing the welfare of free-roaming wild animals, using the Five Domains Model. Summarizes the principles of interpreting indicators of biological function and behaviour in terms of the mental experiences that those indicators reflect (Stage 1 of my Protocol), and how the Five Domains Model is used for assessing welfare (Stage 2 of my Protocol).
3	Literature review: A review of the species-specific information required to enable assessment of wild horse welfare.	The current status of wild horse knowledge is summarized in a novel holistic and multidisciplinary framework drawing together the relevant literature on horses across each of the four physical/functional domains of the Five Domains Model. It addresses Stage 3 of my Ten-Stage Protocol.
4 Published	**Addressing the knowledge gap of gastrointestinal parasitology in free-roaming wild horses in south-east Australia.**	**An example of the type of detailed original research required for addressing any knowledge gaps identified in Stage 3. This chapter describes a detailed parasitological investigation of 293 faecal samples collected from 6 wild horse populations. It describes results of faecal egg counts, larval cultures and molecular diagnostics.**
5 Published	Use of remote camera traps to evaluate animal-based welfare indicators in individual free-roaming wild horses.	A large body of original research investigating for the first time, both the use of remote cameras for identifying individual horses across a range of habitats, and for acquiring data on an extensive range of animal-based welfare indicators. It addresses Stages 4-6 of my Ten-Stage Protocol.
6	Scientific validation of welfare indicators for using the Five Domains Model to grade the welfare status of individual free-roaming wild horses.	Addressing Stage 7 of my Ten-Stage Protocol, this chapter evaluates the scientific evidence linking the described objective measurable/observable welfare indicators to physical/functional impacts in Domains 1-4, and the relationship between those impacts and the mental experiences that are inferred in Domain 5. This concludes with the formulation of a Five Domains Model wild horse specific welfare grading scheme.
7	Dynamic changes in wild horse social organisation and habitat use revealed with remote camera trap monitoring.	With the aim of evaluating traditional ecological metrics alongside welfare status, this chapter describes original research using the remote camera trapping methodology described in chapter 5, to evaluate population dynamics, and temporal and spatial changes in social organization and habitat use of a wild horse population over a 15 month period.
8	The cascading influence of resource availability on the welfare status of wild horses, and association with population demographics, social organization and habitat use.	This original research applied the methodology from all preceding chapters to assess welfare status and changes in welfare status in individual wild horses over a 15 month period, addressing stages 8 -10 of my Ten-Stage Protocol. It further evaluates drivers of change in welfare status and correlations between welfare status, and the population dynamics, social organization and habitat use described in chapter 7.
9	Conclusions, applications and future directions.	Summarizes overall conclusions, and contributions to the fields of wild animal welfare, wild horse ecology and welfare, wild horse management, and general horse welfare. Highlights ongoing work, some of which I have already partially completed that was not included in the main body of the thesis.

Chapter 4. Addressing the knowledge gap of gastrointestinal parasitology in free-roaming wild horses in south-east Australia

In Chapter 3 I identified particular knowledge gaps in the area of 'wild horse health'. I chose one area, gastrointestinal parasitology, to investigate further in detail, in order to address this knowledge gap. Anecdotally, wild horse populations have been said to have high gastrointestinal parasite burdens, but to date has received little research attention. This Chapter is a specific example of the type and detail of multidisciplinary research that may be required to advance species specific knowledge. Specifically, this is the first report globally, demonstrating the high persisting prevalence of the pathogenic parasite *Strongylus vulgaris*, in wild horse populations. The importance of this work extends beyond the health of the wild horses themselves, since this finding also demonstrates that wild horses may be an important reservoir of infection for domestic horses, where *Strongylus vulgaris* has been considered to be virtually 'extinct' in recent years. This research therefore has broad implications for any horse populations worldwide where there is overlap between wild and domestic populations.

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Abstract

Australia has over 400,000 wild horses, the largest wild equid population in the world, scattered across a range of different habitats. We hypothesised that wild horse populations unexposed to anthelmintics would have a high prevalence of *Strongylus vulgaris* infections. Verminous endarteritis and colic due to migrating *S. vulgaris* larvae is now absent or unreported in domestic horses in Australia, yet wild horses may pose a risk for its re-emergence. A total of 289 FECs were performed across six remote wild horse populations in south-east Australia, of varying densities, herd sizes and habitats. Total strongyle egg counts ranged from 50 -3,740 eggs per gram (EPG, mean 1,443) and 89% (257/289) faecal samples had > 500 EPG, classifying them as 'high level shedders'. There were significant differences in mean total strongyle FECs between different locations, habitats and population densities. Occurrence of *S. vulgaris* was not predictable based on FECs of total strongyle eggs or small (< 90 μm) strongyle eggs. A high prevalence of *S. vulgaris* DNA in faecal samples was demonstrated across all six populations, with an overall predicted prevalence of 96.7%. This finding is important, because of the ample opportunity for transmission to domestic horses. The high prevalence of *S. vulgaris* suggests vigilance is required when adopting wild horses, or when domestic horses graze in environments inhabited by wild horses. Appropriate veterinary advice is required to minimize disease risk due to *S. vulgaris*. Monitoring horses for *S. vulgaris* using larval culture or qPCR remains prudent. Gastrointestinal parasites in wild horse populations may also serve as parasite *refugia,*

thus contributing to integrated parasite management when facing emerging anthelmintic resistance.

1. Introduction

Australia is home to the largest population of free-roaming wild horses in the world, estimated to be between 400,000 - 1 million (Dobbie et al. 1993; Dawson et al. 2006). Whilst population ecology of Australian wild horses has been the focus of some research (Drying 1990; Berman 1991; Dawson 2005, 2009; Dawson & Hone 2012; Cairns & Robertson 2015; Zabek 2015), gastrointestinal parasites have not previously been investigated. There are very few studies of gastrointestinal parasites in wild horses worldwide (Rubenstein & Hohmann 1989; Slivinska et al. 2006, 2009; Debaffe et al. 2016; Cain et al. 2018).

Gastrointestinal parasites of horses may cause significant intestinal pathology, adversely affecting gastrointestinal function and potentially impacting on horses' body condition, health, reproduction and longevity (McCraw & Slocombe 1976; Love et al. 1999; Anderson et al. 2014; Debaffe et al. 2016; Pihl et al. 2017, 2018). Year-round management of gastrointestinal parasites of owned horses, through routine anthelmintic administration, has significantly reduced the impact of parasites worldwide (Herd 1990; Gomez & Georgi 1991; Geary 2005; Gokbulut & McKellar 2018; Nielsen & Reinemeyer 2018). The introduction of macrocyclic lactones in the 1980s has dramatically reduced the prevalence of verminous endarteritis due to migrating *Strongylus vulgaris* larvae (Herd 1990; Geary 2005; Nielsen et al. 2014; Pihl et al. 2018).

While clinical disease due to *S. vulgaris* has largely disappeared, the ubiquitous cyathostomines (small strongyles) remain a problem in most if not all domestic horse populations, particularly due to their increasing resistance to anthelmintics (Herd 1990; Prichard 1990; Love et al. 1999; Kaplan 2002; Kaplan & Nielsen 2010; Nielsen et al. 2014; Scott et al. 2015; Becher et al. 2018; Bellaw et al. 2018; Salas et al. 2018; Scare et al. 2018). Absence of clinical disease caused by *S. vulgaris* may either mean that (i) the parasite is no longer prevalent and potentially extinct or that (ii) the parasite remains prevalent, but is controlled by current strategies including the use of macrocyclic lactones (Nielsen et al. 2012; Kaspar et al. 2017). Sustained prevalence may indicate the presence of a reservoir such as untreated but owned horses or wild / free roaming horses that have never received anthelmintics (Cain et al. 2018).

Higher strongyle faecal egg counts (FECs) were found in wild horses on Shackleford Banks (North Carolina, USA), Sable Island (Nova Scotia, Canada) and Fort Polk (Louisiana, USA) compared to domestic horses (Rubenstein & Hohmann 1989; Debaffe et al. 2016; Cain et al. 2018). Shackleford Banks horses were shown to shed *S. vulgaris* based on larval culture (Rubenstein & Hohmann 1989) and over two thirds of the Fort Polk horses' fecal samples were positive for *S. vulgaris* DNA (Cain et al. 2018). Furthermore, *S. vulgaris* has been identified in a very small wild horse population in Poland (Slivinska et al. 2009) and in free-ranging Przewalski horses in Ukraine (Slivinska et al. 2006).

The aim of this study was to investigate the prevalence of *S. vulgaris* in free-roaming wild horses in south-east Australia. To do so, we performed FECs across six remote wild horse populations of varying densities, herd sizes and habitats. Presence of *S. vulgaris* was demonstrated using larval culture and diagnostic *S. vulgaris* specific real-time PCR (qPCR). We hypothesised that (i) wild horse populations never exposed to anthelmintics would have a high prevalence of *S. vulgaris* infection, and that (ii) FECs would vary significantly both within and between the different populations.

2. Materials and Methods

2.1. Study sites

The populations sampled were part of a larger study concerning the population ecology and welfare of wild horses (University of Technology Sydney Animal Ethics Research Authority 2015000490, Parks Victoria scientific license 10008189, National Parks and Wildlife scientific license 101626, WaterNSW access license D2015/128332). Study sites comprised five sites across the Australian Alps in both New South Wales and Victoria, and one site in Blue Mountains National Park (Figure 1). These wild horse populations have never been in active anthelmintic management programs.

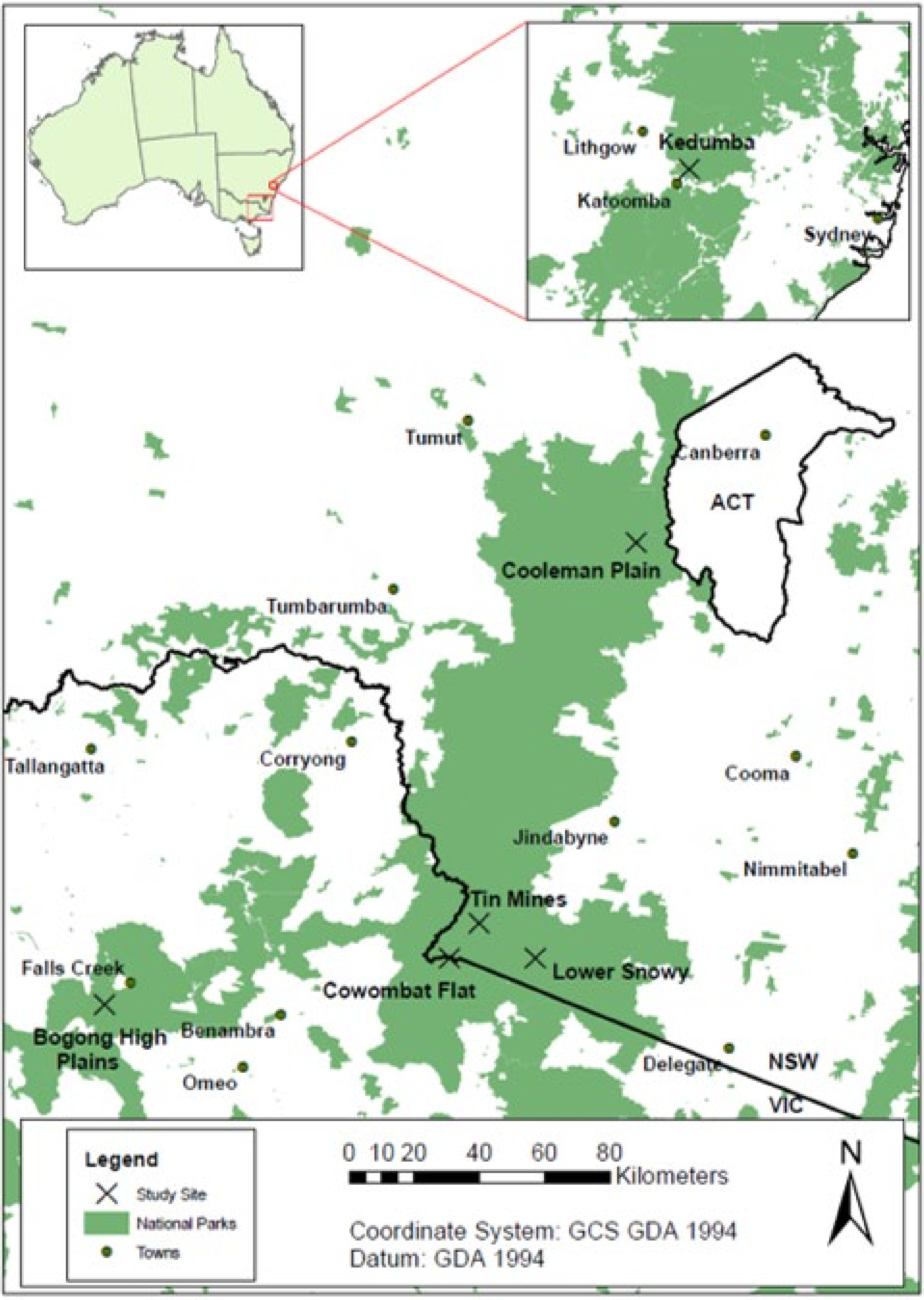

Figure 1. Map illustrating location of sampling sites.

Table 1. Details of study sites, number of samples collected from each site at each different time points, and predicted means and standard errors for *Strongyle* and *Parascaris* spp. faecal egg counts in each population.

Site	Horse density	Estimated total no. of horses	Mean herd size (range)	Habitat type	Date collected	No. samples collected	Mean total strongyle EPG (SE)	Mean EPG eggs < 90 µm (SE)	Mean EPG eggs ≥ 90 µm (SE)	Mean *Triodontophorus*-like EPG (SE)	Mean *Parascaris* spp. EPG (SE)
Kedumba valley	Low	28	4 (2-7)	Eucalyptus woodland	02/2016	9	466 (27)	80 (7)	368 (21)	N/A	N/A
					03/2016	14					
					04/2016	5					
					10/2016	8					
					01/2017	6					
					02/2017	6					
Lower Snowy River	Low	~ 80	3 (2-5)	Eucalyptus woodland	12/2017	11	1,352 (67)	148 (14)	1,185 (56)	16 (3)	46 (41)
					02/2018	10					
Bogong High Plains	Low	~ 50	4 (3-6)	Alpine heathlands	01/2017	2	1,992 (151)	202 (31)	1780 (128)	14 (7)	10 (13)
					03/2017	4					
Cowombat Flat	Medium	85	6 (3-10)	Open grassland	01/2017	60	1,746 (36)	281 (9)	1,434 (29)	23 (2)	66 (13)
					03/2017	35					
Tin Mines	Medium	~ 100	5 (2-6)	Alpine heathlands	12/2017	9	2,016 (93)	220 (20)	1,745 (78)	28 (6)	107 (97)
					02/2017	7					
Cooleman Plain	High	390	10 (4-25)	Open grassland	12/2016	70	1,287 (29)	179 (7)	1,085 (24)	18 (2)	30 (9)
					02/2017	37					

SE Standard Error

EPG Eggs per gram

Low density = estimated < 1 horse/km^2, medium density = estimated 1-2 horses/km^2, high density = estimated > 2 horses/km^2 (Cairns & Robertson 2015, Watts 2017)

Sites comprised of differing habitats, horse population densities and herd sizes (Table 1). Predominant habitat types were taken from previous vegetation surveys (McRae 1989) and verified by on-ground surveys during the study period. Horse densities for the Kosciusko National Park (KNP) sites and Bogong High Plains are from estimations from previous aerial surveys (Cairns & Robertson 2015), and population size estimates and mean herd sizes for Cooleman Plain and Cowombat Flat are from a recent population survey of on-ground observations and camera trapping (Butler 2016-17, personal communications; Watts 2017). Population and herd size estimates for Kedumba Valley, Tin Mines and Lower Snowy River were performed as part of a wider ecological study, based on mark-recapture surveys from direct observations and camera trap images (Harvey 2015-17, unpublished data).

2.2. Sample collection and storage

Faecal samples were collected from Kedumba Valley at seven different time points between February 2016 and April 2017, and from the other study sites at two different time points between December and March of 2016 or 2017.

As horses in these populations are wild, and not habituated to human presence, they could not be approached closely, with recorded flight distances varying from 40-217 metres (Watts 2017). Observations of horses were, therefore, most commonly performed from 200-500 metres away using binoculars and a spotting scope. Consequently, it was rarely possible to identify individuals that the samples were collected from. Most of the samples were collected immediately after observing a known herd grazing, and thus, such samples were known to have been passed within one hour prior to collection. Some samples deemed to have been recently passed, based on warmth and moistness, were collected opportunistically.

Samples collected were from a minimum of three different herds in Kedumba Valley, 15 herds in Cooleman Plain, 12 herds in Cowombat Flat, one herd in Bogong High Plains, four herds in Tin Mines and five herds in the Lower Snowy River region.

Within half an hour of collection samples were placed in a 4°C portable refrigerator for storage until FECs were performed. Faecal sample aliquots (200-400 mg) were placed in 1.5 mL Eppendorf tubes and frozen at -20° C for later DNA extraction.

2.3. Faecal egg counts

Faecal egg counts (FECs) were performed within two weeks of sample collection. Three grams of faeces were dissolved in 60 mL of saturated salt solution (NaCl, specific gravity 1.20). A Whitlock universal 4 chamber worm egg counting slide (J.A.Whitlock & Co, Eastwood, Australia) was used, counting eggs in four 0.5 ml compartments (detection limit 10 $eggs.gram^{-1}$) according to described methodology (Gordon & Whitlock 1939). No larvated eggs were observed. All strongyle eggs were included in the total strongyle egg count. Strongyle eggs were further subdivided into strongyle eggs < 90 μm length and ≥ 90 μm length, to investigate the historically assumed association of smaller eggs with presence of large strongyles such as S. vulgaris (Thienpont et al 1979). Stronglye eggs > 120 μm length were recorded as

Triodontophorus-like eggs. Parascaris spp. and Anoplocephala spp. eggs were also counted and recorded (Figure 2).

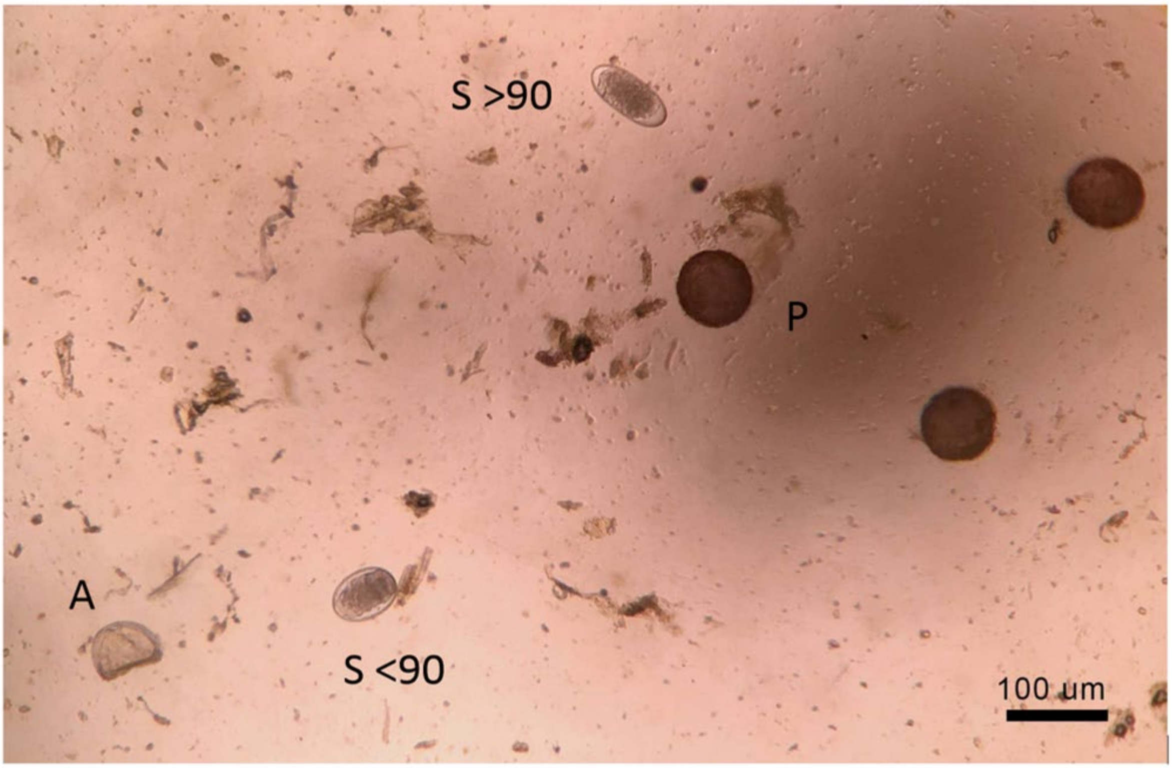

Figure 2. Microscopic view of the different eggs. **A** = *Anoplocephala* spp. eggs, **S** < **90** = strongyle eggs < 90µm length, **S** > **90** = strongyle eggs ≥ 90µm length, **P** = *Parascaris* spp. eggs.

2.4. Larval culture

Faecal samples that had stronglye eggs < 90 µm with counts above 200 EPG were selected for larval culture (n = 20). Samples were cultured within 10 days of sample collection. Larval culture was performed using described methodology (Dunn 1978). Following 10 days of incubation, larvae were retrieved and 100 larvae from each sample were identified (limit of detection 1%), using an identification key (Thienpont et al. 1979), to determine the proportions of *S. vulgaris, S. equinus, S. edentatus, Triodontophorus* and *Trichonema* larvae.

2.5. Strongylus vulgaris diagnostic qPCR

DNA was isolated from 134 faecal samples across the six populations using the MagAttract PowerMicrobiome DNA/RNA Kit (Cat No./ID: 27600-4-KF, Qiagen, Australia) optimised for KingFisher® Duo. Each sample was homogenized for 40 seconds at 6.0 m/s on a FastPrep-24 benchtop homogeniser (MP Biomedicals, Australia). Samples were extracted in batches of 12 including an extraction blank per batch. DNA was eluted into 100 µL stored at -20°C.

The diagnostic *S. vulgaris* qPCR was performed using described methodology (Nielsen et al. 2008). The qPCR reaction included 2 µL of DNA template in a total volume of 20 µL using the SensiFAST™ Probe No-ROX Kit (Cat No.: BIO-86020, Bioline, Australia). The oligonucleotides used in a final concentration of 400 nM for primers and 100nM for the probe per reaction were: Forward primer 5′-GTA TAC ATT AAA TAG TGT CCC CCA TTC TAG-3′; Reverse primer 5′-GCA AAT ATC ATT AGA TTT GAT TCT TCC G-3′; Probe 5′-FAM-TGG ATT TAT TCT CAC TAC TTA ATT GTT TCG CGA C-BHQ3-3′. Primers and probe were synthesized by Macrogen (Seoul, Korea). The qPCR conditions included 95°C for 3 min, followed by 40 cycles of 95°C for 10 s and 60°C for 30 s. The qPCR assay was run on CFX96 TouchTM Real-Time PCR Detection System (BioRad, Australia), with samples run in duplicate. Real-time PCR results were analysed using BioRad CFX Manager 3.1 (BioRad, Australia). Positive results were determined if one or more repeats yielded C_t values < 40.00 and negative results were determined if both repeats did not cross the threshold ($C_t \geq 40$).

Each qPCR run included positive control from DNA extracted from known adult *S. vulgaris* (courtesy of Ian Beveridge, University of Melbourne), negative control (water), plus the DNA extraction blank control.

2.6. Statistical analysis

All statistical analyses were conducted using GenStat v. 17 (VSNi). A Restricted maximum likelihood model (REML) was used to analyse continuous variables. An Anderson-Darling Test for normality was conducted for each variable, with those failing either square root or natural log transformed to assume a normal distribution. Outcome variables were the counts of total strongyle eggs (eggs per gram; EPG), strongyle eggs < 90µm (EPG), strongyle eggs ≥ 90µm (EPG), *Triodontophorus*-like eggs (EPG), *Parascaris* spp. (EPG) and *S. vulgaris* qPCR C_t values. Mean FECs were calculated based on FEC of all samples. Fixed effects of Location, Density and Habitat along with the random effect of Sample were included in the model. As there were limited cases where *Anoplocephala* spp. eggs were recorded, this variable was converted to a binary outcome, where *Anoplocephala* spp. were present or absent. *Anoplocephala* spp. and *S. vulgaris* PCR results were analysed separately using a General Linear Mixed Model with a binomial distribution. Fixed effects were Location, Density and Habitat, with the random effect of Sample.

3. Results

3.1 Strongyle faecal egg counts

A total of 293 faecal samples were collected (Table 1, Figure 1). The most intensively sampled localities were Cooleman Plain (n = 107) and Cowombat Flat (n = 95), sampled over two time points, and Kedumba Valley (n = 48), which was sampled over six time points (Table 1).

Strongyle eggs were detected in 100% of faecal samples (n = 289). Four samples from Kedumba Valley were omitted because the consistency suggested that these were not fresh faecal samples. Total strongyle FEC ranged from 50-3,740 EPG, with an overall mean of 1,443 EPG (Table 1, Figure 3). There were significant differences between the populations, classed by location (Table 1, Figure 3), density and habitat type ($P < 0.001$), with the highest FECs in medium density populations and alpine healthland habitats.

If the arbitrary strongyle FEC values used in domestic horse populations for classifying 'low' (< 200 EPG), 'moderate' (200-500 EPG) and 'high' (> 500 EPG) level shedders are applied, then for Kedumba Valley 5/44 (11%) samples were from low level shedders, 21/44 (48%) samples were from moderate level shedders, and 18/44 (41%) samples were from high level shedders, for Cooleman Plain 5/107 (5%) samples were from moderate level shedders and 102/107 (95%) samples were from high level shedders, from Cowombat Flat 1/95 (1%) were from moderate level shedders and 94/95 (99%) from high level shedders, and for Bogong High Plains, Tin Mines and the

Lower Snowy River 100% of samples (6/6, 16/16, 21/21 respectively) were from high level shedders.

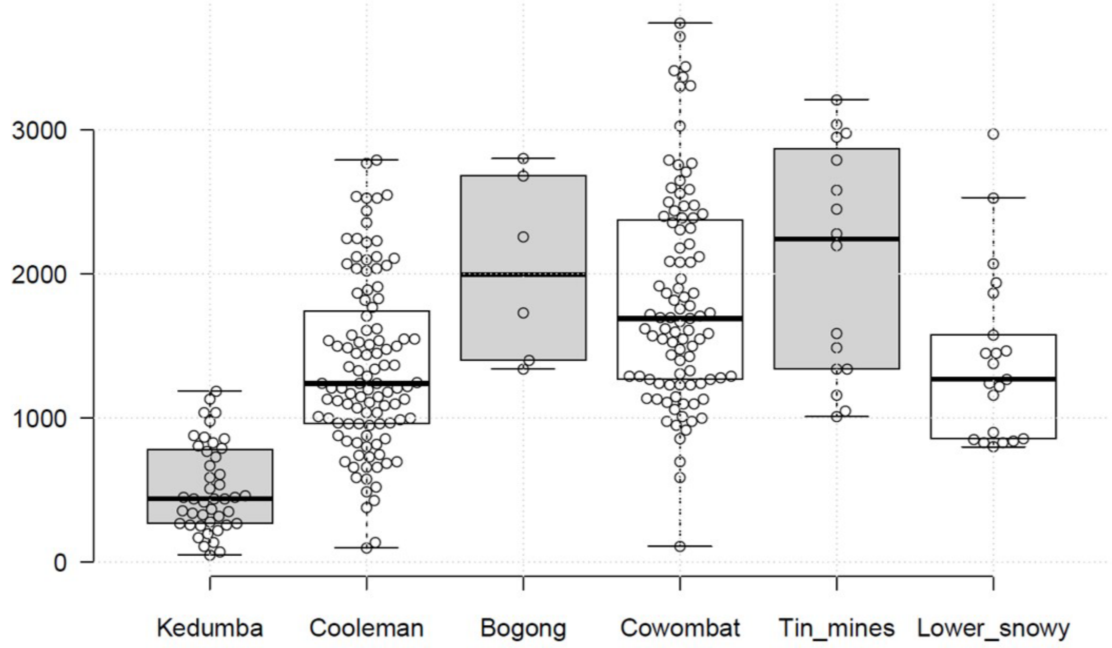

Figure 3. A box and whisker plot (with individual data points) of the total strongyle egg counts across the different populations, showing the highest FECs were from samples from Bogong High Plains and Tin Mines, both alpine heathland habitats. Overall 89% of samples had FECs > 500 EPG, classed as 'high level shedders'.

When strongyle eggs were divided into those < 90μm and ≥ 90μm, the FEC for eggs < 90μm ranged from 0-1,310 EPG (mean 229) and for eggs ≥ 90μm ranged from 30-2,900 EPG (mean 1,205) (Table 1). There were significant differences between the different populations, classed by location (Table 1), density and habitat type ($P < 0.001$). The highest FECs for eggs <90μm being in medium density populations and open grassland habitat, and the highest FECs for ≥90μm being in medium density populations and alpine heathland habitat (Table 2). *Triodontophorus*-like FEC ranged 0-190 EPG (mean 11) (Table 1) with the only significant difference ($P = 0.045$) being between population densities, the highest FEC occurring in medium density populations (Table 2).

Table 2. Significant differences in Australian wild horse parasite faecal egg counts between the locations, population densities and habitat type.

Variable	Total strongyle EPG	EPG eggs < 90 µm	EPG eggs ≥ 90 µm	EPG *Triodontophorus*-like	EPG *Parascaris* spp.	EPG *Anoplocephala* spp. (binary)
Location	<0.001*	<0.001*	<0.001*	0.145	0.07	0.001*
Population density	<0.001*	<0.001*	<0.001*	0.045*	0.011*	0.237
Habitat	<0.001*	<0.001*	<0.001*	0.225	0.209	0.032*

EPG – eggs per gram; **significant differences, P < 0.05*

3.2 *Parascaris* spp. and *Anoplocephala* spp. faecal egg counts

Parascaris spp. eggs were present in 25% (74/289) of samples, with FEC ranging from 0 – 1750 EPG (mean 31). There was a significant difference in *Parascaris* spp. FECs between the different populations, when classed by population density (P = 0.01), with the highest FECs being in medium density populations. There were no significant differences when classed by location and habitat type (Table 1). The prevalence of *Anoplocephala* spp. eggs across all populations was low with only 26 of the 289 samples testing positive for *Anoplocephala* spp. eggs (10-30 EPG). Statistical modelling indicated a 2% prevalence of *Anoplocephala* spp. eggs in all populations.

3.3. Strongylus vulgaris qPCR

S. vulgaris DNA was detected in 123/134 (92%) samples (C_t values ranged from 21.35-39.12, mean 26.58). The blank controls remained negative. A high prevalence of *S.*

vulgaris DNA in faecal samples was demonstrated across all six populations, with an overall predicted prevalence of 96.7% (Table 3). No significant differences between populations were detected when classed by location, density or habitat type. There was no significant correlation between *S. vulgaris* C_t values and either total strongyle FEC or FEC of eggs < 90 μm, illustrating that presence of *S. vulgaris* cannot be predicted based on FECs of strongyle eggs < 90 μm.

Table 3. Summary of qPCR results for *Strongylus vulgaris* DNA in Australian wild horses.

Location	**qPCR *S. vulgaris DNA* positive***	**Predicted prevalence**	**Predicted mean C_t value (SE)**
Kedumba valley	96% (23/24)	95.8%	26.80 (0.68)
Lower Snowy River	90% (10/11)	90.9%	26.12 (1.03)
Bogong High Plains	100% (6/6)	99.9%	29.95 (1.3)
Cowombat Flat	91% (32/35)	91.4%	26.89 (0.57)
Tin Mines	89% (8/9)	88.9%	27.24 (1.23)
Cooleman Plain	90% (44/49)	89.8%	25.76 (0.49)

* Number of samples testing positive/tested samples; SE - Standard error

3.4 Larval culture

Presence of *S. vulgaris* was confirmed by larval culture, with *S. vulgaris* larvae identified in 13/20 samples. Out of 100 larvae from each sample, 1 – 4 (mean 2.8) were identified as *S. vulgaris.* All 20 samples were positive for *S. vulgaris* DNA. Other larvae identified included *S. equinus* in 5/20 samples, *S. edentatus* in all 20 samples, *Triodontophorus*-like spp. in 19/20 samples, as well as cyathostomines (‘*Trichonema*’ spp. or small strongyles) in all 20 samples.

4. Discussion

It is widely accepted that *S. vulgaris* is the most pathogenic gastrointestinal parasite in horses, with an infective L_3 stage, and a migratory arterial L_4 stage, which may cause arteritis, hypertrophy and fibrosis of mesenteric arteries, and thrombosis (McCraw & Slocombe 1976; Ogbourne & Duncan 1985; Nielsen et al. 2016; Pihl et al. 2018). *Strongylus vulgaris* infection has been rarely reported since the 1960's following the advent of effective anthelmintics (Herd 1990; Geary 2005; Kaplan & Nielsen 2010). We demonstrate a high predicted prevalence (88.0 – 99.9 %; overall 96.7%) of *S. vulgaris* DNA in six wild horse populations in south-east Australia. Such a high prevalence across large wild horse populations has not been reported previously.

As a result of the rising resistance of cyathostomins (small strongyles) to anthelmintics, recommended anthelmintic strategies have changed to focus on increased parasite surveillance through FECs, and reduced anthelmintic treatment intensity (Duncan & Love 1991; Kaplan 2002; Nielsen et al. 2014; Becher et al. 2018). In Denmark, this has even led to anthelmintics becoming prescription only, with most veterinarians only prescribing anthelmintics if FECs are above a certain cut- off value (Nielson et al. 2006). This approach, however, raises concerns about the potential re-emergence of *S. vulgaris* (Nielsen et al. 2008, 2012, 2014, 2016).

Historically, non-invasive diagnosis of *S. vulgaris* infection has been challenging. Eggs cannot be definitively distinguished from other strongyle eggs, larval culture is time consuming, false negative results are common, expertise is required for species identification, and freshly collected faeces are required (Nielson & Reinemeyer 2018). The requirement for fresh faeces is challenging when collecting samples in remote locations. The qPCR assay used in this study enabled easier, more reliable, species-specific detection of *S. vulgaris* DNA in faeces (Nielsen et al. 2008, 2012; Kaspar et al. 2017). Applying this qPCR, 20/84 (24%) horses tested in Denmark were positive for *S. vulgaris*, validating concerns about its re-emergence with changes in anthelmintic strategies (Nielsen et al. 2008, 2012, 2014, 2016). Our study found 92% of samples from wild horses to be qPCR positive for *S. vulgaris* DNA. This suggests that if domestic horses graze on habitat occupied by infected wild horses, re-emergence in domestic horses is likely, if they are not routinely receiving anthelmintics.

Egg shedding patterns in domestic horses have been described to almost always follow the 20/80 rule, meaning that 20% of the horses' harbor 80% of the parasites in any given population (Kaplan & Nielsen 2010; Nielsen & Reinemeyer 2018). Even in the absence of anthelmintics, only a small proportion of 'high level shedders' (20-30%) produce the moderately large numbers of eggs responsible for environmental contamination (Nielsen et al. 2006; Becher et al. 2010; Kaplan & Nielsen 2010). Whether similar egg shedding patterns occur in wild horse populations has not been previously described. In the populations of horses in our study, the 20/80 rule was not followed. 257/289 (89%) wild horses from six different regions would be considered 'high level shedders' of strongyle eggs, if the cut-offs used in domestic horses are applied (Neilsen & Reinemeyer 2018). The FECs are likely to be representative, as studies have shown that egg-shedding of individual horses stays consistent over time (Scheuerie et al. 2016). Our findings are consistent with those reported in Sable Island (Nova Scotia, Canada) wild horses, where a mean total strongyle FEC of 1,543 EPG (SD 209.94) was identified across 447 horses (Debeffe et al. 2016), and in Fort Polk (Lousiana, USA) where wild horses had higher FECs than domestic horses, with 63.4% being high level shedders (Cain et al. 2018).

It is not well understood why some domestic horses shed higher numbers of eggs and others shed low numbers, but there are several factors known to influence shedding (Nielsen & Reinemeyer 2018). Host immunity plays a complex role in transmission and progression of parasite infections, and is itself influenced by other factors such as age (Chapman et al. 2003; Nielsen et al. 2006; Becher et al. 2010; Nielsen & Lyons 2017), genetics (Stear et al. 1984; Gasbarre et al. 1990; Kornas et al. 2015), and a range of stressors including nutritional, reproductive, thermal, exertional, social and health stressors (Segerstrom & Miller 2004; Nielsen & Reinemeyer 2018). Grazing behaviour and parasite factors also play a role (Herd & Willardson 1985; Nielsen & Reinemeyer 2018). We can, therefore, hypothesise that perhaps differences in the age distribution, genetics, stressors, and grazing behaviours of wild horses may account for the differences in egg shedding compared to domestic populations. A major limitation of this study, which makes interpretation of FECs challenging, is attributable to the nature of the wild horses, meaning that information such as age, health and reproductive status, was not able to be obtained or linked to the individual from which each sample was collected.

In our study, mean total strongyle FEC was significantly higher in medium density populations and alpine heathland habitats. The aforementioned limitations of this study make it difficult to speculate about the reasons for these differences. The alpine heathland habitats are moister with higher rainfall, at higher elevations and lower temperatures. FECs are also affected by seasonality, influenced by temperature and rainfall, with higher FECs expected during spring and summer (Herd & Willardson 1985; Wood et al. 2012, Misuno et al. 2018). In this study, all samples for most sites were collected during summer. Some samples for Kedumba were collected during autumn and spring, which could in part contribute to the lower mean FECs at this site.

The clinical significance of the 'high' FECs in these wild horses is difficult to determine, since FECs are not necessarily correlated with the size of worm burdens (Nielsen et al. 2010). The cut-offs of low/moderate/high level shedders are arbitrary values used in domestic horses simply to characterize the level of environmental contamination from that individual and thus guide targeted anthelmintic treatment, rather than estimating the impact of the parasite burden on an individual horse (Nielsen & Reinemeyer 2018). Nevertheless, it is likely that the consistently high FECs in the horses in this study are associated with burdens of small strongyles that may be causing significant intestinal disease (Love et al. 1999; Debaffe et al. 2016).

Most horses develop very strong acquired immunity to *Parascaris* spp., and egg shedding eventually ceases (Donoghue et al. 2015; Fabiani et al. 2016). Consequently, infections are most commonly observed in foals, weanlings and yearlings, and only occasionally seen in horses over 18 months-old (Fabiani et al. 2016). It is challenging, however, to interpret the clinical significance of *Parascaris* spp. FECs as developing immunity can result in a negative egg count even in the presence of a potentially pathogenic adult burden (Nielsen et al. 2015).

FECs have a very low sensitivity for detecting *Anoplocephala* spp. infection, as, unlike nematodes, they do not regularly release eggs. The predicted prevalences based on FECs are, therefore, likely to be substantially underestimated (Nielsen & Reinemeyer 2018).

Further work to assess parasite burdens and associated pathology in wild horses is needed to determine the clinical significance of the high prevalence of *S. vulgaris* DNA, the apparently high strongyle FECs, and the pathology and clinical significance associated with *Parascaris* spp. and *Anoplocephala* spp. infections, and other gastrointestinal parasites not detected with these diagnostic techniques.

On a positive note for domestic horses, small and large strongyles, as well as ascarids from wild horse populations serve as parasite *refugia* for strains that are not under anthelminthic selection pressure. Parasites that have never been exposed to anthelmintics, and thus have escaped selection for resistance, are known to delay the development of resistant parasites by outcompeting the resistant strains (Prichard

1990; Van Wyk 2001; Waghorn et al. 2015). Maintenance of diverse horse parasites under no anthelminthic pressure is an important component of integrated parasite management when facing the emerging anthelmintic resistance in horses (Nielsen et al. 2014; Nielsen & Reinemeyer 2018).

5. Conclusions

Despite 30 years of macrocyclic lactone anthelmintic use in domestic horses throughout Australia, *S. vulgaris* remains endemic in wild populations. There is ample opportunity for transmission to domestic horses, since wild horses from studied regions are regularly trapped, rehomed and domesticated. Furthermore, domestic horses are ridden through some of these wild horse habitats and allowed to graze during rest periods. It is important for horse owners who may adopt wild horses, or whose domestic horses may graze in wild horse habitats, to be educated about *S. vulgaris*, and advised appropriately to minimize the risk of their horses acquiring *S. vulgaris* infection. Monitoring domestic horse populations for re-emergence of *S. vulgaris* is prudent, particularly those in rural and remote locations that may not receive routine anthelmintic treatment. Since strongyles and ascarids from wild horse populations have not been exposed to anthelmintics, they may also become useful for tackling the emerging issues with anthelmintic resistance in domestic horses.

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Thesis outline summarizing chapter titles and contents		
Chapter	**Chapter title**	**Brief description**
1	Introduction: The wild horse controversy & the importance of incorporating animal welfare science in decision making.	Summary of the history of wild horses, their cultural significance, environmental impacts and controversies in wild horse management. Introduces the importance of animal welfare science in wild animal controversies.
2 Published	A Ten-Stage Protocol for assessing the welfare of individual non-captive wild animals: free-roaming horses as an example.	Presentation of a novel conceptual framework that I designed in order to guide a systematic and scientific approach to assessing the welfare of free-roaming wild animals, using the Five Domains Model. Summarizes the principles of interpreting indicators of biological function and behaviour in terms of the mental experiences that those indicators reflect (Stage 1 of my Protocol), and how the Five Domains Model is used for assessing welfare (Stage 2 of my Protocol).
3	Literature review: A review of the species-specific information required to enable assessment of wild horse welfare.	The current status of wild horse knowledge is summarized in a novel holistic and multidisciplinary framework drawing together the relevant literature on horses across each of the four physical/functional domains of the Five Domains Model. It addresses Stage 3 of my Ten-Stage Protocol.
4 Published	Addressing the knowledge gap of gastrointestinal parasitology of free-roaming wild horses in south-east Australia.	An example of the type of detailed original research required for addressing any knowledge gaps identified in Stage 3. This chapter describes a detailed parasitological investigation of 293 faecal samples collected from 6 wild horse populations. It describes results of faecal egg counts, larval cultures and molecular diagnostics.
5 **Published**	**Use of remote camera traps to evaluate animal-based welfare indicators in individual free-roaming wild horses.**	**A large body of original research investigating for the first time, both the use of remote cameras for identifying individual horses across a range of habitats, and for acquiring data on an extensive range of animal-based welfare indicators. It addresses Stages 4-6 of my Ten-Stage Protocol.**
6	Scientific validation of welfare indicators for using the Five Domains Model to grade the welfare status of individual free-roaming wild horses.	Addressing Stage 7 of my Ten-Stage Protocol, this chapter evaluates the scientific evidence linking the described objective measurable/observable welfare indicators to physical/functional impacts in Domains 1-4, and the relationship between those impacts and the mental experiences that are inferred in Domain 5. This concludes with the formulation of a Five Domains Model wild horse specific welfare grading scheme.
7	Dynamic changes in wild horse social organisation and habitat use revealed with remote camera trap monitoring.	With the aim of evaluating traditional ecological metrics alongside welfare status, this chapter describes original research using the remote camera trapping methodology described in chapter 5, to evaluate population dynamics, and temporal and spatial changes in social organization and habitat use of a wild horse population over a 15 month period.
8	The cascading influence of resource availability on the welfare status of wild horses, and correlation with demography, social organization and habitat use.	This original research applied the methodology from all preceding chapters to assess welfare status and changes in welfare status in individual wild horses over a 15 month period, addressing stages 8 -10 of my Ten-Stage Protocol. It further evaluates drivers of change in welfare status and correlations between welfare status, and the population dynamics, social organization and habitat use described in chapter 7.
9	Conclusions and future directions.	Summarizes overall conclusions, and contributions to the fields of wild animal welfare, wild horse ecology and welfare, wild horse management, and general horse welfare. Highlights ongoing work, some of which I have already partially completed that was not included in the main body of the thesis.

Chapter 5. Use of Remote Camera Traps to Evaluate Animal-Based Welfare Indicators in Individual Free-Roaming Wild Horses

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Simple Summary

Knowledge of the welfare status of wild animals is critical for informing debates about how we interact with them. However, methodology to enable assessment of the welfare of free-roaming wild animals has been lacking. In this study, we assessed the use of remote camera traps to non-invasively identify individual free-roaming wild horses and evaluate an extensive range of welfare indicators. Camera trapping was successful in detecting and identifying horses across a range of habitats including woodlands where horses could not be directly observed. Twelve indicators of welfare were assessed with equal frequency using both still images and video, with an additional five indicators assessed on video. This is the first time such a methodology

has been described for assessing a range of welfare indicators in free-roaming wild animals. The methodology described can also be adjusted and applied to other species, enabling significant advances to be made in the field of wild animal welfare.

Abstract

We previously developed a Ten-Stage Protocol for scientifically assessing the welfare of individual free-roaming wild animals using the Five Domains Model. The protocol includes developing methods for measuring or observing welfare indices. In this study, we assessed the use of remote camera traps to evaluate an extensive range of welfare indicators in individual free-roaming wild horses. Still images and videos were collected and analysed to assess whether horses could be detected and identified individually, which welfare indicators could be reliably evaluated, and whether behaviour could be quantitatively assessed. Remote camera trapping was successful in detecting and identifying horses (75% on still images and 72% on video observation events), across a range of habitats including woodlands where horses could not be directly observed. Twelve indicators of welfare across the Five Domains were assessed with equal frequency on both still images and video, with those most frequently assessable being body condition score (73% and 79% of observation events, respectively), body posture (76% for both), coat condition (42% and 52%, respectively), and whether or not the horse was sweating excessively (42% and 45%, respectively). An additional five indicators could only be assessed on video; those most frequently observable being presence or absence of weakness (66%), qualitative behavioural assessment (60%), presence or absence of shivering (51%), and gait at walk (50%). Specific behaviours were identified in 93% of still images and 84% of video events, and proportions of time different behaviours were captured could be calculated. Most social behaviours were rarely observed, but close spatial proximity to other horses, as an indicator of social bonds, was recorded in 36% of still images, and 29% of video observation events. This is the first study that describes detailed methodology for these purposes. The results of this study can also form the basis of application to other species, which could contribute significantly to advancing the field of wild animal welfare.

1. Introduction

Knowledge of the welfare status of wild animals is critical for informing ethical, legal, and political debates about the ways we interact with them. The importance of developing scientific methods for capturing data to enable the assessment of the welfare of free-roaming wild animals has recently been highlighted (Harvey et al. 2020). Many studies have evaluated wild horse behaviours, time budgets, home ranges, body condition scores and social organisation (e.g., Klingel 1975; Duncan 1979; Garrott & Taylor 1990; Berman 1991; Linklater 1998; Linklater et al. 2000; Cameron et al. 2003; Linklater et al. 2004; Dawson 2005; Cameron et al. 2008; Grange et al. 2009; Ransom & Cade 2009; Ransom et al. 2010; Scorolli & Cazorla 2010; Dawson & Hone 2012; Zabek 2015; Zabek et al. 2016; Scorolli 2020), but to date, an extensive range of welfare indicators has apparently not been assessed.

We recently published a Ten-Stage Protocol for scientifically assessing the welfare of individual non-captive wild animals, using free-roaming horses as an example (Harvey et al. 2020). Table 1 lists these ten stages.

Table 1. The Ten-Stage Protocol for assessing the welfare of non-captive wild animals (Harvey et al. 2020).

1. Acquire an understanding of the principles of Conservation Welfare.
2. Acquire an understanding of how the Five Domains Model is used to assess welfare status.
3. Acquire species-specific knowledge relevant to each Domain of the Model.
4. Develop a comprehensive list of potential measurable/observable indicators in each physical domain, distinguishing between welfare status and welfare alerting indices.
5. Select a method or methods to reliably identify individual animals.
6. Select methods for measuring/observing the potential welfare indices and evaluate which indices can be practically measured/observed in the specific context of the study.
7. Apply the process of scientific validation for those indices that can be measured/observed and insert validated welfare status indices into the Five Domains Model.
8. Using the adjusted version of the Model that includes only the validated and practically measurable/observable welfare status indices, apply the Five Domains grading system for grading welfare compromise and enhancement within each Domain.
9. Assign a confidence score to reflect the degree of certainty about the data on which welfare status has been graded.
10. Including only the practically measurable/observable welfare alerting indices, apply the suggested system for grading future welfare risk within each domain.

The protocol uses the Five Domains Model for assessing animal welfare (Mellor & Reid 1994; Mellor et al. 2009; Mellor & Beausoleil 2015; Mellor 2017; Mellor et al. 2020). The Five Domains Model comprises four interacting physical/functional domains of welfare—'Nutrition', 'Physical Environment', 'Health' and 'Behavioural Interactions'—and a fifth domain of 'Mental State'. Following measurement of animal-based indices within each physical/functional domain, the anticipated negative or positive affective consequences are cautiously assigned to Domain 5. Three of the ten stages of the Ten-Stage Protocol relate specifically to the Model: Stage 4, develop a comprehensive list of potential measurable or observable indicators of welfare in each physical/functional domain; Stage 5, select methods to reliably identify individual animals; and Stage 6, select methods for measuring or observing potential welfare indices, then evaluate which indices can be practically measured or observed in the specific context of the study. This last stage requires further investigation to identify which indices can be measured or observed using the selected methods, before detailed studies to assess welfare can be conducted. That is the primary focus of this paper.

Current knowledge of wild horse ecology and behaviour has historically relied on direct observations of horses (e.g., Klingel 1975; Duncan 1979; Garrott & Taylor 1990; Berman 1991; Linklater 1998; Linklater et al. 2000; Cameron et al. 2003; Linklater et al. 2004; Dawson 2005; Cameron et al. 2008; Grange et al. 2009; Ransom & Cade 2009; Ransom et al. 2010; Scorolli & Cazorla 2010; Dawson & Hone 2012; Zabek 2015; Zabek et al. 2016; Scorolli 2020). The disadvantages of direct observations for assessing indicators of welfare include practical limitations on the number of observations of individual animals that can be made, and usually the need to observe them from long distances. This may make evaluation of some welfare indicators challenging, and it restricts observations to only those animals that can be seen directly, primarily in open areas. Horses residing in woodland habitats and/or challenging terrain, or those that become separated from their band, for example due to debilitation or injury, are less likely to be observed directly and hence may be underrepresented in resulting datasets.

Camera traps have been widely used to study wildlife in a variety of habitats, e.g., (Cutler & Swann 1999; Silveira et al. 2003; O'Connell et al. 2010; Meek et al. 2015), focusing on a range of variables such as abundance and density (e.g., Silver et al. 2004) and demographic measures (e.g., Karanth et al. 2010; Smit et al. 2017). More recently, they have been used to collect behavioural data from a range of captive and wild animals (e.g., Duggan et al. 2016; Rose et al. 2018; Wooster et al. 2019; Fazio et al. 2020) and to evaluate leprosy-like skin lesions in wild chimpanzees (Hockings et al. 2020). They have also been used to collect data on some aspects of behaviour of wild Przewalski horses (Zhang et al. 2015; Schlichting et al. 2020). However, to date, there are apparently no published reports of camera trap use directed specifically at evaluating a comprehensive range of animal-based welfare indicators in any mammalian species, whether captive or free-roaming.

The aims of this study were to evaluate the use of camera traps, both still images and video, across a range of habitats, to assess (a) whether free-roaming wild horses could be detected and individually identified, (b) which animal-based welfare indicators could be practically and reliably evaluated, (c) the reasons for not being able to identify horses or assess welfare indicators, and (d) whether behaviour could be quantitatively assessed. We specifically describe our methodological information in detail in order to enable other researchers to replicate these methods. Furthermore, we sought to identify advantages and limitations of the use of camera traps for these purposes.

2. Materials and Methods

2.1. Study Overview

We made camera trap and direct observations of a small and geographically constrained population of free-roaming wild horses in Australia over a 15 month period.

Selection of Welfare Indicators

In line with Stage 4 of the published Ten-Stage Protocol (Table 1) for assessing welfare (Harvey et al. 2020), we developed an extensive list of potential measurable/observable indicators in each physical/functional domain, based on a literature search (using the terms 'horse welfare', 'equine welfare', 'welfare indicators') for previously reported animal-based welfare indicators in domestic horses (Henneke et al. 1983; Carroll & Huntington 1988; McDonnell & Haviland 1995; Wemelsfelder et al. 2001; McDonnell & Poulin 2002; McDonnell 2003; Ashley et al. 2005; Wemelsfelder 2007; Ransom & Cade 2009; Samuel et al. 2012 ; Dalla Costa et al. 2014a, 2014b; Hockenhull & Whay 2014; Mullan et al. 2014; Gleerup 2015; Dalla Costa et al. 2016; Hintze et al. 2017; Czycholl et al. 2018; Minero et al. 2018; Somerville et al. 2018; Sigurjónsdóttir & Haraldsson 2019; Torcivia & McDonnell 2021). As already noted, the physical/functional domains of the model are: 1. Nutrition; 2. Physical Environment; 3. Health; and 4. Behavioural Interactions (Mellor et al. 2020). From this list, indicators were divided into specific measures of current welfare status, and alerting measures that draw attention to potential welfare risks (Harvey et al. 2020). We then selected measurable or observable welfare indicators that may be able to be captured in free-roaming horses using camera trap still images and/or videos or by direct observation (Table 2).

Table 2. List of selected welfare indicators in each domain.

Domain		Animal-Based Indices	
		Still Images and Video	**Video Only**
1.	Nutrition	Body condition score	
2.	Physical Environment	Sweating Wet from rain Huddling together with other horses	Shivering
3.	Health	Body posture Hoof condition Coat condition Wounds or other injuries Limb pathology Skin lesions Nasal discharge Ocular discharge Blepharospasm Quidding Food pouching Facial grimace	Gait at walk Gait at trot Gait at canter Weakness Respiratory rate and effort
4.	Behavioural Interactions	Specific quantifiable behaviours (feeding, resting, maintenance, locomotory and social behaviours)	Qualitative assessment of behaviour (dull, relaxed, alert, apathetic, curious, anxious, playful)

The Study Area and Horse Population

Data were collected between December 2015 and March 2017 in the Kedumba Valley, an isolated section of approximately 130 km^2 within the Warragamba Special Area of the Blue Mountains National Park, being part of the Greater Blue Mountains World Heritage Area in New South Wales, Australia. The horse population there was known to be small and geographically constrained since immigration and emigration are inhibited by natural physical boundaries, with Lake Burragorang to the south, high cliff faces to the north and east, and a river and steep terrain covered with dense bush to the west (Figures 1 & 2). In the past, some population exchange has been known to occur when a low water level in Lake Burragorang allowed horses to cross to and from the other side, but this was not possible during the study period. Two dirt roads provided access to the valley at the northern and western borders, locked gates preventing their use by horses. No management interventions had occurred in the preceding eight years. This study was part of a larger investigation of the population ecology and welfare of free-roaming horses conducted over a 15 month period (National Parks and Wildlife scientific license 101626, WaterNSW access license D2015/128332).

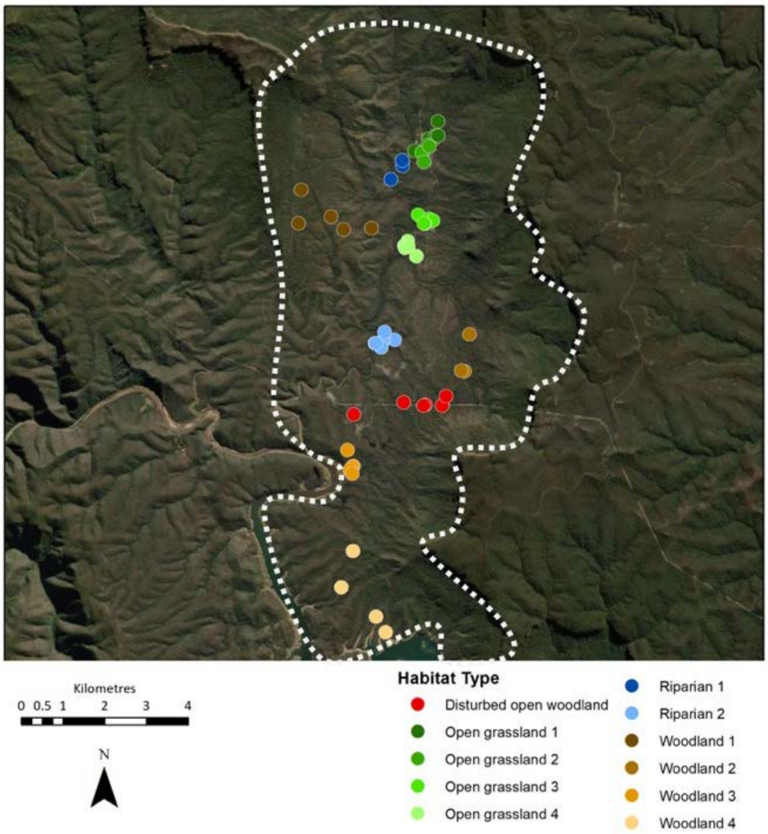


Figure 1. Topographical map of the study area illustrating camera locations and habitat types in the 11 areas. The white dashed line represents the geographic boundaries that inhibit immigration and emigration of this horse population.

Figure 2. View of Kedumba valley facing south. The grassland areas visible near the middle of the picture are the regions named Open grassland 1 and 2. Image A.M. Harvey.

Initial Surveys

Extensive on-ground surveys were performed with a 4WD vehicle and on foot to become familiar with all regions of the valley in order to assess how resources varied spatially and to identify locations of horse tracks and/or faeces. Avenza maps (Avenza Systems Inc., Toronto, Canada) were used on an iPad (Apple Inc., California, United States) to navigate around the area and record GPS locations of the presence and abundance of horse faeces, horse sightings, water access points and habitat details. There was evidence of horses inhabiting eleven distinct areas dispersed within the valley, comprising four different habitat types; four small areas of open grassland with scattered woodland; two riparian areas; one area of disturbed open woodland situated below powerlines; and four woodland areas where water was accessible within 2 km. Figure 3 illustrates these eleven distinct areas. The remaining habitat comprised eucalyptus woodland and shrubs across undulating and often very steep terrain. Riparian corridors throughout these areas were surrounded by a high cliff, making them inaccessible to horses (Figure 4).

Direct Observations

Direct observations were performed (by A.M.H.) on a total of 29 days, over 14 field trips of 1–3 days each, spread over the 15 month period. During each field trip, all eleven areas were surveyed, with direct observations occurring opportunistically whenever horses were sighted. The duration of direct observations depended mainly on how long horses stayed within sight, and so was not standardized. When horses stayed within sight for more than 1–2 min, they were observed using 10 × 42 binoculars (Bushnell Powerview FOV 293FT, Bushnell Corporation, Overland Park, KS, USA). A 900 m laser range finder was used to measure distances to horses, thereby enabling estimation of the maximum distances at which each welfare indicator could be assessed. Where possible, magnified photos and video footage (Canon EOS 70D Digital SLR with Canon EF100–400 mm lens) were taken of horses so that welfare indicators could be evaluated more closely at a later stage.

Camera Trap Placement and Settings

Initial on-ground surveys and direct observations were used to identify suitable camera locations to maximise image capture of horses, for example, near drinking locations, pathways to a drinking location or prime grazing area, dirt tracks, horse trails, river crossing points, stallion faecal piles, and open grassland grazing areas where there was an abundance of horse faeces.

Forty-seven cameras (Bushnell Aggressor; Bushnell Corporation, Overland Park, KS, USA) were used throughout the study period, placed in a total of 58 different locations. Thirty-five cameras remained in the same location for the entire study period, five camera locations were changed slightly to improve the viewing angle of horses or to overcome false triggers, and seven cameras were moved to a different area to improve capture of horses in a particular area. Of the 58 different camera locations, 23 were in grassland habitats, 17 in woodland habitats, 13 in riparian habitats, and 5 in a disturbed open woodland habitat (Figure 1). Cameras were placed at a height of 70–110 cm, depending on the terrain. On dirt tracks and creek crossings, cameras were positioned with the aim of capturing the front of the horse approaching the camera, whilst on open grassland areas they were positioned to capture as much of the grassland area as possible. Initially all cameras were set to take still images only. During various periods over the whole study, 18 of the cameras (nine in open grassland, four in riparian habitats, four in woodland habitats and one in the disturbed open woodland habitat) were set to take hybrid video records, which comprised a still image followed by a video clip. Once triggered, videos ran for a specified duration; video clip duration was set at 5, 10, 15, 20, or 30 s. Camera settings were as follows: 14M pixel image size or 1920 × 1080 video resolution; video sound on; capture number (i.e., number of still images taken in a sequence once the camera was triggered) ranged from one-to-three; camera time delay interval before responding to another trigger varied between 1 and 5 s; light-emitting diode (LED) control high, sensor level auto, night vision shutter speed medium, and camera mode 24 hrs. An identifying number for each camera, date, time and GPS co-ordinates, were entered into each camera. Camera locations were marked on Avenza maps to assist with relocating them. Each camera was checked, and batteries and secure digital (SD) cards changed, during each of the 14 field trips.

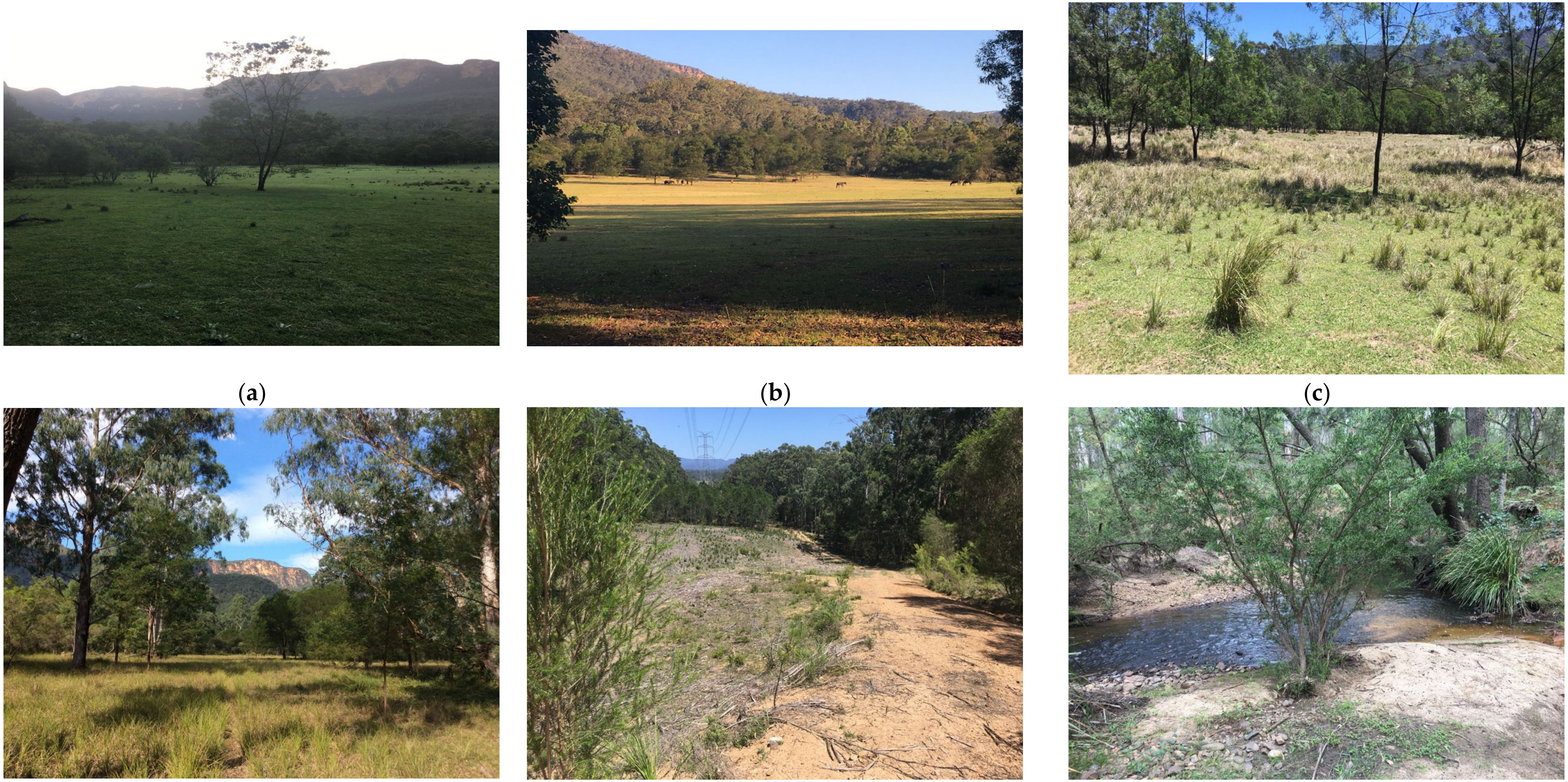


Figure 3a-k. The eleven distinct areas of four main habitat types where horses resided. **(a)** Open grassland 1. **(b)** Open grassland 2. **(c)** Open grassland 3. **(d)** Open grassland 4. **(e)** Disturbed open woodland. **(f)** Riparian 1.

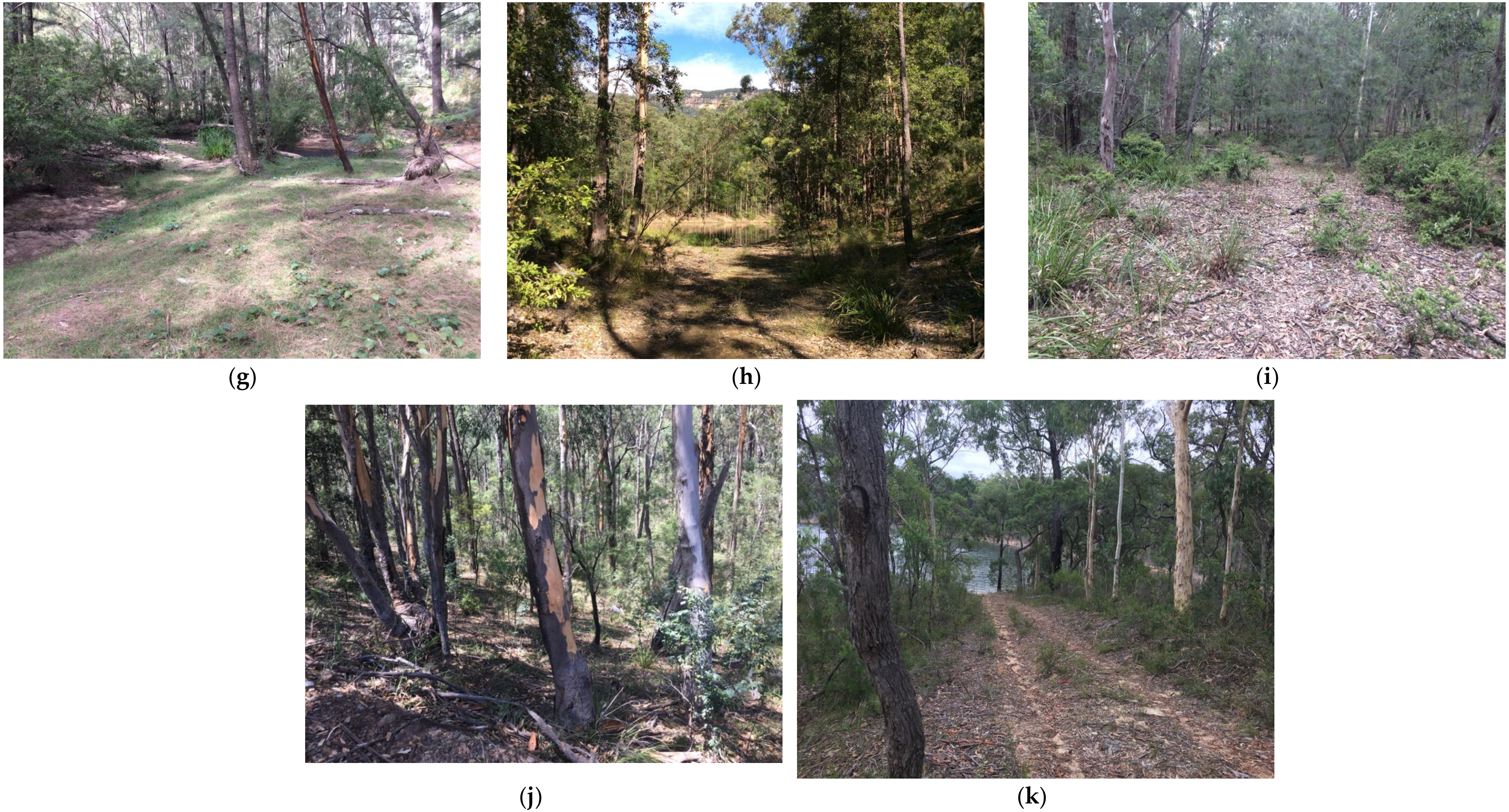


Figure 3a-k (cont'd), (g) Riparian 2. **(h)** Woodland 1. **(i)** Woodland 2. **(j)** Woodland 3. **(k)** Woodland 4. Images A.M. Harvey.

(**a**)

(**b**)

Figure 4. The predominant habitat and topography of Kedumba Valley: **(a)** Most of the region consisted of eucalyptus woodland across undulating and often steep terrain. **(b)** Most riparian corridors comprised steep cliffs making those sections of rivers inaccessible to horses. Images A.M. Harvey.

Acquisition of Demographic Details and Identification of Individual Horses

Camera trap images were used together with resighted identification marks of horses under direct observation, in order to obtain unique identifying features for each horse, in addition to its sex, age category, and familial relationships. Horses observed early in the study period close together on multiple occasions, in the same geographical area, but distant (>1 km) from other horses were considered to constitute a distinct band.

A list of identifying features was made for each horse, including body coat colour, limb coat colour (if different from the body), mane and tail colour (if different from the body), facial markings (blaze, stripe, star, snip) and their size and shape, and limb markings (including the length the marking extended up the limb). An identification diagram was also completed for each horse to record the presence, size, and shapes of facial and limb markings. A unique identifier was then assigned to each horse.

Assessment of Camera Trap Images and Videos

Images and video footage from all cameras over the 15 month study period were viewed and all 'sightings' where at least one horse could be identified were recorded. Each unique combination of horses captured within a duration of one second on a particular camera on a particular day constituted a separate sighting. From these data, a unique camera-day was defined as each camera-date combination where at least one horse was seen.

A stratified-random subset of camera-days from the full dataset of 2071 camera-days was used for detailed evaluation of whether individual horses could be identified and whether selected welfare indicators (Table 2) could be assessed. To do so, the study was divided into five 90-day time periods, broadly aligned with seasons (period 1: summer; period 2: autumn; period 3: winter, period 4: spring; period 5: summer) and for each camera method (still images or video), from each time period, one camera-day was selected from each camera that recorded sightings in the 90-day period, using computer-generated random numbers.

Within each selected camera-day, every observation event was assessed (by A.M.H.). An observation event was defined as a series of consecutive still images or video clips of the same horse on the same camera on the same day, with a maximum of a 3-min interval between consecutive images or video clips where the horse was visible. The observation event ended when the horse left the camera's field of vision and did not return within 3 min. Thus, one horse could have multiple observation events on the same camera on the same day. Other horses could be in view for part or all of another horse's observation event; each horse in view was treated as a separate observation event. Table 3 lists the details recorded for each observation event. For the purposes of this study it was only recorded whether or not it was feasible to assess each indicator, with regard to whether the relevant part(s) of the horse were captured at an appropriate distance to the camera, or for indicators such as gait evaluation and weakness, requiring dynamic records, whether enough of the horse was captured moving within video clips. Precise descriptions that enable the particular indicators to be categorized in terms of potential welfare compromise in each physical/functional domain will be provided in subsequent manuscripts.

Table 3. Details recorded from each observation event.

• Date, method (still image vs. video), camera number
• Identification of the individual horse when possible, if not recorded as unidentified
• Number of images of video clips in the observation event (and video duration setting)
• Start and finish time of the observation event
• Number of images (or for video, time) until the horse identity was determined
• Reason for being unable to identify an individual at all
• Whether the image or video was taken in the daylight or at night
• For each welfare indicator listed in Table 2, whether it was able to be assessed or not, and for those indicators whose status can differ between left- and right-hand sides of the same horse, whether they could be assessed on the left, right, or both sides
• Reason(s) why any of the welfare indicators could not be assessed
• Where there were two or more video clips within an observation event, each video clip was also individually assessed for all of the above

Qualitative behaviour assessment (QBA) (Wemelsfelder et al. 2001; Wemelsfelder 2007; Hintze et al. 2017; Minero et al. 2018) was performed on video-observation-events, by an experienced observer (A.M.H.), using the fixed descriptors of 'dull', 'relaxed', 'alert', 'apathetic', 'curious', 'anxious', 'playful'. If such assessment was not possible, the reasons were recorded. In fact, QBA scores have not been reported here because the present purpose was restricted to evaluating whether or not video-observation events captured enough of the 'whole horse' behaviour to enable a QBA to be conducted.

Observations of specific behaviours were also quantified. Behaviours were assigned to five main categories using a previously defined ethogram for free-roaming horses Ransom & Cade 2009). Categories were: locomotion (subcategorised as walking, trotting, cantering); standing resting; grazing; maintenance behaviours (including grooming, nursing, rolling, lying down, standing alert, drinking, urinating, defaecating); and social behaviours (included allogrooming, play behaviour, nuzzling, sexual interactions, and other affiliative and agonistic social interactions).

A behaviour event was defined as an occurrence or period in which an individual horse performed a particular behaviour without interruption. The duration of these behaviour events was recorded in terms of the number of images and/or times when the behaviour was observed. The end of a behaviour event occurred either when the horse left the camera's field of view or changed to a different behaviour. Table 4 lists additional information recorded from each behavioural event.

These results were used to evaluate the relative proportions of the different behaviours captured by camera traps. For still images, the relative proportions of different behaviours were calculated separately, based on the number of images, and the period of time for which the same behaviour was observed (Table 4).

Table 4. Information recorded from each behavioural event.

- The identifiable behaviour (or recorded as unidentifiable and reasons for that)
- Number of images per behaviour (for still images)
- Start and finish times of the behaviour (for still images this was recorded as the time of the first and last images in a series of consecutive images in which the behaviour was captured)
- The habitat in direct field of view of the camera (i.e., grassland, dirt track, riparian, river crossing, waterhole, horse track through woodland, rest area beneath trees, woodland clearing)

Social bonds were also analysed by reference to 'close spatial proximity' (Wolter et al. 2018). For the purposes of this study, this was defined for each observation event as occurring if other horses were present at the same time in any image or video frame in addition to the focal horse for that observation event, or in a subsequent image taken within one second of the previous image. Since close spatial proximity could occur concurrently with a range of other behaviours, proximity was analysed separately to other social behaviours. Furthermore, for less frequently performed social behaviours, such as allogrooming, play behaviour, nuzzling, sexual interactions, and other affiliative and agonistic social interactions, an 'all occurrence' method was used where the entire dataset of 2071 camera-days (still images and video clips pooled) was evaluated, and the behaviour recorded each time it was captured on images or videos.

All data were entered into standardized Excel 2011 spreadsheets (Microsoft, Washington, United States) with separate spreadsheets for data from still images, from video, and for quantifying behavioural observations.

2.2. Data Analyses

Statistical analyses were performed using Stata (version 16.1; StataCorp, Texas, TX, USA). The numbers of images required for a horse to be identified via still-image observation events was assessed as time-to-event data using Kaplan–Meier survivor and failure functions (Goel et al. 2010), where the 'failure' event in this context was identification of the horse. This approach allowed inclusion of observation events where the horse was not identified. For these events, the record was right-censored at the maximum image number for the horse in that observation event. In time-to-event analyses, right-censoring is used when a subject leaves the study before an event occurs (in this case before the horse is identified). The Kaplan–Meier failure function value at a specified number of images is equivalent to the cumulative percentage of observation events where the horse was identified by that number of images if none-were right-censored. Survivor functions were compared using log rank tests, by day/night, habitat, horse coat colour, facial marking and limb marking categories. For horse-level factors, only observation events where the horse was identified were used. The same methods were used to determine the times at which the horse was identified for still-image observation events. Stata's-*sts list*-and *sts test* commands were used.

Separately for each welfare indicator, proportions of observation events where an indicator could be assessed were compared between habitats and between methods (still images or video) using logistic regression, with both variables fitted simultaneously. Stata's *logistic* command was used. For bilateral welfare indicators (e.g., wounds, limb pathology, and skin lesions), each observation event was classified as one of: could be assessed on both sides/one side/neither side. Then, separately for each welfare indicator, generalised ordered logit models were fitted using the *gologit2* command in Stata. Where the *p*-value for the proportional odds/parallel lines assumption was > 0.05, proportional odds were assumed and ordered logit models were fitted using Stata's *ologit* command. Likelihood ratio test *p*-values were used.

Reasons why welfare indicators could not be assessed were compared between habitats and between methods using the same approach as that for evaluating whether non-bilateral welfare indicators could be assessed (i.e., those welfare indicators that could be assessed without seeing both sides of the horse), with one exception. Blurred images were not an impediment in any of the video-observation events, so methods were compared using exact logistic regression with Stata's *exlogistic* command. Habitat was not fitted, and sufficient statistics were used, rather than the other main statistical alternatives of conditional scores tests or conditional probabilities tests. For all analyses, observation events were treated as if they were statistically independent of each other after accounting for the variables fitted in each model.

3. Results

3.1. Demographic Details of the Population

Twenty-nine horses were identified in the population during the study period. These horses were distributed across five bands, with a total of 5 stallions, 16 mares, 3 fillies, 4 colts and one foal of unknown sex (Table 5).

3.2. Direct Observations

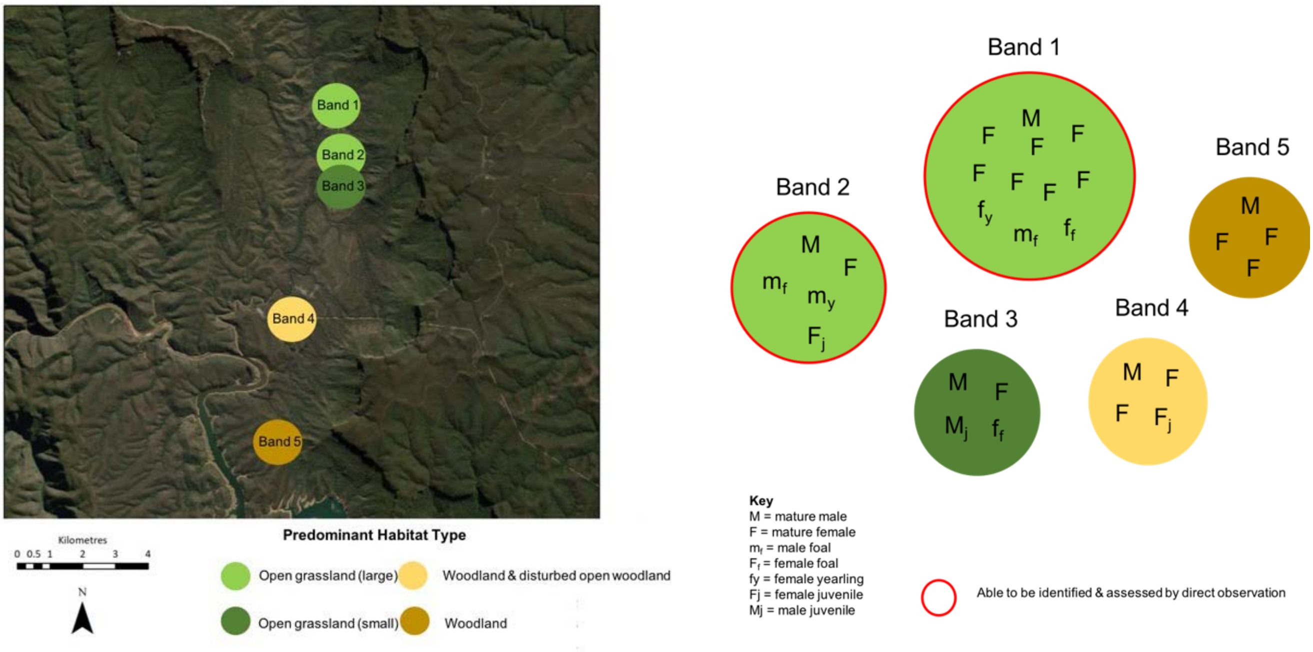
All individuals in Bands 1 (11 horses) and 2 (5 horses), who predominantly frequented open grassland habitats, were identified during the first two of the 14 field trips. The remaining 13 horses in the population were never identified by direct observations during the 15 month study. Figure 5 illustrates the band compositions, their habitats and whether or not they were directly observed.

Horses were observed and identified in open grassland habitats during all 14 field trips and in riparian habitats during 4/14 field trips. Horses were observed in woodland and disturbed open woodland habitats during 4/14 field trips, but only 3 horses were identified (on a single field trip) during observations in woodland habitats, and none were identified in disturbed open woodland. On all other occasions in the woodland and disturbed open woodland habitats, horses moved out of sight quickly, preventing their identification or assessment of welfare indicators.

Table 5. Details of the population and unique identifying features.

Unique Identifier[1]	Sex[2]	Age Group	Reproductive Status	Coat Colour	White Facial Markings	White Limb Markings[3]
1A	M	Mature	Band stallion	Black	Star and snip	None
1B	F	Mature	Foaled year 2	Chestnut	Star and stripe	BLH socks
1C	F	Mature	No foal	Chestnut	None	BLH and RF fetlocks
1D	F	Mature	No foal	Black	Small star	BLH pastern spots
1E	F	Mature	No foal	Black	None	BLH and LF pastern
1F	F	Mature	Yearling (1G) at foot	Black	Large star	BLH fetlocks
1G	F	Yearling	Yearling of 1F	Brown	Blaze	BLH socks
1H	F	Mature	Foal (1K) at foot	Black	None	BLH pasterns
1I	F	Mature	Foal (1J) at foot	Dark bay	Star and stripe	BLH fetlocks and LF pastern
1J	M	Foal	Foal of 1I	Black	Large stripe	LH pastern, small spot medial RH pastern
1K	F	Foal	Foal of 1H	Black	Small star and snip	None
1L	Unknown	Foal	Foal of 1B (year 2)	Bay	Star and snip	None
2A	M	Mature	Band stallion	Flaxen chestnut	Blaze	LH stocking
2B	F	Mature	Foal (2C) at foot	Flaxen chestnut	Blaze	None
2C	M	Foal	Foal of 2B	Flaxen chestnut	Blaze	None
2D	M	Yearling	Dam unknown	Flaxen chestnut	Thick blaze	BLF socks, LH stocking
2E	F	Juvenile	No foal	Black	Large star and stripe	LF pastern, BLH fetlocks
3A	M	Mature	Band stallion	Brown	Stripe	BLH socks
3B	F	Mature	Foal (3C) at foot	Black	Star and stripe	BLH and RF pasterns
3C	F	Foal	Foal of 3B	Black	Large stripe	BLH and LF fetlocks
3D	M	Juvenile	Colt with another stallion in Band	Flaxen chestnut	Blaze	LH sock
4A	M	Mature	Band stallion	Black	None	LH fetlock, RF coronet
4B	F	Juvenile	No foal	Black	Very small star	RH fetlock, LF pastern
4C	F	Mature	No foal	Black	Interrupted stripe	LH pastern, RH coronet
4D	F	Mature	No foal	Chestnut	Stripe	RH fetlock
5A	M	Mature	Band stallion	Black	Star	LF pastern, BLH fetlocks
5B	F	Mature	No foal	Chestnut	Very small star	None
5C	F	Mature	No foal	Black	Large star	LH fetlock, RH coronet
5D	F	Mature	No foal	Black	Large star	LH fetlock, RF coronet

[1]Number = band number; Letter = sequential letter within band. [2]M = male; F = female.
[3]LH = left hindlimb; RH = right hindlimb; LF = left forelimb; RF = right forelimb; BLH = bilateral hindlimb; BLF = bilateral forelimb.



(**a**) (**b**)

Figure 5. Illustration of **(a)** the geographical location and predominant habitat type of the different bands, and **(b)** band compositions. Only horses in Bands 1 and 2 that resided on larger open grassland habitats could be identified and assessed by direct observation. Horses in all other bands and habitats could only be identified and assessed with remote camera traps**.**

Welfare indicators could only be assessed directly in the 16 horses (Bands 1 and 2) in the larger open grassland habitats, the primary determinants of success being how long each horse remained in sight and how close it was to the observer. Proximity influenced whether or not individual horses could be identified, and which welfare indicators could be assessed (Figure 6). Using 10 × magnification binoculars, the distances where specific attributes were observable were as follows: horses within approximately 150 m could be identified as individuals and their body posture and behaviour assessed; at approximately 100 m, all indicators other than facial indicators (i.e., facial grimace, blepharospasm, ocular discharge, nasal discharge, food pouching and quidding), and hoof condition could be assessed, although closer distances enabled more accurate assessment and detection of more subtle abnormalities; and within 50 m, facial indicators and hoof condition could be assessed. Photographs/videos were acquired with a 400 mm lens, which provided 8 × magnification, enabling welfare assessments at similar distances to dynamic live direct observations with binoculars, but with the benefit of less hurried deliberation.

Although welfare indicators may be assessed at longer distances using more powerful magnification devices, the observer needs a clear line of sight, unobscured by trees and undulating terrain. In this study, the maximum such distance was approximately 300 m, which only occurred on one open grassland location occupied by Band 1. Horses were mostly observed from distances of 50–100 m, and so the facial indicators and hoof condition could not always be assessed. Horses in Bands 1 and 2, which were observed regularly, became more habituated to observer presence throughout the study. By the end of the study, all of their welfare indicators could be assessed for longer periods at distances of 20–30 m. In all other habitats, the unobscured line of sight was rarely greater than 20 m, but none of this sub-population of horses would allow such close proximity.

(**a**)

(**b**)

(**c**)

(**d**)

Figure 6a-j. Examples of identification of horses and assessment of welfare indicators with direct observations (with 8–10x magnification), and some reasons for not being able to assess indicators. **(a)** Behaviour can be assessed but other indicators are obscured by vegetation. **(b)** Close spatial proximity with other horses can be assessed, but other indicators are obscured by vegetation. **(c)** At >100m, behaviour is being observed without disturbing the horses, but the distance is too great to assess other welfare indicators. **(d)** At 100m from the horse most welfare indicators can be assessed, but in this case the orientation of the horse prevents assessment of facial features and the right side of the horse.

(e) (f) (g) (h)

Figure 6a-j (cont'd). (e) At approximately 50m, most welfare indicators can be assessed, but at this distance the observer will be seen by the horse, altering its behaviour. Distal limbs are obscured by vegetation. **(f)** Facial indicators can be assessed from approximately 50m. **(g)** The detail of facial features is much greater < 50 m to the horse but it is rare to be able to approach them so closely. **(h)** Trot is more readily assessed with direct observations.

(i)

(j)

Figure 6a-j (cont'd). (i) Canter is more readily assessed with direct observations. **(j)** A good example of where most welfare indicators can be assessed on the right side of the horse, but at such a close distance behaviour is impacted by the observer's presence.
Images A.M.Harvey.

3.3. Camera Trapping

General Statistics

Over the 15 month period, a total of 220,836 image/video files (a file constitutes a single image or video clip) were obtained. Of these, 42,925 (19%) image/video files contained horses whilst the remainder contained only other animals (e.g., macropods) or were false triggers due to wind-blown vegetation moving in front of the camera.

One or more horses were seen and identified via at least one camera on 428 days (95%) of the study period. For individual cameras, one or more horses were seen and identified on a mean of 9.5% of the camera's days (range 0–45.9%). By habitat, of the cameras located in open grasslands, the corresponding average was 12.5% of the camera's days (range 0–45.5%), which was similar to cameras located in disturbed open woodland (mean 14.4%; range 0–45.9%). In contrast, horses were seen and identified on lower proportions of days by cameras located in riparian (mean 6.8%; range 1.7–21.6%) and woodland habitats (mean 3.6%; range 0–21.2%).

In all, 199 camera-days were randomly selected from the 2071 camera-days that had detected horses, of which 158 days were still images and 41 were videos. Within the 158 camera-days of still images, there was a total of 538 observation events (range 1–23 observation events per camera day, mean 3.4, SD 3.44, median 2). Within the 41 camera-days of video clips, there was a total of 119 observation events (range 1–11 observation events per camera-day, mean 2.9, SD 3.85, median 1).

For still images, there was a range of 1–36 images per observation event (mean 4.2, SD 4.75, median 3) with the duration of observation events ranging from <1–257 s (mean 47, SD 113, median 4). For the 411 observation events with more than one image, the time between images ranged from <1–163 s (mean 14.5 secs, median 4.3, SD 25.76).

For videos, the duration of observation events ranged from 1 to 252 s (mean 29.3, SD 46.3, median 5). The most common total observation event durations were 5 s in 40/119 (33.6%), 10 s in 12/119 (10.1%), and 30 s in 38/119 (31.9%). Observation events comprised between 1 and 11 separate video clips with 26/119 (20.2%) involving more than one clip (mean 1.63).

Longer video clip durations resulted in the horse being visible for longer (Table 6), such that each additional 5 s added, on average, 3.1 s where the horse was visible. For the 5 s duration, the horse was usually visible for the entire 5 s, whereas, for the 10-, 15- and 30 s durations, the horse was visible for 50–61% of the time (Table 6).

Table 6. Video clip durations and the period during which the horse was visible in the video clip.

Video Clip Duration Setting (Seconds)	Number Of Video Clips	Number of Video Clips That a Horse Was Visible for Full Video Duration	Mean Period during Which a Particular Horse Was Visible in Video Clip (Seconds)
5	110	106 (96%)	4.9
10	20	10 (50%)	7.3
15	18	11 (61%)	11.2
20	6	1 (17%)	8.3
30	40	22 (55%)	20.6

Identification of Individual Horses

Of the 29 horses in the study population, 27 (93%) had at least one randomly selected still image observation event, with the number of such events for each of those horses ranging from 2 to 33 (mean 14.9). Twenty horses (69%) had at least one randomly selected video observation event, with the number of observation events per horse ranging from 1 to 11 (mean 3.2).

For still images, the individual horse was identified in 405 of 538 (75%) observation events. In 62% (333/538; 95% CI, 58% to 66%) of observation events, the horse was identified from the first image. The Kaplan–Meier failure function (indicating successful identification) rose to 83% (95% CI, 78% to 87%) by five images. Increases with further images were only small. By 16 images, the Kaplan–Meier failure function was 93% (95% CI 86% to 97%). For the time until identification, the horse was identified immediately in 333/538 (62%) observation events. The Kaplan–Meier failure function was 70% by 10 s (95% CI, 66% to 75%) and 77% (95% CI, 73% to 82%) by 30 s.

Identification of individual horses was more rapid with still images taken during daylight than with images taken at night ($p < 0.001$ for both number of images and time to identification). During the day, 70% of horses were identified with one image, and 88% of horses were identified after 4 images, whereas at night only 42% of horses were identified after one image, and 61% after 4 images (Figure 7). Patterns of time to identification were similar (Figure 7).

There were no substantial differences in the numbers of images or times to identification between horses with different coat colours or markings.

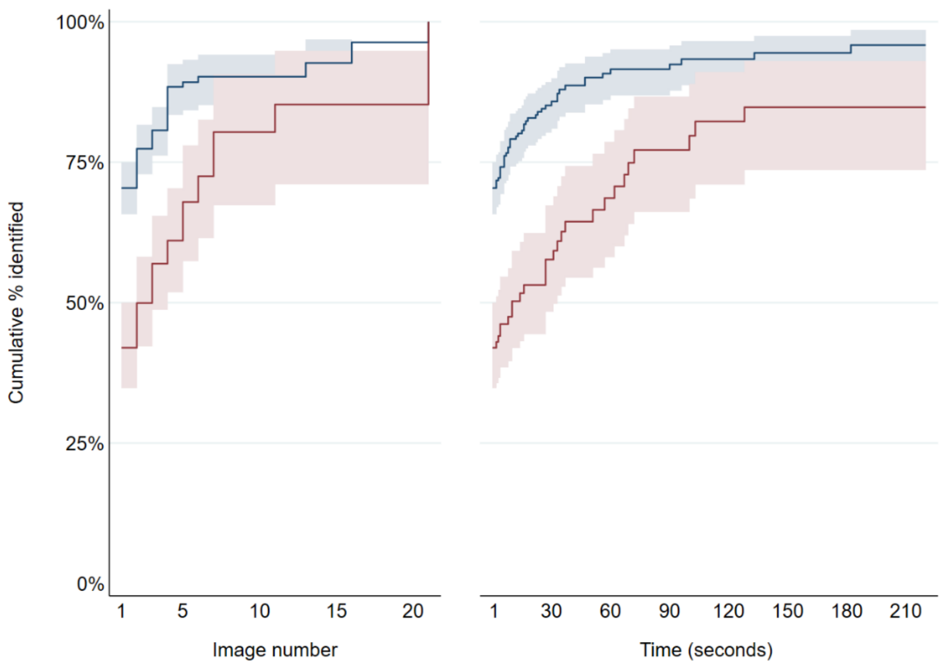


Figure 7. Cumulative percentages of observation events where the horse was identified by number of images (left-hand graph) and time from start of each observation event (right-hand graph) from still image observation events in the daytime (blue) and night-time (red). Kaplan–Meier failure functions are graphed; these are equivalent to cumulative percentages of observation events where each horse had been identified by the specified x-axis if no observation events had been right-censored. Shaded areas are point-wise 95% confidence intervals.

Of the 538 observation events, 300 occurred in open grassland habitats, 99 in riparian areas or at river crossings, 93 in woodland habitats, and 46 in disturbed open woodland habitats. Numbers of images until the horse was identified varied by habitat (overall p = 0.03), with the greatest numbers of images required for identification in observation events in open disturbed woodland habitats (Figure 8).

The most common reason for not being able to identify a horse in the first image was being unable to see facial or limb markings in the image (n = 31). Other reasons were only a small part of the horse being in the image, for example only the back, side, neck, shoulder or head side on (n = 24), the horse being too distant +/− at night (n = 5) and horse obscured by another horse or vegetation (n = 2).

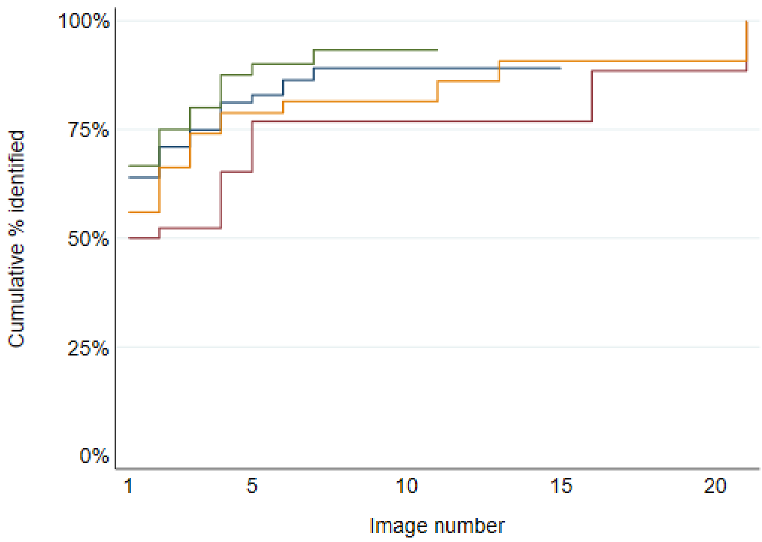


Figure 8. Cumulative percentages of observation events where the horse was identified by number of images from still image observation events in various habitats: riparian (green); open grassland (blue); woodland (orange); open disturbed woodland (red). Kaplan–Meier failure functions are graphed; these are equivalent to cumulative percentages of observation events where the horse was identified by the specified x-axis value if no observation events had been right-censored.

The reasons for not being able to identify a horse at all for a whole observation event were only a small part of the horse being in the image (n = 57), the images either being too dark, blurred and/or the horse too distant (n = 31), being unable to see facial or limb markings in the image (n = 25), and the images captured at night (n = 24). Some examples are shown in Figure 9.

(**a**)

(**b**)

(**c**)

(**d**)

Figure 9a-t. Examples of identification of horses, and assessment of welfare indicators with camera trap images, and common reasons for not being able to identify horses or assess indicators. **(a)** This horse was too close to the camera resulting in only the top half of the horse being in the image, therefore it was not possible to identify or assess welfare indicators other than body condition on this particular image. **(b)** Although this image was taken during daylight, shadowing from trees resulted in the image being too dark to identify the horse or welfare indicators. **(c)** A night-time image illustrating the difficulty in identifying horses or indicators in night-time images. **(d)** These horses' distinctive markings make them identifiable, and it can be determined that both horses are cantering, however blurring of the image means no other welfare indicators are able to be assessed.

(e)

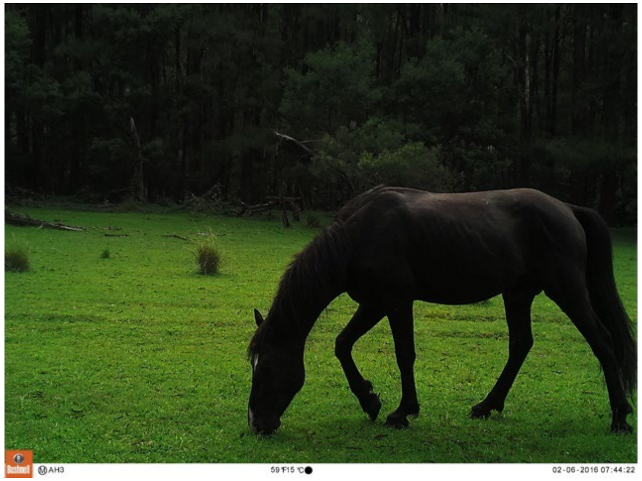
(f)

(g)

(h)

Figure 9a-t (cont'd). (e) This horse can be identified from its colour and hind limb markings, and it is evident that the horse is grazing, but no other welfare indicators can be assessed due to only the back of the horse being visible. **(f)** This horse can be identified, and it is evident that the horse is walking; body posture, body condition, hoof condition and presence of limb pathology can be assessed, but other welfare indicators are unable to be assessed as the image is too dark. **(g)** This horse cannot be identified on this single image as the shape of the blaze is not evident from the side-on image, and the limb markings are obscured by the water. Body condition and presence of wounds on the right side can be assessed. **(h)** The horse on the left cannot be identified as only the rump and tail are in the image, however it is enough to ascertain that the horse on the right is not alone. The horse on the right can be identified from its limb markings, and is observed to be grazing but the image is too dark and the horse too distant from the camera to be able to assess any other welfare indicators.

(i)



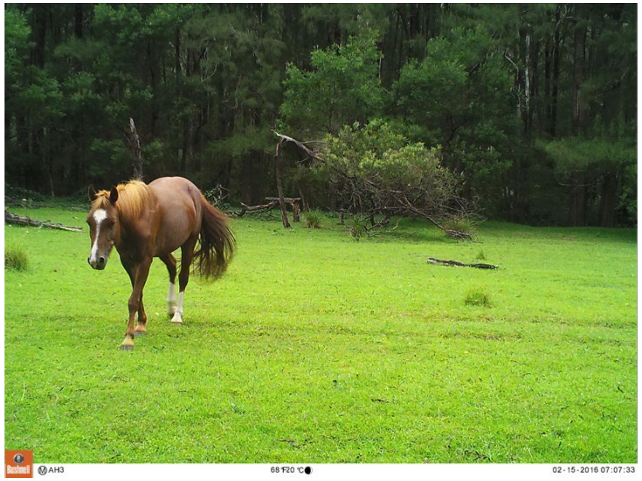
(j)

(k)

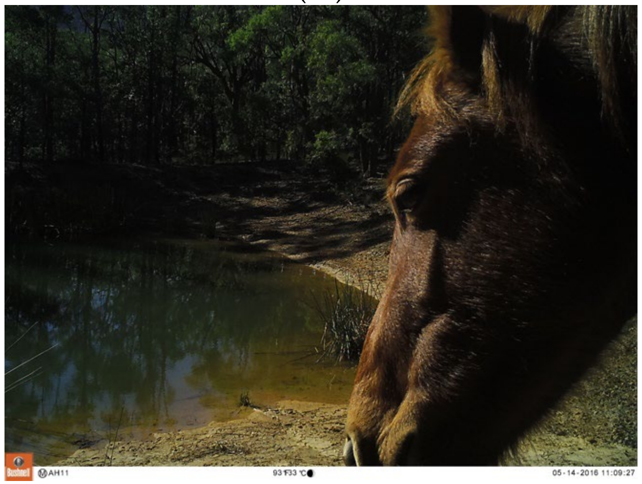
(l)

Figure 9a-t (cont'd). (i) This horse can be identified from his colour, limb markings, sex, and length of tail suggesting he is juvenile. It is evident that he is grazing, but he is too distant from the camera to enable assessment of other welfare indicators. **(j)** A close-up image of a horse just at the perfect distance and orientation to the camera to enable both its identification and assessment of all welfare indicators on the left side. **(k)** This horse is identifiable from her colour and distinctive facial marking, and it is evident that she is grazing. An appreciation of body condition is possible although an accurate assessment of body condition score cannot be made with the limited proportion of the body captured in the image, and she is too distant to the camera to be able to assess facial indicators accurately. No other indicators are able to be assessed from this image. **(l)** This horse is identifiable from her facial marking, and it is evident that she is cantering. Body posture and body condition can be assessed, but she is too distant from the camera to enable assessment of other welfare indicators.

(**m**)



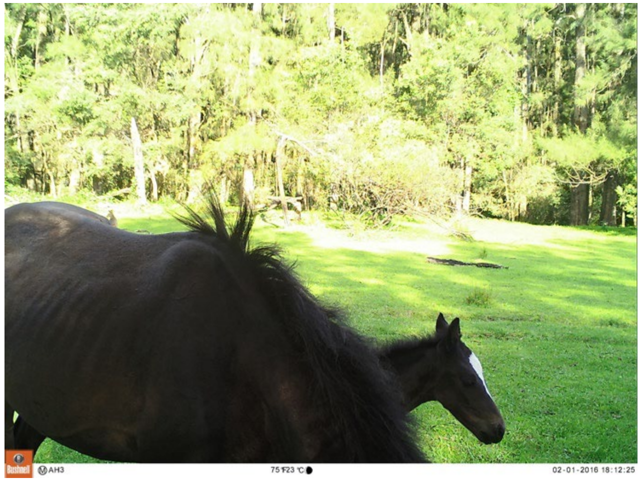
(**n**)



(**o**)

(**p**)

Figure 9a-t (cont'd). (m) This image captures four horses from Band 2 cantering together and illustrates close spatial proximity between them all. Most welfare indicators can be assessed from the left side of the horse in the centre of the image, and a variable number of indicators can be assessed for the other horses due to distance from the camera or only half the horse being in the image. **(n)** This horse is at a good orientation to and distance from the camera to enable most indicators to be assessed on the left side. **(o)** A close up facial image enables assessment of facial indicators on the left side of this horse. **(p)** Although a night-time image, this horse is can be identified, and body condition and body posture assessed.



(**q**)

(**r**)

(**s**)

(**t**)

Figure 9a-t (cont'd). (q) Only parts of the horses are visible in this image hindering assessment of welfare indicators, however the distinct facial marking on the foal enables his identification; facial indicators on the right side can be assessed in the foal, whilst body condition can be assessed in the mare. **(r)** This image shows close spatial proximity between two identifiable horses, with the horse on the left grazing and the horse on the right walking in a relaxed posture. Body condition, coat condition, body posture, limb pathology, presence of wounds on the left side, and, in the chestnut horse, some facial indicators on the left side can be assessed. **(s)** The orientation and distance of this horse from the camera enable most indicators to be assessed on the left side, except hoof condition since the front hooves are missing from the image. **(t)** This image shows close spatial proximity between three horses with the two on the right grazing and the chestnut on the left standing alert. Body posture and body condition can be assessed, but distance from the camera precludes assessment of other welfare indicators.

For camera trap video, horses were identified in 72% (86/119) of observation events, and when this was the case, it was always possible to identify horses at the beginning of the observation event. Reasons for the horses not being identifiable were only a small part of the horse being in the video (n = 18), the horse being too far from the camera (n = 12), and an inability to see facial markings (n = 1).

3.4. Assessment of Welfare Indicators

The welfare indicators that can be assessed most frequently on both still camera trap images and video, were body condition score (73% and 79% of observation events, respectively), body posture (76% for both), coat condition (42% and 52%, respectively), and whether or not the horse was sweating excessively (42% and 45%, respectively) (Table 7). Coat condition was the only welfare indicator where there was evidence of differences between still images and videos, where it could be assessed more often via video (Table 7).

Body condition score was less likely to be assessable in open grassland (67% of observation events) than in other habitats (80% to 85%; overall p-value for habitat adjusted for method <0.001). Percentages of observation events where body posture could be assessed were similar for each habitat (74% to 78%) and the p-value for differences between habitats was high (0.860). Hoof condition, coat condition, sweating, and facial grimace were all less able to be assessed in open grassland habitats (for each, $p < 0.001$).

Table 7. Welfare indicators assessed with camera trap still images and video.

Welfare Indicator	Able to Be Assessed With: Still Images: Number of Observation Events (% of 538 Observation Events)	Video: Number of Observation Events (% of 119 Observation Events)	*p*-Value for Comparison of Ability to Assess Indicator between Still Image and Video Observation Events[1]
Body posture	411 (76%)	91 (76%)	0.972
Body condition score	392 (73%)	94 (79%)	0.237
Coat condition	225 (42%)	62 (52%)	0.053
Sweating	226 (42%)	54 (45%)	0.587
Facial grimace	102 (19%)	20 (17%)	0.441
Hoof condition	92 (17%)	25 (21%)	0.382
Wounds:			0.227
Both sides could be assessed	24 (4%)	8 (7%)	
Only one side could be assessed	198 (37%)	49 (41%)	
Limb pathology:			0.611
Both sides could be assessed	144 (27%)	40 (34%)	
Only one side could be assessed	36 (7%)	0 (0%)	
Skin lesions:			0.538
Both sides could be assessed	23 (4%)	7 (6%)	
Only one side could be assessed	180 (33%)	42 (35%)	
Nasal discharge:			0.604
Both sides could be assessed	21 (4%)	15 (13%)	
Only one side could be assessed	89 (17%)	13 (11%)	
Ocular discharge and blepharospasm:			0.550
Both sides could be assessed	17 (3%)	15 (13%)	
Only one side could be assessed	107 (20%)	14 (12%)	
Quidding or food pouching:			0.220
Both sides could be assessed	4 (0.7%)	5 (4%)	
Only one side could be assessed	80 (15%)	8 (7%)	
Gait at walk	NA[2]	60 (50%)	NA
Gait at trot	NA	0 (0%)	NA
Gait at canter	NA	0 (0%)	NA
Weakness	NA	78 (66%)	NA
Respiratory rate and effort	NA	43 (36%)	NA
Shivering	NA	61 (51%)	NA
Qualitative behavioural assessment	NA	71 (60%)	NA

[1]*p*-value for method after adjustment for habitat. [2]NA: not assessed-we considered a priori that it would never be possible to assess these indicators in still image observation events.

The most common reasons for not being able to assess particular welfare indicators were images/videos being captured at night and the horse being too distant from the camera, such as in open grasslands (Table 8). Assessment of hoof condition was prevented when hooves were obscured by vegetation, mud, or water. Although night-time commonly prevented a range of welfare indicators from being identified, body condition score (68% and 75%, respectively) and body posture (75% for both) could be assessed as frequently in night-time as in daylight observation events, whereas coat condition could only be assessed in 22% of night-time observation events compared with 51% of those in daylight. Some examples are shown in Figure 9.

An additional seven welfare indicators were assessed only in video observation events as we considered a priori that it would never be possible to assess these with still image observation events. Of these, the indicators that were able to be assessed most frequently were presence or absence of weakness (66%), qualitative behavioural assessment (60%), presence or absence of shivering (51%) and gait at walk (50%, Table 7). Gait at trot and canter could not be assessed in any observation events, but respiratory rate and effort could be assessed in 36% of video observation events (Table 7). For assessments of hoof condition and facial grimace in video observation events, numbers were sufficient to assess the usefulness of additional video clips within the observation event. For 15 and 20 observation events, respectively, these indicators could not be assessed in the first video clip nor in any of the further 1 to 10 video clips.

Gait at walk (overall p = 0.010), respiratory rate and effort (overall p = 0.006), and shivering (overall = p 0.018) were less likely to be assessable in open grassland habitats than in woodlands. The most common reasons for not being able to assess video-specific welfare indicators were: only a small part of the horse being in the camera's field of view (n = 28); the horse not moving substantially during the video recording (n = 18); the horse being too distant from the camera (n = 16); and the horse not being in the camera's field of view for long enough (n = 11).

Table 8. Reasons for being unable to assess welfare indicators on camera trap still images and video.

Reason for Being Unable to Assess Welfare Indicators	Still Images: Number of Observation Events (% of 538 Observation Events)	Video: Number of Observation Events (% of 119 Observation Events)	*p*-Value for Comparison of Reason for Being Unable to Assess Welfare Indicator between Still Image and Video Observation Events[1]
Night-time images	172 (32%)	31 (26%)	0.345
Only top half of horse in the camera's field of view	96 (18%)	26 (22%)	0.273
Only front of horse in the camera's field of view	26 (5%)	1 (1%)	0.022
Only back of horse in the camera's field of view	59 (11%)	20 (17%)	0.090
Only head in the camera's field of view	35 (7%)	2 (1%)	0.020
Obscured by another horse	13 (2%)	1 (0.8%)	0.213
Hooves obscured by vegetation, mud or water	129 (24%)	18 (15%)	0.037
Horse too distant from the camera	178 (33%)	36 (30%)	0.845
Image too dark	190 (35%)	7 (6%)	<0.001
Image blurred	57 (11%)	0 (0%)	<0.001 [2]

[1]Likelihood ratio test *p*-value for method after adjustment for habitat. [2] Exact *p*-value for method; no adjustment for habitat.

3.5. Quantifying Behavioural Observations

From the random selection of camera days, 601 behaviour events were identified on still images, the specific character of which could be identified in 560/601 (93%) of these (Table 9). On video clips, 213 behaviour events were identified, the specific character of which could be identified in 178/213 (84%) of these. Behaviours were unidentifiable if the horse was still and the head and/or limbs were not in the camera's field of view.

The most commonly observed behaviours were grazing and walking, with grazing occupying a larger proportion of time, being 60% when based on duration of time taken from still images, 43% when derived from number of still images, and 51% when timed from video clips (Table 9). Social behaviours were rarely observed, with only two such events detected, namely, a horse sniffing a stallion faecal pile, and one horse sniffing another.

Table 9. The number and durations of identifiable behaviours calculated from camera trap still images and videos.

Behaviour	Number of Behaviour Events from Still Images (n = 560)	Number of Behaviour Events from Video (n = 178)	Number (%) of Still Images (n = 2159 Images[1]) With This Behaviour	Duration (%) of Time from Still Images (n = 36,711 s[1]) With This Behaviour	Duration (%) of Time from Video (n = 1683 s[1]) With This Behaviour
Locomotion total	341	83	902 (42)	6916 (19)	465 (28)
Walking	325	83	872 (40)	6900 (19)	465 (28)
Trotting	8	0	14 (<1)	8 (<1)	0
Cantering	8	0	16 (<1)	8 (<1)	0
Standing resting	56	31	276 (13)	7669 (20)	299 (18)
Grazing	148	56	938 (43)	22025 (60)	851 (50)
Maintenance behaviours	13	7	39 (2)	99 (<1)	65 (4)
Social behaviours	2	1	4 (<1)	2 (<1)	3 (<1)

[1]Total numbers are for pooled behaviour events where the behaviour was identified.

In addition, close proximity of the focal horse to other horses was recorded in 236/657 (36%) of observation events (202/538 (38%) for still image and 34/119 (29%) for video observation events). Using the 'all occurrence' approach to further evaluate uncommonly observed behaviours, specific social interactions between two or more horses were only recorded on 39 occasions within the full dataset of 42,925 image/video files. These interactions were mostly affiliative (35 affiliative interactions including allogrooming, being herded or herding other horses, trotting, cantering or galloping with other horses, frolicking, nuzzling, playing, physical contact with another horse whilst walking), with three occasions of reproductive behaviours (mating/being mated, flehmen response, winking), and one agonistic event (chasing another horse).

4. Discussion

This paper describes, for the first time, camera trapping methodology that can be successfully applied across a range of habitats in order to identify individual horses, and to non-invasively measure or observe a range of animal-based welfare indicators. Furthermore, it demonstrates the advantages and limitations of this methodology.

Methodological information is often lacking in camera trap publications (Meek et al. 2014). We have sought to describe methods in sufficient detail to enable other researchers to easily replicate them. Further, as a result of this study we can make recommendations for others wishing to use camera trapping for this purpose. Firstly, extensive ground surveys to facilitate precise and strategic camera placement are key to optimising image quality and detection of horses at appropriate angles and distances from the camera in order to both identify individual horses and assess a range of welfare indicators. Deploying cameras on tracks, grazing areas, and drinking locations within the same region assists in capturing the full range of listed welfare indicators, including a wider range of behaviours. Secondly, the choice of camera settings is important to enhance the data obtained whilst also minimising battery usage and SD card storage. We recommend using an image capture number of one (i.e., the number of still images taken in a sequence once the camera is triggered), and a camera time delay interval of 1 s for cameras placed on tracks where horses may pass quickly, and 3 s for cameras overlooking grazing or drinking locations. For videos, a duration of 10 s is recommended. Other settings recommended are as described in the Materials and Methods. Whether or not to use both day-time and night-time settings is dependent on the precise purpose of the study; although fewer horses and welfare indicators can be identified on night-time images, meaningful information can still be obtained. Use of video will increase the range of welfare indicators that can be assessed, but does significantly increase battery usage, SD card storage, and the time required for processing and analysing the data. Whilst there are strategies for increasing battery power (e.g., solar panels) and storage (higher memory SD cards), these can be costly, and despite security measures, vandalism and theft can be an issue. We therefore recommend prioritising use of video in habitats where horses cannot be reliably observed directly, in regions where repeated capturing of non-target species is less likely, and where cameras are able to be checked within a 1 to 2 month period. In other situations, still images may suffice and complement direct observations.

In woodland habitats, direct viewing of horses is challenging and relying on this would have significantly underestimated the population size in this study. A major advantage of camera trapping is the ability to identify individual horses in any habitat. Furthermore, demographic information such as herd size and reproductive rates varied spatially and across different habitats, so such data obtained from direct observation alone would not have represented the whole population. Understanding population dynamics and the processes that influence them is critical for management of populations. In Australia, there are few demographic studies on wild horses (Berman 1991; Dawson 2005; Dawson & Hone 2012), particularly within woodland habitats (Zabek 2015; Zabek et al. 2016). In south-east Australia, although free-roaming horses commonly reside in woodland habitats (eucalyptus forests) and undulating and often steep terrain, population and demographic data for horses in these habitats are lacking. Our study suggests that utilising camera trapping methodology could address some of these knowledge gaps.

Practical limitations in the frequency with which direct observations can be performed is another challenge in assessing the welfare of any free-roaming wild species. Both the severity and the duration of welfare impacts are important in assessing welfare (Mellor & Reid 1994; Mellor et al. 2009, 2020; Mellor & Beausoleil 2015; Mellor 2017; Harvey et al. 2020), so repeated assessments are advantageous. Beneficially, the continuous collection of data by camera traps permits more frequent assessments to be made. Further advantages are that the horses are not disturbed, and are captured close-up, thereby enabling a range of welfare indicators to be assessed and informative behaviours to be quantified, even in horses that cannot be observed directly.

To date, published studies have predominantly used invasive measures of health that require physically capturing the animal to perform procedures such as physical examination and blood collection (reviewed by Kophamel et al. 2021). Whilst these measures are informative, if available, it is preferable to use the least invasive methods for welfare assessments. Our study shows that a wide range of indicators of different aspects of welfare can be evaluated non-invasively using camera traps, and for some horses also by distant direct observations.

Twelve indicators of welfare aligned with the first four domains of the Five Domains Model, were able to be assessed with equal frequency on both still images and video, with an additional five indicators able to be assessed on video. The most practically measurable and reliable animal-based welfare indicators able to be detected were: in Domain 1 (Nutrition), body condition score; in Domain 2 (Physical Environment), presence of sweating or shivering; in Domain 3 (Health), body posture, coat condition, gait at walk, and presence/absence of weakness; and in Domain 4 (Behavioural Interactions), qualitative behavioural assessment and assessment of close spatial proximity for evaluation of social bonds. Spatial proximity of horses may have been overestimated on grasslands due to the greater field of vision of cameras in this habitat, compared to woodlands. In future studies, we therefore recommend defining close spatial proximity as animals standing within two body lengths of each other (Wolter et al. 2018), rather than animals simply being in the same image or video frame. Facial indicators and hoof condition could only be assessed infrequently due to the need for clear close-up images/videos of these body parts, the correct angle of view and an absence of obscuring vegetation. Some indicators were less likely to be detectable in open grassland habitats than in other habitats, because the horses were often further away from the camera in grassland habitats. Additionally, for bilateral indicators, most commonly only one side of the horse could be assessed.

Quantification of specific behaviours was also achievable by evaluating the proportions of time that each behaviour was captured, or the proportion of still images demonstrating a particular behaviour. Since camera traps only capture the behaviour being performed at the precise time that the horse is within camera view, these proportions do not necessarily accurately reflect continuously recorded time budget behaviours (Duncan 1979; Berman 1991; Ransom & Cade 2009; Ransom et al. 2010). Camera location may also bias the behaviour detected, e.g., grazing is more likely to be detected on open grassland and walking more often captured on tracks. However, if cameras remain in the same location over time, useful information may be obtained regarding temporal changes in the proportions of different behaviours being performed.

Despite the many advantages of remote camera traps, there are limitations that need to be considered when interpreting data. Information can only be collected when horses are in the camera's field of view, which is heavily dependent on camera placement and the number of cameras deployed. Problematically on still images, it can be difficult to assess the motivation for a behaviour, and whether it reflects a positive or negative mental state (Domain 5). For example, rolling can be both a maintenance behaviour (McDonnell 2003; Ransom & Cade 2009) and an indication of abdominal pain (Ashley et al. 2005; Torcivia & McDonnell 2021); lying in lateral recumbency is commonly linked to rest (McDonnell 2003; Ransom & Cade 2009), but can also be due to abdominal pain (Ashley et al. 2005; Torcivia & McDonnell 2021); and rubbing/scratching is a normal maintenance behaviour (McDonnell 2003; Ransom & Cade 2009), but when performed excessively can be an indicator of pruritus (Fadok 1995; Metz et al. 2011). Similarly, although some features of 'facial grimace' (Dalla Costa et al. 2014a; Gleerup 2015) could sometimes be identified on still images, the significance of this may be over interpreted when based on a single still image. When these events are observed directly or with video, additional indicators and the context of the behaviour usually assist interpretation.

5. Conclusions

As far as the authors are aware, this is the first study that describes in detail a remote camera trapping methodology that enables identification of individual free-roaming wild horses across a range of habitats and the assessment of an extensive range of animal-based welfare indicators. Camera trap images and video provided valuable information about the horses, particularly those that could not be sighted regularly, sighted for a long enough duration, or approached closely enough to enable direct assessment of welfare indicators, as was the case in woodland habitats.

The next phases of this research include applying the same methodology to larger populations across different geographical areas, in addition to incorporating these methods into a welfare assessment protocol to objectively evaluate how the welfare of wild free-roaming horses varies spatially and temporally. The described methodology can also form the basis of applications to other species.

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Thesis outline summarizing chapter titles and contents		
Chapter	**Chapter title**	**Brief description**
1	Introduction: The wild horse controversy & the importance of incorporating animal welfare science in decision making.	Summary of the history of wild horses, their cultural significance, environmental impacts and controversies in wild horse management. Introduces the importance of animal welfare science in wild animal controversies.
2 Published	A Ten-Stage Protocol for assessing the welfare of individual non-captive wild animals: free-roaming horses as an example.	Presentation of a novel conceptual framework that I designed in order to guide a systematic and scientific approach to assessing the welfare of free-roaming wild animals, using the Five Domains Model. Summarizes the principles of interpreting indicators of biological function and behaviour in terms of the mental experiences that those indicators reflect (Stage 1 of my Protocol), and how the Five Domains Model is used for assessing welfare (Stage 2 of my Protocol).
3	Literature review: A review of the species-specific information required to enable assessment of wild horse welfare.	The current status of wild horse knowledge is summarized in a novel holistic and multidisciplinary framework drawing together the relevant literature on horses across each of the four physical/functional domains of the Five Domains Model. It addresses Stage 3 of my Ten-Stage Protocol.
4 Published	Addressing the knowledge gap of gastrointestinal parasitology in free-roaming wild horses in south-east Australia.	An example of the type of detailed original research required for addressing any knowledge gaps identified in Stage 3. This chapter describes a detailed parasitological investigation of 293 faecal samples collected from 6 wild horse populations. It describes results of faecal egg counts, larval cultures and molecular diagnostics.
5 Published	Use of remote camera traps to evaluate animal-based welfare indicators in individual free-roaming wild horses.	A large body of original research investigating for the first time, both the use of remote cameras for identifying individual horses across a range of habitats, and for acquiring data on an extensive range of animal-based welfare indicators. It addresses Stages 4-6 of my Ten-Stage Protocol.
6	**Scientific validation of welfare indicators for using the Five Domains Model to grade the welfare status of individual free-roaming wild horses.**	**Addressing Stage 7 of my Ten-Stage Protocol, this chapter evaluates the scientific evidence linking the described objective measurable/observable welfare indicators to physical/functional impacts in Domains 1-4, and the relationship between those impacts and the mental experiences that are inferred in Domain 5. This concludes with the formulation of a Five Domains Model wild horse specific welfare grading scheme.**
7	Dynamic changes in wild horse social organisation and habitat use revealed with remote camera trap monitoring.	With the aim of evaluating traditional ecological metrics alongside welfare status, this chapter describes original research using the remote camera trapping methodology described in chapter 5, to evaluate population dynamics, and temporal and spatial changes in social organization and habitat use of a wild horse population over a 15 month period.
8	The cascading influence of resource availability on the welfare status of wild horses, and association with population demographics, social organization and habitat use.	This original research applied the methodology from all preceding chapters to assess welfare status and changes in welfare status in individual wild horses over a 15 month period, addressing stages 8 -10 of my Ten-Stage Protocol. It further evaluates drivers of change in welfare status and correlations between welfare status, and the population dynamics, social organization and habitat use described in chapter 7.
9	Conclusions, applications and future directions.	Summarizes overall conclusions, and contributions to the fields of wild animal welfare, wild horse ecology and welfare, wild horse management, and general horse welfare. Highlights ongoing work, some of which I have already partially completed that was not included in the main body of the thesis.

Chapter 6. Scientific validation of welfare indicators for using the Five Domains Model to grade the welfare status of individual free-roaming wild horses

Abstract

The Five Domains Model for assessing welfare aligns with the understanding that physical and mental states are linked. Following measurement of indices within each physical/functional Domain, the anticipated negative or positive affective consequences are cautiously inferred, and assigned to Domain 5. Those inferences derive credibility from validated knowledge of the underlying systems physiology, neurophysiology and affective neuroscience.

Any indices used for assessing welfare need to be scientifically validated, in line with Stage 7 of my Ten Stage Protocol (Chapter 2, Harvey et al. 2020). This requires scientific evidence of the links between a measurable/observable indicator and the physical/functional impact (in Domains 1 to 4), and of the relationship between the physical/functional impact and the mental experience it is proposed to reflect (in Domain 5).

Current knowledge of free-roaming wild horses within each of the four physical/functional domains (Stage 3 of the Ten Stage Protocol), was reviewed in Chapter 3. This knowledge informed Stages 4-6 of the Ten Stage Protocol which was addressed in Chapter 5 where novel methods for practically measuring/observing a range of animal-based welfare indicators in free-roaming horses were described

(Harvey et al. 2021). This chapter has now used those indicators of physical/functional states in Domains 1 to 4, that were shown to be feasible for assessing in free-roaming wild horses, and evaluated the scientific evidence linking them to inferred mental experiences in Domain 5. It further provides a guide for grading welfare compromise, enhancement and future risk (based on welfare alerting indices), which can be applied to individual free-roaming wild horses.

This is the first time that the scientific evidence validating a comprehensive range of welfare indicators has been synthesised in this way. The material in this chapter may also act as a guide for others on scientifically supporting key aspects of welfare assessment protocols they may wish to apply to other species in a range of situations. The adjusted version of the Five Domains Model presented in this Chapter can now be applied to grade welfare in individual free-roaming wild horses, in accordance with Stages 8 and 9 of my Ten Stage Protocol. Further, the new grading scheme devised for grading future welfare risk can also be applied as per Stage 10 of my Ten Stage Protocol, described in Chapter 2 (Harvey et al. 2021). These applications are demonstrated in Chapter 8.

1. Introduction

As described in detail in Chapter 2 (Harvey et al. 2020; Stages 1 and 2 of my Ten Stage Protocol), contemporary animal welfare science aims to interpret indicators of biological function and behaviour in terms of the mental experiences that those indicators are likely to reflect. The Five Domains Model for assessing welfare status was described in detail in Chapter 2, and is illustrated again here (Figure 1). It structurally represents the understanding that physical and mental states are linked, and facilitates the assessment of welfare based on the current understanding of the functional bases of negative and positive subjective experiences that animals may have. Following measurement of indices within each physical/functional Domain, the anticipated negative or positive affective consequences are cautiously inferred, and assigned to Domain 5. It is these mental experiences that contribute to descriptions of the animal's welfare state.

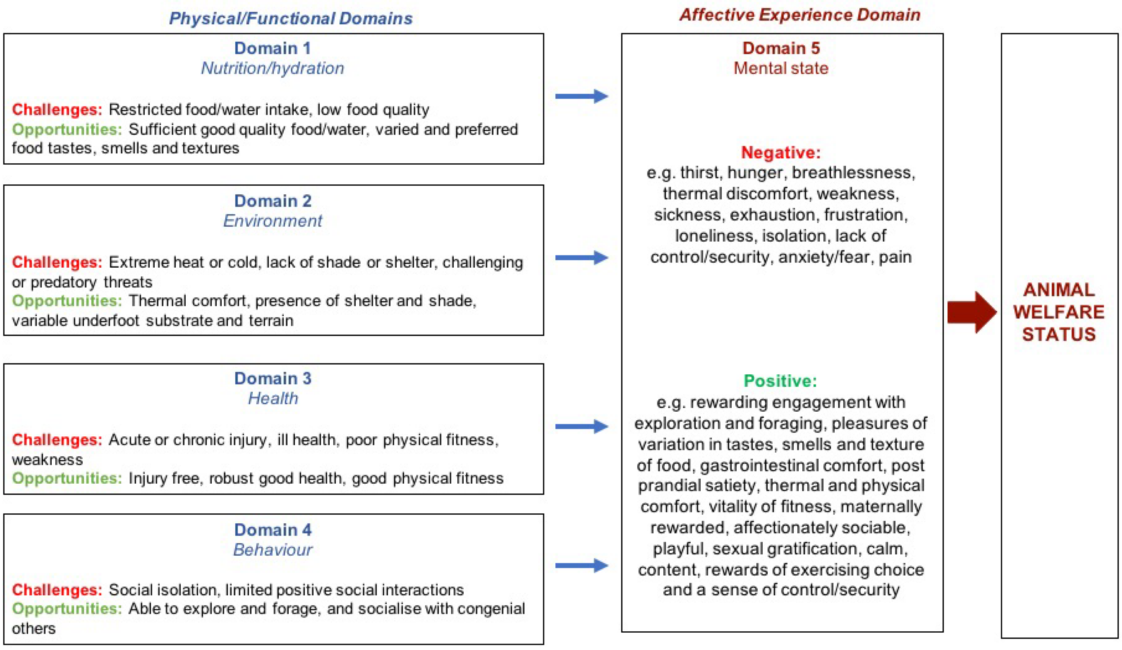

Figure 1. Reproduced from Harvey et al. 2020 (Chapter 2). An abbreviated schema of the Five Domains Model (adapted from Littlewood & Mellor 2016), showing negative and positive physical/functional states or situations (Domains 1-4) and examples of their associated negative and positive mental experiences or affects (Domain 5), relevant to free-roaming wild horses. Taken together, these mental experiences represent the overall welfare state of the animal.

Although mental experiences are subjective, those inferences derive credibility from validated knowledge of the underlying systems physiology, neurophysiology and affective neuroscience, as also from the caution exercised when inferring the presence of particular affects (Mellor & Beausoleil 2015; Mellor 2017; Mellor et al. 2020).

Current knowledge of free-roaming wild horses within each of the four physical/functional domains (Stage 3 of the Ten Stage Protocol), was reviewed in Chapter 3. This knowledge informed Stages 4-6 of the Ten Stage Protocol which was addressed in Chapter 5 where novel methods for practically measuring/observing a range of animal-based welfare indicators in free-roaming horses were described (Harvey et al. 2021).

Stage 7 of the Ten Stage Protocol then involves applying the process of scientific validation to those indicators of welfare. This requires scientific evidence of the links between a measurable/observable indicator and the physical/functional impact (in Domains 1-4), and of the relationship between the physical/functional impact and the mental experience it is proposed to reflect (in Domain 5).

Only those indices that are scientifically supported are then inserted into the Five Domains model for assessing and grading welfare status (Stage 8 of the Ten Stage Protocol), with confidence scores assigned which reflect the degree of certainly about the data upon which the grade was based (Stage 9). In addition to these welfare status indices, other indices that do not directly reflect an animal's mental state, but which indicate that welfare may be adversely affected if the circumstances they reflect persist, may be regarded as 'welfare alerting indices', as described in Chapter 2

(Harvey et al. 2020), and can be evaluated and graded separately to welfare status indices (Stage 10).

The aims of this chapter are (i) to apply the process of scientific validation to the practically measurable/observable welfare indices described in Chapter 5 (Harvey et al. 2021), and then (ii) to use those scientifically validated indicators to create a welfare status grading scheme based on the Five Domains Model that is specific for free-roaming wild horses, and (iii) define welfare alerting indices and create a separate scheme for grading future welfare risks.

2. Selection of welfare indicators

Based on information presented in Chapters 2 to 5 (Harvey et al. 2019, 2020, 2021), a list was made of potential welfare status and welfare alerting indices, that I have shown can be feasible to assess in free-roaming wild horses (Table 1). For ease of reference, I have included in Table 1, under welfare status indices only those that *were* able to be scientifically validated. This Chapter presents the scientific evidence used for validating the links between those physical/functional indicators and the specific mental experiences they are proposed to reflect. It is beyond the scope of this chapter to discuss the scientific evidence in depth for every indicator, but I have aimed to demonstrate the process, summarise the most important evidence and direct the reader to appropriate further resources. Also, I have focused on indices that were most often able to be assessed, as demonstrated in Chapter 5 (Harvey et al. 2021).

Table 1. Welfare status and alerting indices that are feasible to assess in free-roaming wild horses (Harvey et al. 2021; Chapter 5) and have been scientifically validated in this Chapter.

Domain	Welfare status indices	Welfare alerting indices
1. Nutrition	Body condition score Forage and water availability*	Forage and water availability Reproductive status
2. Physical Environment	Sweating Ambient temperature and other weather conditions (e.g. humidity, sun exposure, wind, rain) in combination with shelter/shade* Shivering	Ambient temperature Other weather conditions (e.g. humidity, sun exposure, wind, rain, snow, hail) Shelter/shade Body condition score
3. Health	Wounds Quidding/food pouching Blepharospasm Lameness Body posture Facial grimace Weakness Qualitative assessment of behaviour (relaxed, alert, dull, apathetic) Body condition score Respiratory rate & effort	Body condition score Hoof condition Coat condition Skin lesions Nasal discharge Ocular discharge Faecal egg count Limb pathology
4. Behavioural Interactions	Close spatial proximity with other horses & other affiliative interactions	Aspects of population dynamics and social organization

* resource-based indicators that can be cautiously used as welfare status indicators

For welfare status indices, animal-based indicators are preferred as these most directly reflect the animal's inferred experiences. However, in some circumstances there are scientifically robust established links to support the use of an indirect indicator, which may be resource-based (Chapter 2, Harvey et al. 2020). This may be used when an animal-based indicator may not be practical to measure or observe in free-roaming animals. For example, drinking behaviour and indicators of cold discomfort may not be observable, whilst distance to water, ambient temperature and weather conditions are measurable. Resource-based measures for evaluating compromises in Domains 1 and 2 are also commonly used in domestic horse assessments (reviewed by Hockenhull & Whay 2014). The strength of evidence for the link between an indicator and the mental experience that it infers should be reflected in the confidence score, which may be lower if only resource-based indicators are used. In some situations it is the *combination* of animal-based and resource-based measure(s) that are most informative.

Note that some welfare status indices may also be an alerting indicator to the risk of a physical/functional impact in another domain. For example, a very low body condition score increases the risk of hypothermia (Domain 2) in the presence of low ambient temperatures due to impacts on thermoregulatory mechanisms (discussed in Chapter 3). Note that body condition score as a welfare status indicator appears in both Domains 1 (Nutrition), and Domain 3 (Health), since at extremes of body condition (very underweight or very overweight), the associated pathophysiologic changes may give rise to negative affective experiences in Domain 3 (Mellor et al. 2020).

Different indices may also have different temporal relationships to the mental experience generated. For example, body condition indicates what the animal may have experienced for a prolonged duration, whereas shivering indicates current cold discomfort and provides no information on previous or ongoing thermal challenge (Beausoleil & Mellor 2017b).

3. Scientific validation of welfare status indicators

3.1 Indicators of welfare compromise (negative mental experiences)

A process for organising the scientific evidence linking indices in Domains 1 to 4 to mental experiences (Domain 5), involving two steps that align with the Five Domains Model has previously been described (Beausoleil & Mellor 2017b). This is summarised in Table 2.

Table 2. Description of the steps in scientifically validating animal-based indicators of welfare compromise (adapted from Beausoleil & Mellor 2017b)

Step 1: Validation of the links between observed indicators and physical/functional impacts (Domains 1-4)	Step 2: Validation of the links between physical/functional impacts (Domains 1-4) and particular mental experiences (Domain 5)
Scientific understanding of the pathophysiology and aetiology of disease	Scientific understanding of the neurophysiological mechanisms underlying the mental experience in species with similar neurological capacity, i.e., affective neuroscience evidence
Scientific understanding of the mechanisms related to deficiency, dysfunction, disruption or homeostatic imbalance	Comparison with mental experiences reported by humans in similar situations or with similar physical/functional impacts
Absence of elimination of the indicator using a method known to prevent or remove the underlying causative process (i.e. physical/functional impact)	Elimination or reduction of a mental experience reported by humans using a method known to prevent or alleviate the physical/functional impact
Coherence between multiple indicators in different modalities (e.g., behavioural, physiological) measured in the same situation	

The precise nature of an indicator that was linked to a physical/functional compromise in Domains 1 to 4 was first defined (Table 3). For example, with body condition, it is a lower than optimal body condition score that indicates undernutrition and a subsequent long term energy deficit. Conversely, a higher than optimal body condition score indicates overnutrition, and each of these infer different mental experiences. I then noted the mental experience or experiences inferred to be aligned with the physical/functional impact. These are detailed in Table 3.

Table 3. Table showing potential physical/functional negative impacts/compromises (Domains 1-4) and the associated inferred negative mental experience (Domain 5) associated with each welfare indicator.

Domain	Welfare compromise indicator	Potential physical/functional impacts (Domains 1-4)	Potential negative mental experience inferred (Domain 5)
Domain 1: Nutrition	Low body condition score (+/- Food seeking behaviour)	Longer term energy deficit	Longer term hunger Physical exhaustion
	*Low food availability	Short term energy deficit	Short term hunger
	*Water seeking behaviour/water availability	Dehydration	Thirst
Domain 2: Physical Environment	Sweating *Ambient temperature above lower critical temperature +/- low water availability +/- humidity, +/- insufficient shade	Hyperthermia	Heat discomfort
	Shivering *Ambient temperature below lower critical temperature, +/- rainfall/wind, +/- lack of shelter, +/- low food availability and other factors impacting thermoregulation (e.g. very low body condition, wet coat)	Hypothermia	Cold discomfort
Domain 3: Health	Wounds	Disruption of integument/muscle/joints or bones	Skin/muscle/orthopaedic pain
	Quidding/food pouching	Oral cavity pathology	Mouth pain
	Blepharospasm	Ocular pathology	Ocular pain
	Lameness	Limb pathology and consequent impaired ambulatory ability	Limb pain
	Head-lower-than-withers body posture	Impaired musculoskeletal activity	Malaise, pain, exhaustion
	Facial grimace	N/A	Any pain
	Weakness	Impaired musculoskeletal activity	Malaise, exhaustion
	Reduced alertness, dull, apathetic	N/A	Malaise, pain, exhaustion
	Very thin/emaciated body condition	Muscular weakness, metabolic disturbances	Malaise, exhaustion
	Increased respiratory rate +/or effort	Respiratory dysfunction/impairment	Breathlessness
Domain 4: Behavioural Interactions	Isolation from other horses, no or minimal affiliative interactions	Social isolation	Loneliness, insecurity, anxiety

*Resource-based indicators where there is scientific evidence to support their cautious use to assess welfare status, when animal-based indicators of that status are not possible or practical to assess.

As there may be more than one indicator associated with a particular mental experience, I have presented the scientific evidence linking each indicator to each inferred mental experiences.

Domain 1: Nutrition

Indicators of thirst

Detection of raised plasma osmolarity (i.e. dehydration) by osmoreceptors increases water-seeking and drinking behaviour, and drinking eliminates water-seeking behaviour (Denton et al. 2009), validating the link between water-seeking behaviour/drinking, with raised plasma osmolarity (dehydration). Specifically in horses, drinking has been shown to be elicited by inducing increases in plasma osmolarity, decreases in blood volume, and increases in plasma sodium concentration (Sufit et al. 1985). Plasma osmolality has been used as a reference to assess dehydration (Pritchard et al. 2008). Affective neuroscience provides evidence of the link between water seeking behaviour/drinking, and the mental experience of thirst, via neurohormonal pathways transmitting afferent inputs from osmoreceptors to higher brain centres associated with emotions (Denton et al. 2009).

However, water seeking or thirst behaviours can be difficult to observe in free-roaming wild animals. In domestic horses that can be approached a 'drink test' can be performed; a bucket of water is offered to the animal, and it is noted how eagerly the animal drinks and whether they drink none, some or most of the water (Mullan et al. 2014). Given that this cannot be performed in unapproachable wild animals, dehydration may be indirectly assessed based on the resource-based indices of how available water sources are in relation to required frequency of drinking, based on the best available data for horses. Even in domestic horses, using the resource-based indicator of water availability is regarded as a valid, most reliable and feasible indicator for on-farm assessment (Dalla Costa et al. 2014b).

In free-roaming horses, drinking frequency depends on water availability (Chapter 3). Therefore, although travelling long distances to water and drinking as little as once every 24-48 hours may be routine for some wild populations (Chapter 3), these are not optimal conditions and it is likely that such horses will, at least occasionally, experience more severe thirst and/or thirst for longer periods, than horses that typically drink several times daily from readily available water sources (Chapter 3). Factors other than location of water sources, also needs to be considered as difficulty in accessing water may occur for other reasons, such as illness or injury.

Experimentally, drinking occurred in ponies after an elevation in plasma osmolality of 3mmol/L, which was induced after 19 hours of water deprivation (Sufit et al. 1985), suggesting for these animals that the mental experience of thirst became significant after 19 hours of water deprivation. However, the environmental conditions during this study were not described, so it is not clear what influence ambient temperature had on the speed of onset and magnitude of dehydration. As the onset of dehydration is faster when ambient temperatures are high and during exercise and lactation (Chapter 3), these factors need to be considered when evaluating the impacts that the frequency with which horses can access water may have on the intensity of the thirst they may experience when that access is impeded by distance or water shortages.

Indicators of hunger

Hunger is the negative mental experience that motivates food seeking and ingestive behaviours (McKiernan et al. 2008). A low body condition score (BCS), i.e. being thin or emaciated, is widely used as a welfare indicator in many species including horses (Carrol & Huntington 1988; Burkholder 2000; Pritchard et al. 2005; Burn et al. 2009, 2010). The validation of low BCS as an indicator of hunger has been discussed in detail elsewhere (Beausoleil & Mellor 2017b). Since it takes some time of food deprivation for animals to lose significant body fat, it is not an indicator of short term hunger, but is an indicator of longer-term hunger. Body condition scoring has been discussed in depth in Chapter 3. Step 1 in validation is to explore the link between BCS and energy reserves/nutritional status. BCS has been shown to accurately reflect the physical bulk of fat and muscle cover, thereby providing a repeatable indication of available energy reserves related to an animal's nutritional status, evaluated among the physical/functional impact in Domain 1 (Henneke et al. 1983; Carrol & Huntingdon 1988; Dugdale et al. 2012). In other species, animals with lower BCS also had low blood glucose, leptin, insulin-like growth factor 1 and higher free fatty acid concentrations indicating mobilization of fat reserves and catabolism of muscle (reviewed by Beausoleil & Mellor 2017b).

Step 2 is evaluating evidence between the link of energy/nutritional status and the mental experience of hunger. The strength of motivation to obtain food is one measure of this, with the expectation that animals will work harder to obtain food, the hungrier they are (Kirkden & Pajor 2006). Obtaining sufficient energy has been shown to be one of the main motivators of food intake (Toates 1986). Animals in low body condition exhibit higher feeding motivation (e.g., walk further to access food), consumed more food and spent more time eating (reviewed by Beausoleil & Mellor 2017b).

A low BCS represents undernutrition, which can be caused by insufficient quantity and quality of food available. However, other factors can contribute to it even when sufficient food is available. For example, inability to ingest food normally due to oral cavity disease, interference with digestion due to intestinal disease, and increased metabolic demands resulting from disease or the heightened physiological loads of thermoregulation and lactation (Chapter 3). Therefore, factors such as these also need to be considered when assessing nutritional status. Food availability and motivation to obtain food may also provide more of an indication of acute hunger and can be informative to evaluate alongside BCS as a *welfare status* indicator. However, given that food availability is challenging to accurately assess with free-roaming animals utilising large areas, taken alone it would be regarded as an *alerting* index.

Domain 2: Physical Environment

Indicators of cold and heat discomfort

Detection of low or high body temperature by thermoreceptors stimulates shivering or sweating respectively (Alexander 1979; Cymbaluk & Christison 1990). Acute cold exposure in climatic chambers also causes horses to shiver (Cymbaluk 1994). This validates the link between the observed indicators of shivering or sweating, and the physical/functional impact of hypo- or hyperthermia. Humans report cold discomfort during shivering, and heat discomfort during sweating, mental experiences that are eliminated when body temperature is normalised, validating the link between the physical impact of hypo- or hyperthermia and the mental experiences of cold or heat discomfort respectively.

The context and environmental conditions also need to be considered, as tremoring with anxiety or fear can resemble shivering. Any process increasing adrenaline can result in sweating (Evans & Smith 1956), including pain (Ashley et al. 2005). These indicators are also relatively short lived so provide little or no indication of previous welfare challenges related to the physical environment or ongoing welfare risk.

Given the knowledge of thermoneutral zones and thermoregulatory mechanisms as discussed in Chapter 3, resource-based indicators can be cautiously used in this domain to suggest the possible presence of hypo- or hyperthermia. With warm temperatures, cold-seeking behaviours such as moving to the shade, or self-immersion in cool water may also be observed. With cold temperatures, heat and shelter seeking, and huddling may also be observed (Mejdell & Bøe 2005; Heleski & Murtazashvili 2010). In rainy, windy conditions, horses used shelters more, particularly if ambient temperature was less than –1°C (Heleski & Murtazashvili 2010)

Domain 3: Health

Indicators of health compromise has not been a focus in wild horse research to date (Hausberger et. al. 2016). Whilst some indicators may be extrapolated from studies in domestic horses, most health problems encountered in domestic horses are considered to be 'diseases of domestication', related to social and spatial restriction, a high energy diet and demanding work (Hausberger et. al. 2016). As discussed in Chapter 3, little is known about the health status of wild horses and so optimal indicators of compromises in this domain may need to be refined as knowledge advances. The mental experiences that can be best validated are different types of pain, malaise/exhaustion and breathlessness.

Indicators of pain

Pain has been defined as 'an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage' (IASP 1983). Equine neuroanatomy and neurophysiology have long suggested that horses are able to experience physical and mental impacts of pain (Guthrie 1980; Murrell & Johnson 2006). The mental experience of pain is also wide ranging in quality, intensity, frequency and duration. Complex interactions between different mental experiences may also occur. For example, 'pain induced distress' (Mellor et al. 2000) may give rise to a behavioural response to pain that may also include behaviours caused by distress (Ashley et. al. 2005).

Indicators of localised pain

Cutaneous pain: wounds

A wound is any damage or break in the surface of the skin, so in this case the indicator defines the physical/functional impact, although depending on the location, size and extent of the wound, additional impacts on muscle, joints, bone and internal organs can also be present. The presence of pain associated with wounds has been validated with nociceptive threshold testing (Redua et al. 2002), and subsequent amelioration of behavioural responses with analgesia (Olbrich & Mosing 2003), providing the link between the physical/functional impact and the mental experience of pain.

Mouth pain: Quidding and food pouching

Mouth pain would commonly be related to dental disease in domestic horses (Lane 1994; Easley 1999; Graham 2002). Little is known about the prevalence of dental pathology encountered in wild horses. It is postulated that due to more time spent chewing a higher fibre diet, with the head in a natural grazing position, that dental disorders may be less common in wild horses compared with domestic horses, however this has not been studied. Problematically significant dental disease can be present in horses without externally observable clinical signs. However, presence of quidding (dropping partially chewed food from the mouth) and food pouching in the cheeks are clear externally observable indicators of dental disorders that frequently cause secondary bruising, ulceration and laceration of soft tissues in the mouth (Lane

1994; Easley 1999; Graham 2002). This information provides a link between quidding and food pouching as indicators of the physical/functional impact of oral cavity disease.

Physiological foundations of mouth pain in horses has recently been reviewed in detail, in relation to the use of bits in domestic horses (Mellor 2020). Much of the information presented on the foundations of mouth pain are also relevant to dental pain, and some descriptions of the behavioural consequences of bit pain have also been described in association with dental disease (Coneglian et al. 2020). The oral cavity generally has a rich supply of nociceptors susceptible to bruising, laceration and ulceration (Haggard & de Boer 2014). Being modified periosteum, the gums are exceptionally sensitive tissues (Mantyh 2014). Thus, periodontal disease, dental pathology that results in cheek ulceration, as well as dental pathology directly exposing tooth nerve roots would all be anticipated to cause varying types and intensities of pain. The Horse Grimace Scale (Dalla Costa et al. 2014a; Gleerup et al. 2015) has also been used to identify pain related to dental disorders in horses (Coneglian et al. 2020). Combined, this provides the evidence for a link between the physical/functional impact of some oral cavity pathologies and the mental experience of pain.

Ocular pain: blepharospasm

Blepharospasm is an involuntary contraction of eyelid muscles (blinking) in response to ocular pain that may arise with a range of ocular pathologies or injuries. The cornea is the most densely innervated tissue in the body, resulting in it being 300-600 times more sensitive than skin (Yang et al. 2018). Thus, a very tiny corneal injury/ulcer can cause intense ocular pain. Corneal injury is the most common cause of blepharospasm in horses (Brooks & Matthews 2007). Stimulation of corneal nerves induce blinking, which is resolved with analgesia, providing evidence of a link between corneal pathology and ocular pain.

Limb/foot pain: lameness

Lameness is an abnormal distribution of weight between limbs, and is usually associated with a change in vertical head motion, in an attempt to reduce pain on an affected limb. Features and severity of lameness depends on the pathology and the associated type and severity of pain (Hood et al. 2001; DuPreez 2002; Dyson 2002; Dyson & Marks 2003; Dyson & Murray 2003; Dabareiner & Carter 2003; Hewetson 2003; Bussières et al. 2008). There is extensive literature on the pathophysiology and aetiology of diseases causing lameness, with disorders of the foot being most common (Stashak 2002). Investigation identifying the underlying cause provides a link between the indicator of lameness, and the physical/functional impact of foot/limb pathology. Resolution of lameness with local analgesia in the form of 'nerve blocks' (Owens et al. 1995; Dyson 2002; Olbrich & Mosing 2003) can localise the more precise anatomical location of the pathology, providing evidence of a link between the foot/limb pathology and the mental experience of pain.

Non-specific chronic pain/malaise/fatigue/exhaustion

There is a wealth of research regarding the recognition of acute pain in horses (Ashley et al. 2005; Graubner et al. 2011; Dalla Costa et al. 2014a; de Grauw & van Loon 2016) but much less regarding chronic non-specific pain and malaise (Fureix et al. 2010; Gregory 1998). Non-specific pain refers to presence of pain that cannot be localised to a particular anatomical region or associated with a particular pathological process. Malaise is defined as a feeling of illness or generalised discomfort (Broom 1998), whilst fatigue is a feeling of tiredness resulting from physical exertion or illness, with exhaustion being extreme fatigue.

It is recognised that aspects of behaviour that may be altered by pain include elements of demeanour, posture and gait, as well as interactive behaviours (de Grauw & van Loon 2016). However, behaviour is also influenced by other factors including temperament, sex, age and environment (Wagner 2010). Not showing outwards signs of pain or illness is an important survival strategy for prey species, and therefore behavioural signs of pain or illness may not be as obvious in free-roaming wild horses (Taylor et al. 2002). To the authors knowledge, scientific assessment of pain or illness specifically in wild free-roaming horses has not been attempted.

Body and ear posture

The normal range of behaviours and body postures observed in both free-roaming horses (Ransom & Cade 2009) and domestic horses (McDonnell 2003) have been well described, as reviewed in Chapter 3. The challenges in detecting sickness and discomfort behaviours in horses have previously been discussed in detail, and the importance of body posture highlighted (Hausberger et al. 2016). Ear and tail postures (Kiley-Worthington 1976) and neck height (Wolff et al. 1997; Waring 2003) have been thought to be informative about a horse's internal state for some time (Wolff et al. 1997; Waring 2003). A 'slumped' stance has been associated with exhaustion (Burn et al. 2010) but this posture was not well described.

More specifically, a lower-than-withers-head-carriage has been associated with chronic discomfort (Taylor et al. 2002; Ashley et al. 2005; Bussieres et al. 2008; Hausberger et al. 2016, Torcivia & McDonnell 2021), as well as more acute pain (Price et al. 2003; Pritchett et al. 2003; Graubner et al. 2011), particularly when occurring with the ears pointing backwards (Graubner et al. 2011; Ashley et al. 2014; Dalla Costa et al. 2014a), and with a 'pain face' grimace (Taylor et al. 2002; Dalla Costa et al. 2014a). Ears can intermittently move backwards for a range of reasons including noises and negative social interactions (Hausberger et al. 2016). However, in one large study of horses in calm environments with little external stimulation, they spent long periods with ears back in association with sickness (Fureix et al. 2010; Hausberger et al. 2016).

Recently, specific abnormal body postures associated with discomfort and/or exhaustion in horses have been more extensively defined in a 'horse discomfort ethogram' (Torcivia & McDonnell 2021). As these abnormal body postures were usually recorded in association with a known cause of musculoskeletal impairment, and disappeared when the underlying causative process had resolved (Torcivia & McDonnell 2021), this validates a link between the observed body posture and the physical/functional impact of musculoskeletal impairment. However, lower-than-withers posture, ears back and changes in head and tail postures have all been reported in response to variable sources of pain in domestic horses in hospital settings (Price et al. 2003; Pritchett et al. 2003; Ashley et al. 2005; Bussieres et al. 2008; Graubner et al. 2011; Viñuela-Fernandez et al. 2011), with resolution of these changes in response to analgesia.

Whilst there is substantial evidence that a lower-than-withers posture combined with ears pointing backwards can be associated with both acute and chronic pain, this posture has also been associated with exhaustion. However, these mental experiences may be linked as physical exhaustion may also give rise to musculoskeletal discomfort, and exhaustion has also been described as a form of pain (Fleming 2002). Consequently, when interpreting the lower-than-withers posture with ears back in affective terms, I have not attempted to differentiate between non-specific chronic pain, malaise, fatigue and exhaustion.

Facial grimace

A pain facial grimace scale has been established for horses (Dalla Costa et al. 2014a; Gleerup et al. 2015) as embodying six facial features: stiffly backward ears, orbital tightening, tension above the eye area, prominent chewing muscles, mouth strained, strained nostrils and flattening of the profile. Features of the facial grimace have been associated with post-operative pain (Dalla Costa et al. 2014a), mouth pain (Coneglian et al. 2020) and musculoskeletal pain (Dyson et al. 2007). It may not be possible to observe all these features in free-roaming horses or to score facial grimace, but it is possible to assess some features such as ear position, and in some cases tension above the eye and strained nostrils. Note, however, that these features may not be specific to acute pain, and could also be found in other contexts related to malaise and/or exhaustion.

Reduced alertness/dullness/apathy

Subjective qualitative assessment of demeanour has been described in several studies (Pritchard et al. 2005; Burn et al. 2010; Popescu & Diugan 2013, Wemelsfelder 1997). The characteristic features of 'reduced alertness', 'dullness' and 'apathy', have not been clearly defined, but all them have referred to unresponsiveness to environmental stimuli (Lesimple 2020). Likewise for commonly described postures suggestive of apathy (Pritchard et al. 2005; Burn et al. 2010; Fureix et al. 2010, 2012; Popescu & Diugan 2013).

Apathy has been identified in these terms in working horses in developing countries and riding school horses, and has been correlated with presence of wounds, low body condition and lameness (Pritchard et al. 2005; Popescu & Diugan 2013). In a large study of working equids, lack of alertness was correlated with low body conditions, dermatological lesions and abnormal gaits suggesting as association with chronic pain conditions (Burn et al. 2010). Other studies of working equids have linked apathy to conditions associated with chronic pain or exhaustion (Fureix et al. 2012; Popescu &

Duigan 2013). Reduced alertness has also been observed in combination with the 'pain face' grimace (Taylor et al. 2002; Dalla Costa et al. 2014a).

There is a difference in the assessment of the indices demeanour and weakness using the methods in this study. Body and ear posture could be assessed on still camera trap images, whereas the presence/absence of weakness and assessment of demeanour could only be performed by direct observation or assessment of camera trap videos.

Other indicators of malaise, fatigue, exhaustion

Very thin/emaciated body condition

As discussed in Chapter 3, undernutrition to the stage of starvation results in a range of impairments to health including metabolic changes, muscle wasting, reduced gut mucosa integrity, immunosuppression and increased risk of infectious diseases. Reproductive efficiency and lactation is also reduced (Henneke et al. 1984). In people the feeling of malaise is a non-specific type of discomfort associated with being unwell, and is associated with a wide range of pathologies. Neuroendocrine studies suggest that the feeling of malaise may be associated with cytokines altering brain corticosteroid receptor balance (De Kloet et al. 1994). This can further lead to reduced vigilance, inactivity and feelings of depression in people. In horses thin body condition has been correlated with lack of responsiveness (Burn et al. 2010) and apathy (Pritchard et al. 2005; Popescu & Diugan 2013).

Muscular weakness

Weakness is a loss of muscle strength. This may be observed as the horse having difficulty rising, a slow possibly wobbly gait with difficulty lifting limbs, and difficulty lifting the head. Weakness caused by cardiovascular disease, metabolic disorders, or neurological, neuromuscular or primary muscular disorders cannot be distinguished by direct observation alone, and not all of these will result in feelings of malaise, and/or fatigue/exhaustion. However, interpretation together with other indicators is likely to be helpful. For example, if muscular weakness is evident in an emaciated horse, it is likely to be a result of low muscle mass and associated with physical exhaustion.

Indicators of breathlessness: Increased respiratory rate and/or effort

The pathophysiology and animal welfare significance of breathlessness has been previously reviewed in depth (Beausoleil & Mellor 2015, 2017a). The term breathlessness, rather than dyspnoea and tachypnoea, has been used in animal welfare science to highlight the fact that respiration is associated not only with physical sensations, but also with affective experiences (Beausoleil & Mellor 2015). Whilst this is recognised in human medicine (Lansing et al. 2009; Parshall et al. 2012), typically in the veterinary literature the term dyspnoea is used to describe only the physical signs of 'difficult, laboured breathing' (Mellema 2008).

The physical indicators of respiratory impairment, increased respiratory rate and effort, can arise from a wide range of pathological conditions causing different types of respiratory impairment (Beausoleil & Mellor 2015). Examples include decreased respiratory muscle function (e.g. muscle weakness due to metabolic disorders or severe under-nutrition, thoracic or abdominal pain due to injuries, tissue hypoxia due to anaemia, respiratory muscle paralysis induced by toxins such as snake bite envenomation), increased restrictive loading from conditions causing airway narrowing such as chronic bronchitis, or conformational defects such as laryngeal hemiplegia, or reduced chest wall compliance with pulmonary diseases such as pneumonia (reviewed by Beausoleil & Mellor 2015).

There may or may not be additional physical indicators present suggestive of potential underlying causes for the respiratory impairment, but the resulting affective experience of breathlessness is similar, although in humans different qualities of breathlessness do vary in their unpleasantness (Banzett et al. 2008).

Evidence that breathlessness is an unpleasant affective experience for animals comes from the sense of urgency to engage in specific behaviours, such as withdrawal, escape attempts, struggling and other aversive behaviours to stimuli similar to those that cause dyspnoea in humans (Gilbert & Gofton 1982; Nehashi et al. 2001; Niel & Weary 2007; Denton et al. 2009; O'Donnell et al. 2009; Dalmau et al. 2010; Packer et al. 2012). Electrophysiological and gene expression studies in animals also support the behavioural evidence (Buchanan & Richerson 2009; Davenport & Vovk 2009; Evans et al. 2010). Further, in humans, brain imaging studies demonstrate activation of cortico-limbic regions similar to those activated with other unpleasant experiences such as thirst, hunger and pain (Banzett et al. 2000; Evans 2002; Peiffer et al. 2008; von Leupoldt et al. 2008), which also motivate the behaviours that attempt to rectify the sensation (Davenport & Vovk 2009).

Domain 4: Behavioural interactions

Typically challenges in Domain 4 are considered a result of restrictions imposed by captivity and impoverished environments. However, situations will arise in free-roaming animals that may limit their ability to engage in social interactions with conspecifics, even when there are opportunities to do so. This may consequently give rise to feelings of loneliness and social isolation. A whole branch of neuroscience, 'social neuroscience' is wholly dedicated to understanding the processes of social bonding and associated affective states (McMillan 2016). In behavioural ecology, sociality has long been known to be important for fitness in being associated with increasing resource acquisition, protection from predation, and parental care increasing the survival of young (Alexander 1974). The literature linking sociality to health outcomes in both human and non-human animals has grown rapidly over the last three decades. For example, social isolation produces a reliable stress response such that it has become an experimental model for inducing stress (Cacioppo et al. 2014).

The negative mental experiences that arise from social isolation in animals have been reviewed in detail (McMillan 2016). The negative affects associated with social isolation are often referred to as 'social pain', in line with contemporary definitions of pain including the discomfort of any unpleasant emotional state. Feelings of insecurity and fear can also be components of social pain in humans, and there is behavioural evidence of this in animals as well (reviewed by McMillian 2016). There is also evidence that in some situations animals may prioritise sociality above relieving other mental experiences. Horses expressed a high motivation (Sondergaard et al. 2011) and priority (Schatzmann 1998) for any available type of social contact with conspecifics, whether it be full social contact, head contact, muzzle contact, or simply viewing another horse. From the knowledge of social organisation in horses, as discussed in Chapter 3, it is apparent that horses are highly social animals. Social neuroscience provides evidence of negative affective states that occur when such social animals are placed in situations of social isolation or are separated from familiar conspecifics.

Situations in the external environment that may be perceived as threatening are also aligned with domain 4. These include possible or actual attack by predators, separation from the protection of others, disturbance by humans, and hazardous environmental events such as flood or fire. These are all situations which may generate negative mental experiences (Domain 5) of anxiety, fear, panic and/or nervous vigilance (Panksepp 2005; Boissy et al. 2007; Beausoleil et al. 2018). Indicators of these experiences were not assessed in the current study and none of these circumstances were encountered, but they may certainly be relevant in future assessments.

3.2 Indicators of welfare enhancement (positive mental experiences)

Indicators of welfare enhancement mostly relate to behavioural observations. Positive experiences may result from behaviours that are directed at minimising negative experiences, such as pleasures associated with eating. Other positive experiences may replace negative ones when there are more opportunities to engage in behaviours that the animal finds pleasurable (Mellor 2009; Mellor & Beausoleil 2015; Mellor 2015b, 2016, 2017; Mellor et al. 2020).

In evaluating positive mental experiences the approach is somewhat different, as has been described in detail elsewhere (Mellor & Beausoleil 2015), and summarised in Chapter 2 (Harvey et al. 2000). With welfare enhancement, in each domain, opportunities are first evaluated, which are often resource-based indicators. Utilisation of that opportunity is then evaluated, which involves evidence of the animal using the resource. And finally, a cautious judgement is made about the type of 'positive affective engagement' this resource confers. This is demonstrated in Table 4.

Table 4. Table illustrating the possible opportunities for welfare enhancement in Domains 1-4, indications that these opportunities are being utilised, and the potential associated positive mental experiences (Domain 5).

Domain	Opportunity (resource-based measure)	Utilisation (animal-based observation)	Potential positive mental experience inferred (Domain 5)
1.Nutrition	Sources of good quality water readily available Range of quality vegetation available	Able to access and utilise water sources Observed foraging range of vegetation	Oral wetting and quenching pleasures Rewarding engagement of foraging, pleasures of eating a variety of food, satiety, gastrointestinal comfort
2. Physical environment	Appropriate ambient temperatures for thermal comfort, available shelter and shade Radiant sun e.g. after a cool night. Available water source for immersion on a hot day	Utilising available shelter and shade Observed resting under radiant sun on a cool day, or immersing in water on a hot day	Thermal comfort Pleasures of radiant heat Cooling effects of immersion in cool water
3. Health	Injury free, good health and physical fitness	Observed actively engaging with environment at a range of gaits, alert to surroundings	Vitality of fitness
4.Behavioural interactions	Multiple con-specifics, range of sex and ages	Observed engaging in a range of affiliative social interactions on a regular basis	Affectionately sociable, maternally rewarded, content, sense of security, social comfort

In the evaluation of positive mental experiences, the scientific evidence of those experiences is derived mostly from the combination of extensive knowledge about the behaviour of the species when under optimal conditions (as described in Chapter 3), and affective neuroscience providing evidence of positive emotions associated with those behaviours.

Under optimal conditions where there are no or minimal welfare compromises, we can cautiously assume that free-roaming animals will experience positive affective states. This is because, unlike most domesticated forms of captivity, they have agency to engage in behaviours of their choice; foraging and exploration, social interactions, locomotor activity and rest, behaviours that they are likely to find rewarding (Fraser & Duncan 1998; Ikemoto & Panksepp 1999; Spinka et al. 2001; Scherer et al. 2006; Yeates & Main 2008; Held & Spinka 2011). Thus knowledge of welfare compromise, and the study of free-roaming animals in optimal conditions provides opportunity for learning more about those behaviours that an animal finds pleasurable.

The behaviours in each domain that are likely to be rewarding are summarised in Table 4. Examples of positive associated experiences and the associated evidence from affective neuroscience are as follows:

Domain 1: Oral wetting and quenching pleasures of drinking, masticatory pleasures associated with eating a range of foods with varying smell, tastes and textures (Grill & Norgren 1978; Berridge 1996; Deag 1996; Fraser & Duncan 1998; Steiner et al. 2001; Kelley et al. 2005; Balcombe 2009). Resting behaviour after eating (e.g., standing-sleep) has been described by as a specific indicator of post-prandial satiety in horses (Ninomiya et al. 2007, 2008)

Domain 2: Immersion in cold water and/or its evaporation from the skin, providing pleasurable cooling effects, and radiant heat on the skin providing pleasurable warming effects (Gregory 2004; Cabanac 2005).

Domain 3: Horses are highly motivated to perform active locomotory behaviour (Freire et al. 2009; Chaplin & Gretgrix 2010). When injury free, in good health and physically fit, they are able to engage freely in active locomotory behaviours, which may indicate vitality of fitness.

Domain 4: Engaging in behaviours that are rewarding (Panksepp 2005; Boissy et al. 2007; Spinka & Wemelsfelder 2011; Spinka 2019). For example, the exercise of agency (Spinka & Wemelsfelder 2011; Spinka 2019) including energised exploration of, and interactions with, a stimulus-rich environment (Berridge 1996; Panksepp 2005; Spinka & Wemelsfelder 2011), focused and engaged selective foraging in environments with abundant and varied food sources (Panksepp & Zellner 2004; Panksepp 2005; Spinka & Wemelsfelder 2011). Social interactions including reciprocated affiliative interactions between animals (Nelson & Panksepp 1998; Carter & Keverne 2002; Lim & Young 2006), the dedicated maternal nurturing and care of young (Pfaff 1999; Fisher et al. 2006; Panksepp 2006), the joyfulness of rough-and-tumble play (Vanderschuren et al. 1997; Burgdorf & Panksepp 2006; Held & Spinka 2011) and the eroticism and orgasmic pleasures of sexual activity (Pfaff 1999; Fisher et al. 2006; Balcombe 2009).

The associated positive mental experiences may include, for example, feeling energised, engaged, affectionately sociable, maternally rewarded, nurtured, secure, protected, excitedly joyful and/or sexually gratified (Panksepp 2005; Mellor 2015a, 2015c; McMillan 2019).

4. Welfare alerting indicators

Indices where there is no scientific evidence to link them to a mental experience, may still be useful to consider separately as welfare alerting indices, if they act as a warning regarding possible future welfare risk. For these indices, there should be a link to what that future risk might be. This is detailed in Table 5. Some welfare alerting indices may also provide additional information together with a welfare status indicator, despite not being indicators of welfare status themselves. For example, a high faecal egg count in the presence of diarrhoea may indicate that faecal parasites are a likely cause of the diarrhoea, whilst a high faecal egg count in the absence of other indicators is only welfare alerting. As noted earlier (section 2), some indicators of welfare status in one domain, can also be indicators of welfare risk in another domain.

Table 5. Table of welfare alerting indicators detailing the future welfare risks that they may indicate.

Domain	Welfare alerting indicator	Alerts to the risk of future potential physical/functional impacts (Domains 1-4)
1. Nutrition	Food availability	Energy/nutritional deficit
	Water seeking behaviour/water availability	Dehydration
2. Physical Environment	Sweating	Dehydration if combined with lack of water
	Very low body condition score	Hypothermia if combined with low temperatures
	Low ambient temperature, heavy rain, inadequate shelter	Hypothermia
	High ambient temperature, humid, inadequate shade	Hyperthermia
3. Health	Very low body condition score	Illness, reduced fertility, weakness, exhaustion
	Poor coat condition	Indicators of illness
	Poor hoof condition	Impaired ambulatory ability
	High faecal egg count Positive *S. vulgaris* PCR	Gastrointestinal pathology
4. Behavioural Interactions	Small herd size Low reproductive rate	Social isolation

5. Grading welfare status

The validated welfare status indices were then inserted into the Five Domains Model, using the scientific evidence to inform guidelines for assigning compromise and enhancement grades for the physical/functional indicators in each of Domains 1 - 4, leading to an overall grading of the inferred severity/duration of mental experiences in Domain 5 (Tables 6a-d, and 7a-d). Principles of grading were discussed in Chapter 2. A modified three-tier grading scheme (as previously illustrated in Chapter 2) was chosen because preliminary assessments of welfare indicators in free-roaming horses (Chapter 5) suggested, as recommended by Mellor (2017) for contexts such as this, that when insufficient information was obtainable for confidentially distinguishing between the traditional five tiers.

5.1 Grading welfare compromise

A modified three-tier scheme for grading welfare compromise in each of the physical/functional Domains 1 to 4, and the associated inferred mental experiences in Domain 5, is presented in the following pages in Table 6.

5.2 Grading welfare enhancement

A modified three-tier grading for grading welfare enhancement in each physical/functional Domain 1 to 4, and the associated mental experiences inferred in Domain 5, is presented in the following pages in Table 7.

5.3 Using welfare alerting indices to grade future welfare risk

Using the identified welfare alerting indices, a three-tier grading scheme was created for grading future welfare risk in each physical/functional domain 1-4. This is presented in the following pages in Table 8.

Table 6a-d. Grading welfare compromise.

Table 6a. Grading welfare compromise in Domain 1 (Nutrition), and the negative affective experiences inferred in Domain 5.

Measurable/ observable welfare status indices	Compromise grade		
	A/B (No to low)	**C (Moderate)**	**D/E (Severe)**
Access to water	Able to access water at least every 6-12 hours. May be up to 12 hours interruption in water supply in cool weather.	Able to access water every 12-24 hours. May be up to 12 hours interruption in water supply in hot weather and up to 24 hours interruption in water supply in cool weather.	Unable to access water within 48 hours in cool weather or 24 hours in hot weather.
Domain 5 Negative affective experience inferred: *Thirst*	*No to very low-level thirst*	*Moderate thirst*	*Severe thirst*
Body condition score (BCS) and forage availability	Optimal body condition (5-6/9) with good forage availability	Moderately thin (4/9) to thin (3/9) body condition with poor forage availability	Very thin (2-3/9) to emaciated body condition (1/9) with very poor forage availability
Domain 5 Negative affective experience inferred: *Hunger*	*No to very low-level hunger*	*Moderate hunger*	*Severe hunger, weakness*

Table 6b. Grading welfare compromise in Domain 2 (Physical environment), and the negative affective experience inferred in Domain 5.

Measurable/ observable welfare status indices	Compromise grade		
	A/B (No to low)	C (Moderate)	D/E (Severe)
Presence of shivering and/or horses huddling together	No shivering and/or huddling together observed	Shivering and/or huddling together observed occasionally for short duration	Shivering and/or huddling together observed frequently or for prolonged duration
Ambient temperature and weather conditions	Temperature above lower critical temperature (LCT)[1] (approx. 5 °C) most of the time, good shelter, no severe weather conditions, no interference with normal thermoregulatory effectiveness	Temperature mildly below LCT for moderate durations or significantly below LCT for short durations. May be combined with rain, wind, lack of shelter, and/or loss of normal thermoregulatory effectiveness	Temperature frequently and/or substantially below LCT. May be combined with heavy rain, wind and/or lack of shelter, and loss of normal thermoregulatory effectiveness
Domain 5 Negative affective experience inferred: *Cold discomfort*	*No to very low level cold discomfort*	*Moderate cold discomfort*	*Severe cold discomfort*
Ambient temperature and weather conditions	Temperature below upper critical temperature (UCT) (approx. 25 °C) most of the time, good shade and water available, no interference with normal thermoregulatory effectiveness	Temperature mildly above UCT for moderate durations or significantly above UCT for short durations of time. May be combined with inadequate shade, inadequate water availability and/or loss of normal thermoregulatory effectiveness	Temperature frequently and/or substantially above UCT, combined with inadequate shade, inadequate water availability and/or loss of normal thermoregulatory effectiveness
Presence of Sweating	No sweating observed	Sweating observed occasionally for short duration	Excessive sweating, observed frequently or for prolonged duration
Domain 5 Negative affective experience inferred: *Heat discomfort*	*No to very low level heat discomfort*	*Moderate heat discomfort*	*Severe heat discomfort*

Table 6c. Grading welfare compromise in Domain 3 (Health), and the negative affective experience inferred in Domain 5.

Measurable/ observable welfare status indices	Compromise grade		
	A/B (No to low)	C (Moderate)	D/E (Severe)
Presence of wounds, lameness, blepharospasm, quidding/food pouching	No obvious wounds, no lameness, no evidence of blepharospasm, quidding/food pouching	Small wound, moderate lameness, mild blepharospasm, persistent quidding +/- food pouching	Large wound, severe, non-weight bearing lameness, severe persistent blepharospasm
Domain 5 Negative affective experience inferred: *Localised pain*	*No to very low level localised pain*	*Moderate localised pain*	*Severe localised pain*
BCS, body posture, facial grimace, alertness	BCS > 3/9, normal body posture, no persistent features of facial grimace, alert and relaxed	BCS 3/9, head-lower-than-withers body posture and/or features of facial grimace evident some of the time, reduced alertness	BCS ≤ 2/9, head-lower-than-withers body posture and/or features of facial grimace evident most of the time, dull and/or apathetic, weak
Domain 5 Negative affective experience inferred: *Non-specific/generalised pain/malaise/fatigue/exhaustion*	*No to very low level non-specific pain/malaise/ fatigue*	*Moderate level non-specific pain/malaise/ fatigue*	*High level non-specific pain/ severe malaise/ exhaustion*
Respiratory rate and effort	Normal respiratory rate and effort	Moderately increased respiratory rate and/or effort	Markedly increased respiratory rate and/or effort
Domain 5 Negative affective experience inferred: *Breathlessness*	*No to very low level breathlessness*	*Moderate level breathlessness*	*Severe breathlessness*

Table 6d. Grading welfare compromise in Domain 4 (Behavioural interactions), and the negative affective experience inferred in Domain 5.

Measurable/ observable indices	**Compromise grade**		
	A/B (No to low)	**C (Moderate)**	**D/E (Severe)**
Animal-to- animal interactions: Number of companions, time spent with companions *vs* alone, positive social interactions	Little time alone Many social companions Stable herd of mixed sexes and ages.	Moderate time spent a lone, very few social companions, unstable herd structures,	Most of the time spent alone, no or very few social interactions
Domain 5 Negative affective experience inferred: *Loneliness/isolation, anxiety/fear, restricted choice*	*No to very low-level loneliness, isolation, anxiety*	*Moderate level loneliness, isolation, anxiety*	*High- level loneliness, isolation, fear*

Table 7a-d. Grading welfare enhancement.

Table 7a. Grading welfare enhancement in Domain 1 (Nutrition), and the positive affective experience inferred in Domain 5.

Measurable/ observable indices	**Enhancement grade**		
	0 (None)	**+ (Low to medium)**	**++ (High)**
Water availability and quality. Ability to access source and drink.	Limited water available and limited choice in location and type of water source, poor quality water. Difficulty accessing water.	Multiple water sources or variation in type of water source available and accessible intermittently. Variable quality.	Multiple water sources of varying types e.g., dams, rivers, creeks, all continuously available and accessible, limited competition for access, easy access. Good quality water.
Domain 5 Positive affective experience inferred: *Quenching pleasures of drinking*	*None to very low level quenching pleasures*	*Low to medium level quenching pleasures*	*High level quenching pleasures*
Food availability, quality and variation. Ability to access.	Limited food availability. Quantity and quality of feed meets functional needs only, dietary components and palatability constant over long periods	Quantity and quality of feed meets functional needs, plus moderate choice between varying feeds with different smells, tastes and textures which are accessible	Quantity and quality of food meets functional needs. Widely varied diet enabling much choice between pleasant food smells, tastes and textures in engagingly different locations and easily accessible
Domain 5 Positive affective experience inferred: *Masticatory pleasures of eating a range of foods, post-prandial satiety pleasures of variations in tastes, smells and textures, gastrointestinal comfort*	*No to very low level masticatory pleasures, satiety, gastrointestinal comfort*	*Low to medium level masticatory pleasures, satiety, gastrointestinal comfort*	*High level masticatory pleasures, satiety, gastrointestinal comfort*

Table 7b. Grading welfare enhancement in Domain 2 (Physical environment), and the positive affective experience inferred in Domain 5.

Measurable/ observable welfare status indices	**Enhancement grade**		
	0 (None)	**+ (Low to medium)**	**++ (High)**
Ambient temperature, weather conditions, presence of shelter/shade, water with ability to access	Extremes of temperature and weather very common, limited options for good shelter or unable to access	Extremes of temperature and weather occur rarely, reasonable shelter in more than one location	Minimal extremes of temperature and weather, and ability to obtain excellent shelter from weather extremes available in a variety of locations that are easily accessible
Domain 5 Positive affective experience inferred: *Thermal comfort, pleasurable experiences of warming in the sun, or cooling in shade or water immersion*	*No to very low level thermal comfort*	*Moderate thermal comfort*	*High thermal comfort*

Table 7c. Grading welfare enhancement in Domain 3 (Health), and the positive affective experience inferred in Domain 5.

Measurable/ observable welfare status indices	Enhancement grade		
	0 (None)	+ (Low to medium)	++ (High)
Body condition, musculature, movement in different gaits, alertness	Lack of long term physical fitness, poor body condition, illness, may be injuries or lameness	May be mild or infrequent suboptimal physical fitness, minor short term injury,	Long term physical fitness, well-muscled and in optimal body condition, alert, no wounds/injuries/ lameness, able to move easily and willingly at all gaits
Domain 5 Positive affective experience inferred: *Comfort of good health, high functional capacity, vitality of fitness*	*No to very low level comfort of good health*	*Moderate level comfort of good health, functional capacity and vitality of fitness*	*High level comfort of good health, high functional capacity and vitality of fitness*

Table 7d. Grading welfare enhancement in Domain 4 (Behavioural interactions), and the positive affective experience inferred in Domain 5.

Measurable/ observable indices	Enhancement grade		
	0 (None)	**+ (Low to medium)**	**++ (High)**
Social interactions	*Rarely or never engaging in rewarding behaviours such as maternal care, mutual grooming, play, close spatial proximity*	*Occasionally engaging in rewarding behaviours such as maternal care, mutual grooming, play, close spatial proximity*	*Frequently engaging in rewarding behaviours such as maternal care, mutual grooming, play, close spatial proximity*
Domain 5 Positive affective experience inferred: *Content, engaged, affectionately sociable, maternally rewarded, sexually gratified, playful*	*No to low level contentment*	*Moderate level of contentment, engagement, affectionately sociable, maternally rewarded, sexually gratified, playful*	*High level of contentment, engagment, affectionately sociable, maternally rewarded, sexually gratified, playful*

Table 8. Examples of using welfare alerting indices for grading future welfare risk.

Measurable/ observable welfare alerting indices	Future welfare risk grade		
	No to low	**Moderate**	**High**
Domain 1 (**Nutrition**)	Good or increasing forage and water availability. Older foal likely to be weaned soon thus reducing energy requirements	Low rainfall, low or reducing forage availability	Markedly below average rainfall, very low forage availability & low water availability In foal or young foal at foot increasing energy requirements
Domain 2 (**Physical environment**)	No extreme weather conditions predicted, presence of shade/shelter/ water	Some extreme weather conditions predicted but good shade/shelter, in good BCS and good health status sufficient to cope	Extreme weather predicted, limited shade/shelter or water. Poor BCS and health status with reduced thermoregulatory ability
Domain 3 (**Health**)	Good body condition and health status	Declining body condition, high faecal egg count, poor hoof condition, skin lesions, limb abnormality, ocular or nasal discharge.	Very poor body condition, worsening health status
Domain 4 (**Behavioural interactions**)	Stable band of mixed age and sex composition	Small band size, risk of dispersal or band members dying	Few companions, companions died

6. Discussion

This chapter has used indicators shown to be feasible for assessing in free-roaming wild horses (in Chapter 5). It provides scientific evidence linking specific indicators of physical/functional states in Domains 1 to 4 to inferred mental experiences in Domain 5. It further provides a guide for grading welfare compromise, enhancement and future risk, which can be applied to individual free-roaming wild horses. This is the first time that the scientific evidence validating a comprehensive range of welfare indicators has been synthesised in this way. The material in this chapter may also act as a guide for others on scientifically supporting key aspects of welfare assessment protocols they may wish to apply to other species in a range of situations.

Several reviews have evaluated a range of welfare indicators for use in domestic horses (for example, Dalla Costa et al. 2014b; Hockenhull & Whay 2014; Hausberger et al. 2016; Hausberger et al. 2020; Lesimple 2020), but to date no clear distinction has been made between indicators of welfare status (i.e., those for which there is scientific evidence supports their use for inferring particular mental experiences), and welfare alerting indices. Ensuring that welfare status and welfare risk indicators are evaluated separately improves the discriminating power of the information used to make inferences about the animals' mental experiences. For example, a horse with cracked overgrown hooves that is not lame, could be scored similarly to a lame horse with no visible hoof abnormalities. However, their mental experiences would likely be different, as the first horse is not apparently in pain, whereas the second one is.

To be scientifically valid, indicators also need to be reliable and produce consistently repeatable results. Reliability is established when the same physical states are found and affective states inferred in different individuals of the same species confronted with the same challenge or opportunity, and the repeatability is demonstrated when similar results are found over time and by different observers. I have not evaluated these aspects of validity in this Chapter. However, in Chapter 5 I did evaluate reliability in terms of whether it was feasible to assess each indicator, in the circumstances of my study, using the described methodology. Several reviews have evaluated reliability and repeatability of welfare indicators in domestic horses (Burkholder 2000; Pritchard et al. 2005; Burn et al. 2009, 2010; Viñuela-Fernández et al. 2011; Popescu & Duigan 2013; Dalla Costa 2014b). There are more challenges when assessing these parameters in free-roaming horses. One study attempted this, but there were few welfare challenges in the horses evaluated (Harley et al. 2021). Furthermore, these horses were habituated to people and able to be approached very closely, with only direct observations being made and assessed. This is very different from my study population and many others. Any research involving assessment of welfare indicators in free-roaming wild species needs to include detailed information on the precise circumstances of the assessments, their duration and frequency, and the proximity of the assessor to the animal.

I have restricted the indicators in the suggested Five Domains Model grading schemes to those for which I could convincingly find scientific evidence for links between the indicator and inferred mental experiences. Other indicators might be identified as more welfare assessments in free-roaming animals are performed.

In summary, the adjusted version of the Five Domains Model presented here, specifically designed for grading welfare status in free-roaming wild horses can now be applied to grade welfare in individual horses, in accordance with Stages 8 and 9 of my Ten Stage Protocol. Further, the new grading scheme devised for grading future welfare risk can also be applied as per Stage 10 of my Ten Stage Protocol, described in Chapter 2 (Harvey et al. 2021).

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Thesis outline summarizing chapter titles and contents		
Chapter	**Chapter title**	**Brief description**
1	Introduction: The wild horse controversy & the importance of incorporating animal welfare science in decision making.	Summary of the history of wild horses, their cultural significance, environmental impacts and controversies in wild horse management. Introduces the importance of animal welfare science in wild animal controversies.
2 Published	A Ten-Stage Protocol for assessing the welfare of individual non-captive wild animals: free-roaming horses as an example.	Presentation of a novel conceptual framework that I designed in order to guide a systematic and scientific approach to assessing the welfare of free-roaming wild animals, using the Five Domains Model. Summarizes the principles of interpreting indicators of biological function and behaviour in terms of the mental experiences that those indicators reflect (Stage 1 of my Protocol), and how the Five Domains Model is used for assessing welfare (Stage 2 of my Protocol).
3	Literature review: A review of the species-specific information required to enable assessment of wild horse welfare.	The current status of wild horse knowledge is summarized in a novel holistic and multidisciplinary framework drawing together the relevant literature on horses across each of the four physical/functional domains of the Five Domains Model. It addresses Stage 3 of my Ten-Stage Protocol.
4 Published	Addressing the knowledge gap of gastrointestinal parasitology in free-roaming wild horses in south-east Australia.	An example of the type of detailed original research required for addressing any knowledge gaps identified in Stage 3. This chapter describes a detailed parasitological investigation of 293 faecal samples collected from 6 wild horse populations. It describes results of faecal egg counts, larval cultures and molecular diagnostics.
5 Published	Use of remote camera traps to evaluate animal-based welfare indicators in individual free-roaming wild horses.	A large body of original research investigating for the first time, both the use of remote cameras for identifying individual horses across a range of habitats, and for acquiring data on an extensive range of animal-based welfare indicators. It addresses Stages 4-6 of my Ten-Stage Protocol.
6	Scientific validation of welfare indicators for using the Five Domains Model to grade the welfare status of individual free-roaming wild horses.	Addressing Stage 7 of my Ten-Stage Protocol, this chapter evaluates the scientific evidence linking the described objective measurable/observable welfare indicators to physical/functional impacts in Domains 1-4, and the relationship between those impacts and the mental experiences that are inferred in Domain 5. This concludes with the formulation of a Five Domains Model wild horse specific welfare grading scheme.
7	**Dynamic changes in wild horse social organisation and habitat use revealed with remote camera trap monitoring.**	**With the aim of evaluating traditional ecological metrics alongside welfare status, this chapter describes original research using the remote camera trapping methodology described in chapter 5, to evaluate population dynamics, and temporal and spatial changes in social organization and habitat use of a wild horse population over a 15 month period.**
8	The cascading influence of resource availability on the welfare status of wild horses, and association with population demographics, social organization and habitat use.	This original research applied the methodology from all preceding chapters to assess welfare status and changes in welfare status in individual wild horses over a 15 month period, addressing stages 8 -10 of my Ten-Stage Protocol. It further evaluates drivers of change in welfare status and correlations between welfare status, and the population dynamics, social organization and habitat use described in chapter 7.
9	Conclusions, applications and future directions.	Summarizes overall conclusions, and contributions to the fields of wild animal welfare, wild horse ecology and welfare, wild horse management, and general horse welfare. Highlights ongoing work, some of which I have already partially completed that was not included in the main body of the thesis.

Chapter 7. Dynamic changes in wild horse social organisation and habitat use revealed with remote camera trap monitoring

Abstract

In this chapter I describe the population demographics including foaling rates and mortality, of the small and geographically constrained population of free-roaming wild horses described in Chapter 5. Using a combination of camera-traps and direct observations I evaluated in detail their social organization and habitat use over a 15-month period. Population demographics and habitat use may be important welfare alerting indices, whilst social interactions are an important welfare status indicator. Social networks were analysed for five 90-day time periods (seasons) and habitat use was explored through documenting different geographical regions, habitats and estimated areas utilized during those periods (seasons). Spatial differences across bands residing in different habitats were found in population demographics, social interactions and estimated areas utilised. Horses residing in woodland habitats had smaller band sizes, lower foaling rates, higher mortality, roamed larger areas and had fewer social interactions. Substantial seasonal changes were found in social organisation and habitat use, with bands dispersing during Winter and Spring with fewer social interactions, and utilising larger areas and a wider range of habitats. This is the first study to describe such changes in detail using camera traps. The next stage of this research is (i) to assess the drivers for these changes, (ii) to assess the welfare

status of the horses over this period, and (iii) to evaluate the associations between population dynamics, welfare status, habitat use and social organisation.

1. Introduction

In Chapter 2 (Harvey et al. 2020) I discussed how animal-based indices traditionally collected by wildlife biologists (e.g., population dynamics, home range features and size, reproductive rates and survival rates), do not directly reflect the mental experiences of individuals, but that they provide relevant contextual information. For example, low reproductive success, smaller herd sizes and/or larger home ranges, may reflect physiological states (e.g., chronic undernutrition) that would generate negative affective states of relevance to welfare. Consequently, such indices may provide information about future welfare risks, and thus become important welfare alerting indices.

Some indices traditionally collected in ecological studies may also provide information directly relevant to welfare status. For example, as discussed in Chapter 3, the primary determinant of habitat use in free-roaming horses is availability of preferred forage, and home range size is highly dependent on resource availability, factors which may also impact welfare in Domain 1 (Nutrition). Since the welfare status of free-roaming wild animals has not been scientifically studied to date, neither has the association between such indices and welfare status. Thus, it is important when assessing wild animal welfare, to also assess population dynamics and habitat use.

Sociality, the degree to which animals interact with each other, is an important welfare indicator in Domain 4 (Behavioural interactions). To date, there does not appear to be any published research specifically evaluating relationships between sociality and welfare status in free-roaming wild animals (Brakes 2019). In Chapter 5 (Harvey et al. 2021) I demonstrated how close spatial proximity could be assessed in free-roaming wild horses using remote camera traps, as a measure of social bonds between individuals. How individuals' social bonds differ spatially between different groups of animals across a range of habitats, and change over time, has not been studied in detail before and may be important to their welfare.

In Chapter 5, I also discussed how the current knowledge of wild horse ecology and behaviour (detailed in Chapter 3) has historically relied on direct observations of horses, and I demonstrated how this restricts the data collected only to those animals that can be seen directly, primarily those in open areas (Chapter 5). Assumptions are often made in population modelling that population demographics are similar across different populations and habitats, but preliminary results shown in Chapter 5 suggest that this may not be the case.

The aims of this chapter were to describe (i) population demographics including foaling rate and mortality rates, (ii) social organization within, and (iii) habitat use by, a small and geographically constrained population of free-roaming wild horses in Australia over a 15-month period, and (iv) to evaluate spatial differences across habitats, and temporal changes in social organization and habitat use, using both camera traps and direct observations.

2. Materials and Methods

2.1. Study Overview

I made both camera trap (still images and video) and direct observations of a small and geographically constrained population of free-roaming wild horses in Kedumba Valley of the Blue Mountains National Park, NSW, Australia over a 15-month period. The study area, habitats, geographical regions, and methodology for direct observations of horses, and camera trapping, have already been described in detail (Harvey et al. 2021, Chapter 5), as has the horse population (Harvey et al. 2021, Chapter 5). However, the current chapter provides more details regarding foaling rates, foal and adult mortality and changes in social organization over time. In this chapter the same data as used in Chapter 5 were used for analyzing social organization and habitat use of all the individual horses over the study period, being derived from the full data set of 42,925 image/video files as explained in Chapter 5.

Study periods

The study extended over 450-days, from 18th December 2015 to 11th March 2017 inclusive. Data were initially summarized on a monthly basis. However preliminary analysis revealed minimal changes on that basis, but substantial differences between seasons. Data were therefore collapsed into five 90-day periods for detailed analyses. Each period was broadly aligned with seasons, but the precise start and finish dates were chosen to ensure that each one was 90-days duration so that an equal number of days were being compared (Table 1).

Table 1. Dates for the time periods used for data analysis.

Period	Start date	Finish date	Predominant season[1]
1	18th Dec 2015	16th Mar 2016	Summer
2	17th Mar 2016	14th June 2016	Autumn
3	15th June 2016	12th Sept 2016	Winter
4	13th Sept 2016	11th Dec 2016	Spring
5	12th Dec 2016	11th Mar 2017	Summer

[1] Precise season dates for NSW, Australia (https://www.timeanddate.com/calendar/aboutseasons.html) are as follows: Summer: 22nd Dec – 20th Mar; Autumn: 21st Mar – 20th June; Winter: 21st June – 22nd Sept; Spring: 23rd Sept – 21st Dec

2.2 Population Demographics

Horse sightings

As described in Chapter 5, each of the 29 horses sighted during the study period had a unique combination of coat colour and markings, and was allocated a unique individual identifier based on these visually-assessable attributes. Every camera trap still image and video clip (total 42,925 image/video files) that had captured horses was assessed, and the identifications of individual horses present in images and video clips were recorded for each day of the 450-day study period. Only horses that were identifiable were recorded. In addition, photographs and video footage obtained on hand-held cameras via direct observations were viewed and identities of all identifiable horses recorded for each date, along with the habitat and geographical location where the direct observations were made. From these data, for each horse, the number of horse-days when the horse was sighted were counted, where a horse-day was defined as an individual horse-date combination where the horse was sighted at least once by any method on a particular date.

Horses observed early in the study period close together on multiple occasions, in the same geographical area but distant (> 1km) from other horses were defined as a distinct band. The predominant habitat type and geographical location where each band was most frequently observed was also recorded. Age category (in Period 1), reproductive status, sex, familial relationships of individuals and foaling rate were all recorded. The identification of individuals has been described in detail in Chapter 5 (Chapter 5: Table 5, Figure 5).

The study period ended on 11th March 2017, when a feeding, trapping and removal program was instigated. However, monitoring continued for a further 12 months to assist in obtaining survival data for the remaining horses. Further monitoring was also instigated by National Parks & Wildlife Service at a later stage.

Identification of mortalities and survival analysis

The Kaplan-Meier survivor function was estimated for the study population, assuming all horses were present in the study area from study start date other than the foal that was born early in Period 5. This foal was excluded from calculations of the survival function.

Sightings by any method were used to identify mortalities. For horses seen alive after the study end date (11th March 2017), intervals from the study start date were right-censored on the study end date. Horses not seen alive after the study end date were classified as having died 30 days after their last sighting during the study period, except for one horse whose carcass was found. Based on direct observations, this horse was assessed as having died in early March 2017 and for calculations, its date of death was set at 7th March 2017 (4 days before the study end date). For other horses, 30 days was chosen based on the cumulative distribution of intervals between sightings. Using the 1,470 such intervals that commenced in periods 3, 4 or 5, for 99% of intervals, horses had been resighted within 30 days (Figure 1).

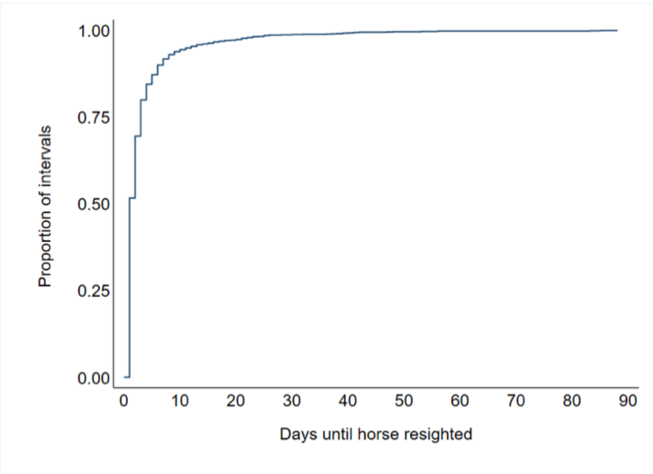


Figure 1. Cumulative distribution of intervals between sightings for 1,470 intervals commencing in Periods 3, 4 or 5.

The assumption that the horses not seen alive after the study end date had died was supported by the high frequency of sightings during the 30 days prior to their last sighting date during the study; these horses were seen on between 3 and 18 days in that period (median 6 days; including the day of their last sighting). For some horses, other observational data supported the assumption that the horse had died. For example, (i) a sudden cessation of stallion faecal piles in the bands habitat, together with the band no longer being observed with a stallion; (ii) a mare being observed only without her foal despite still nursing it on last observations; (iii) extreme emaciation +/- weakness, +/- dyspnoea observed during the last observations.

Age at death

For the horse whose carcass was found, age at death was determined by close evaluation of dentition. Approximate age was known for horses < 1 year of age. For the remaining horses age at death was unknown. Historical information regarding age of death of previous horses in the population was obtained by evaluating the dentition of any skulls that were opportunistically found during ground surveys.

2.3. Social organization

In addition to initial band composition, data on individual horses' close companions was also obtained for each separate period. This information was further utilised to construct social networks for each separate period.

Analysis of close companions

Horse-days when a pair of horses were defined as being in close spatial proximity were identified as previously described (Harvey et al. 2021, Chapter 5), i.e., as occurring if, at least once on that day, both horses were present at the same time in any still image or video clip, or one horse was in one still image and a second horse was sighted in a subsequent still image taken within one second of the previous image.

Any horse recorded in close spatial proximity to another horse, was defined as being a 'close companion' to that horse on that day. A horse could have two or more close companions on the same day if sighted with two or more other horses in the same or subsequent still image taken within one second or video frame and/or if the horse was sighted with another horse on one occasion and a different horse on a separate occasion on the same day.

A horse was regarded to be 'only alone' on any day that the horse was sighted alone, and never sighted with any close companions. A horse could be sighted alone on one or more occasions during the same day, but if also sighted with one or more close companions on the same day, that day was not regarded as 'only alone'.

For each period, I recorded (i) the number of days that each horse was sighted only alone, (ii) the number of days each horse was sighted with close companions (with or without also being sighted alone on the same day), and (iii) how many different close companions it was sighted with on those days, and in addition, (iv) the total number of different close companions that each horse had over the whole period. When analysing social organization, all camera trap data were used. Data from direct observations were also used when photographic or video footage had been recorded during the observations. Some direct observations were excluded if photographic or video footage had not also been obtained, since close companionship data had not been recorded at the time.

Initial bands were defined for each horse as the band that they were identified to be within during Period 1. Analysis was performed by initial band, as well as by study period (season). For statistical analyses, bands were combined into subpopulations based on the habitat type that they were typically seen in during Period 1. Subpopulation A comprised initial bands 1 and 2, the two bands that first resided in large open grassland areas, and subpopulation B comprised initial bands 3, 4 and 5, the three smallest bands that first resided in small grassland, open disturbed woodland or woodland habitats.

Social networks

Social networks were analysed separately for each period (season) using the numbers of days that each horse was identified as a close companion of each other horse.

The basic unit of analysis in social networks is the dyad. A dyad is a pair of nodes (in this case, a pair of horses). If two horses were close companions on at least one day in the period, they were considered to have a direct 'tie'. Tie values for each pair of horses were calculated as the percentage of all days in the period when both horses were seen at least once to have had close contact. For example, if horse A was seen at least once on 30 days, horse B was seen at least once on 20 days, 16 of which were days when horse A was also seen, and of those 16 days, horses A and B were seen in close contact on 10 days, the tie value for that pair of horses would be 10/16 or 63%.

Social networks were plotted for each period using each of modern multidimensional scaling and sociomatrices, employing the -nwplot- command in Stata (version 16, StataCorp, College Station, Texas, USA). Tie values were categorised, and the category of each direct tie indicated. Horses seen at least once in the period but with no direct ties are shown as unconnected nodes on the multidimensional scaling figure, and are listed in the sociomatrix but with a tie value category of 0 for its relationships with each other horse. Horses not seen during the period are shown as unconnected nodes with an asterisk.

2.4. Habitat use

The eleven distinct geographical regions, comprising four different habitat types have previously been described in detail (Harvey et al. 2021; 2.1.3-2.1.5, Figures 1-4; Chapter 5). For ease of reference, Figure 1 in Chapter 5 is repeated here as Figure 2 which outlines the geographical regions, habitat types and camera trap locations.

Geographical regions utilized

From direct observations, and camera trap still images and video recordings, the number of days that each horse was sighted at least once, was recorded for each of the eleven geographical areas, for each period, for each horse. For analysis, the data for individual horses was grouped together in terms of their initial band.

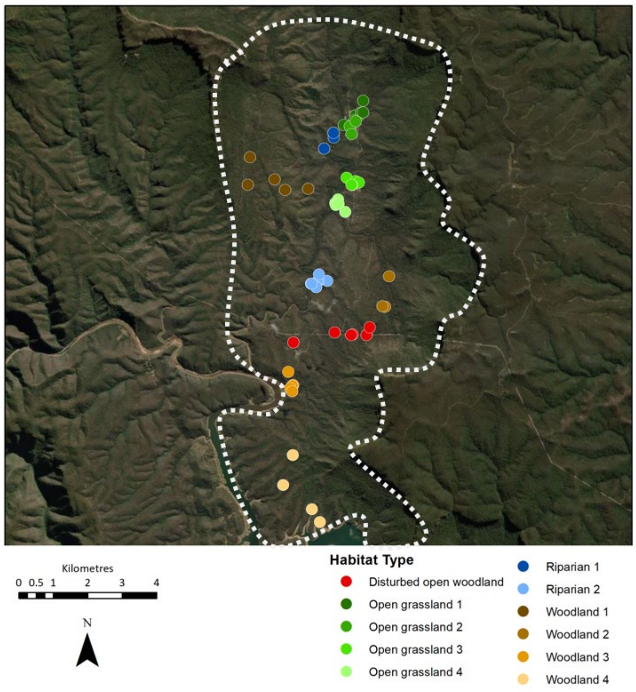


Figure 2. Topographical map of the study area illustrating camera locations and habitat types in the 11 geographical regions. The white dashed line represents the geographic boundaries that inhibited immigration and emigration of this horse population during the study period. Reproduced from Harvey et al. 2021 (Chapter 5).

Estimated area utilised

An Estimated Area Utilised by each horse, for each period, was defined as the size of an area based on the locations of all cameras where the horse was sighted at least once in that period. Only sightings where the horse's identity was unequivocally known were used. Direct observations were not used for these calculations. However, no horse was ever directly observed in a region where it was not also detected on camera traps. Latitude and longitude GPS co-ordinates of each camera location were identified.

The Estimated Area Utilised was calculated as the smallest of the areas of two rectangles. The first rectangle was oriented with sides running North-South and top and bottom running East-West. The width of the rectangle was the distance between the West-most and East-most longitude coordinates of the cameras at the midpoint of the South-most and North-most latitude coordinates of the cameras. The height of the rectangle was the distance between the South-most and North-most latitude coordinates of the cameras at the midpoint of the West-most and East-most longitude coordinates of the cameras. Distances between co-ordinates were calculated using Pythagoras' theorem on an equirectangular projection (https://www.movable-type.co.uk/scripts/latlong.html):

Width or height in km = $6371*\sqrt{((diff_{long}*cosine((lat_{south}+lat_{north})/2))^2+(diff_{lat})^2)}$

where:

6371 = approximate radius of the earth in km

$diff_{long}$ = difference between the West-most and East-most longitude coordinates in radians

lat_{south}+lat_{north} are both the midpoints of the South-most and North-most latitude coordinates in radians

$diff_{lat}$ is difference between the South-most and North-most latitude coordinates in radians

As width was calculated at the midpoint of the South-most and North-most latitude coordinates, when calculating width, $diff_{lat}$ was 0. As height was calculated at the midpoint of the West-most and East-most longitude coordinates, when calculating height, $diff_{long}$ was 0. Area in km^2=width*height (both in km).

The second rectangle was oriented with the sides (2 parallel lines with positive slopes) running 45^0 from the South-North line, from South-West to North-East, and top and bottom (2 parallel lines with negative slopes) running perpendicular to the sides, 45^0 from the West-East line, from North-West to South-East. For each horse, all lines with 45^0 positive slopes through each camera location were assessed and the cameras with the most North-West line and the most South-East line were selected; these lines were the sides of the rectangle. Similarly, all lines with 45^0 negative slopes through each camera location were assessed and the cameras with the most North-East line and the most South-West line were selected; these lines were, respectively, the top and bottom of the rectangle. The width (distance between sides along line parallel to top and bottom) and height (distance between top and bottom along line parallel to sides) of that rectangle were calculated as described in Supplementary material 1. Area in km^2 was then calculated as width x height (both in km).

The Estimated Area Utilised was defined as the smallest of the areas of the first and second rectangles. These methods were checked by mapping camera co-ordinates for selected horses in selected periods using an on-line mapping tool (https://www.google.com/maps/d/), drawing the two rectangular areas using that tool, and comparing the areas as reported to those we calculated. Areas were calculated only for horses seen on at least three cameras in the period.

2.5. Statistical analyses

Statistical analyses were performed using Stata (version 16, StataCorp, College Station, Texas, USA). Odds of a horse being sighted only alone on a day when it was sighted at least once were assessed using logistic regression with horse fitted as a random effect using Stata's -xtlogit- command. The unit of analysis was the horse-day. For comparisons of number of close companions on any day that the horse was sighted, the unit of analysis was also the horse-day. Multilevel mixed-effects Poisson and negative binomial regression models were compared using the likelihood ratio test, with initial subpopulation and period fitted as fixed effects using Stata's -mepoisson- and -menbreg-commands. The same approach was used for comparisons of the number of close companions on any day when the horse was sighted with one or more close companions. For comparison of total number of close companions for the period for each horse, the unit of analysis was the horse-period. Multilevel mixed-effects linear regression models were fitted with horse as a random effect and a first order autoregressive correlation fitted for correlations in residuals between periods within horse. These were fitted using Stata's -mixed- command. Estimated areas utilised were compared using the same approach. For all dependent variables, initial subpopulation and period were fitted as fixed effects. The interaction between these variables was assessed; the p-value for the interaction terms jointly was calculated using the likelihood ratio test.

3. Results

3.1 Population demographics

Horse sightings

There were a total of 3408 horse-days where horses were sighted and identified at least once by any of camera trap still images, camera trap videos, or direct observations. For 3148 horse-days, the horse was sighted only on camera trap images and/or videos, for 147 horse-days, the horse was sighted only by direct observations, and for 113 horse-days, the horse was sighted at least once by both camera trap and direct observation.

Table 2. Number of horses and number of days sighted during each period.

Period (season)	Number of horses sighted	Number of days horses were sighted			
		Range	Mean	Median	SD
1 (Summer 2015/16)	25	1-75	46.4	51	19.3
2 (Autumn 2016)	28	4-44	26.8	26.5	11.9
3 (Winter 2016)	24	1-23	7.5	6.5	5.76
4 (Spring 2016)	23	1-57	25.1	23	15.96
5 (Summer 2016/17)	19	3-57	38.8	43	16.03

Note that in Period 1, there where an additional 3 horses known to be present (since they were sighted in Period 2 and could not have entered the population during the study period). There where an additional 4 horses present in Period 2 that were not seen in Period 3 and an additional 5 horses present in Period 2 that were not seen in Period 4. Results for Period 5 include a new foal (1L), born around the start of Period 5 (22-24th Dec) and there where an additional 10 horses present in Period 2 that were not seen in Period 5.

Combining all methods of sighting horses, 29 horses were each sighted at least once during the 450-day study period, with horses sighted from 7 to 229 days (mean 117.5, median 117, SD 66.2). In total, 29 horses were sighted by camera trap still images on 6 to 216 days (mean 110, median 105, SD 63.1), 26 horses were sighted by camera trap videos on 1 to 16 days (mean 6.5, median 6, SD 4.3), and 25 horses by direct observations on 1 to 20 days (mean 10.4, median 12, SD 5.4). The number of days horses were sighted during each period is shown in Table 2.

Table 3. Details of the population (adapted from Harvey et. al 2021; Chapter 5).

Unique Identifier[1]	Sex[2]	Age group	Reproductive status	Mortality
1A	M	Mature	Band stallion	Alive at study end
1B	F	Mature	Foaled period 5	Alive at study end
1C	F	Mature	No foals during study period	Alive at study end
1D	F	Mature	No foals during study period	Alive at study end
1E	F	Mature	No foals during study period	Alive at study end
1F	F	Mature	Yearling (1G) at foot in period 1	Alive at study end
1G	F	Yearling	Yearling of 1F (estimated birth summer 2014/15)	Alive at study end
1H	F	Mature	Foal (1K) born period 1	Died period 5 (carcass found)
1I	F	Mature	Foal (1J) born period 1	Alive at study end
1J	M	Foal	Foal of 1I born period 1	Alive at study end
1K	F	Foal	Foal of 1H born period 1	Alive at study end
1L	Unknown	Foal	Foal of 1B born period 5	Died period 5
2A	M	Mature	Band stallion	Died period 3
2B	F	Mature	Foal (2C) at foot in period 1	Alive at study end
2C	M	Foal	Foal of 2B (estimated birth early spring 2015)	Died period 4
2D	M	Yearling	Dam unknown, possible 2B (estimated birth early spring 2014)	Alive at study end
2E	F	Juvenile	No foals during study period	Alive at study end
3A	M	Mature	Band stallion	Died period 5
3B	F	Mature	Foal (3C) at foot in period 1	Alive at study end
3C	F	Foal	Foal of 3B (estimated birth early spring 2015)	Died period 5
3D	M	Juvenile	Colt with another stallion in band	Alive at study end
4A	M	Mature	Band stallion	Died period 5
4B	F	Juvenile	No foals during study period	Died period 3
4C	F	Mature	No foals during study period	Died period 4
4D	F	Mature	No foals during study period	Alive at study end
5A	M	Mature	Band stallion	Alive at study end
5B	F	Mature	No foals during study period	Died period 3
5C	F	Mature	No foals during study period	Died period 2
5D	F	Mature	No foals during study period	Died period 3

[1] Number = band; Letter = sequential letter within band

[2] M = Male; F = Female

The band compositions, sex, age distribution, reproductive status and mortalities are shown in Table 3. Excluding the foal born at the end of the study (1L), foals comprised 14% of the population, juveniles 18% and adults 68%. Juveniles were defined as being approximately >1 but < 3 years of age based on physical appearance and behaviour. Adult male to female sex ratio was 0.26. There were five distinct bands at the start of the study period. Band compositions and predominant habitat types and geographical location of bands at the start of the study were detailed in Chapter 5.

Foaling rate

Based on the number of yearlings present at the start of the study period, two surviving foals had been born in 2014 (1G, 2D). During Spring 2015 (prior to study start) two foals had been born that were alive at the start of the study (2C, 3C). A further two foals were born during period 1 (Summer 2015/16). One foal (1L) was born late December 2016 (Period 5; Summer 2016/17). During the study period there were no sightings of any surviving foals from bands 4 and 5.

In total there were 14 mature mares in the population at the start of the study period. Overall foaling rate (number of foals/number of sexually mature mares) for Spring/Summer 2015/2016 was therefore 0.28 (4/14). Foaling rate for Spring/Summer 2016/2017 was 0.07 (1/14). Assuming foals born Spring/Summer 2014/2015 had survived to the beginning of the study period, then foaling rate for that season would have been 0.14 (2/14).

Presumed mortalities and survival analysis

All horses were assumed to have been present in the study area from the study start date, apart from the foal that was born early in period 5 (1L). Of these 28 horses, 17 were seen alive after the study end date. These horses' last sightings were 0 days (n=11 horses), 2 days (n=2 horses), 4 days (n=2 horses), and 98 days (n=2 horses) before the study end date. Eleven horses were assumed to have died during the study; the carcass was found for one, and the other 10 were last seen 19, 69, 91 days (for each, n=1) and 166 to 321 days (n=7 horses) before study end date. The horse whose carcass was found (1H) was last seen alive 18 days before the study end date and 14 days before its estimated date of death. The foal born early in period 5 (1L) was also thought to have died, during Period 5, as it was never sighted again beyond the study period, whilst the dam was observed regularly before and after the study end date.

Therefore, a total of 12 horses were presumed to have died during the study period (Table 3); three foals (1L, 2C, 3C), one juvenile (4B) and eight mature horses (1H, 2A, 3A, 4A, 4C, 5B, 5C, 5D). Thus, the 15-month cumulative incidences of mortality for juveniles and adults were, 0.2 (1/5) and 0.42 (8/19) respectively. Foal mortality was 0.6 (3/5). Of the adults that died, three were stallions, one was a lactating mare and four were non-lactating mares. There was one horse from each of bands 1 and 2, three horses from band 4, and three horses from band 5.

One foal is thought to have died during Spring, Period 4 (2C) and the others during Summer, Period 5. Of the mature horses, one is thought to have died during Autumn; Period 2 (5C), four during Winter; Period 3 (2A, 4B, 5B, 5D), one during Spring; Period 4 (4C), and three during Summer; Period 5 (1H, 3A, 4A).

Survivor functions were calculated using the 28 horses. Of the 10 horses classified as having died 30 days after their last sighting during the study period, three were last seen ≤91 days before the study end date whereas the other seven horses were last seen ≥166 days before the study end date. The sensitivity of the survivor function to the assumed dates of death for these three horses was assessed by right-censoring on their last sighting date (rather than being classified as having died 30 days after their last sighting). Survival functions are shown in Figure 3.

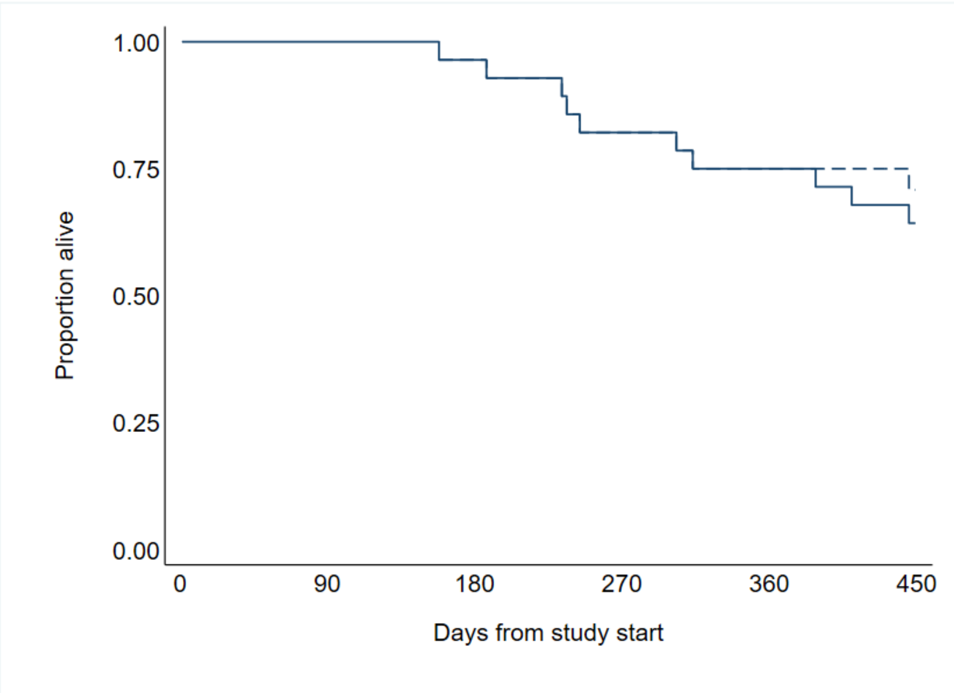


Figure 3. Survivor functions from the study start date for 28 horses. The solid line is when the three horses not seen alive after the study end date and whose carcass was not found that were last seen ≤91 days before the study end date were classified as having died 30 days after their last sighting. Dashed line is when these three horses were instead right-censored on the last date they were sighted.

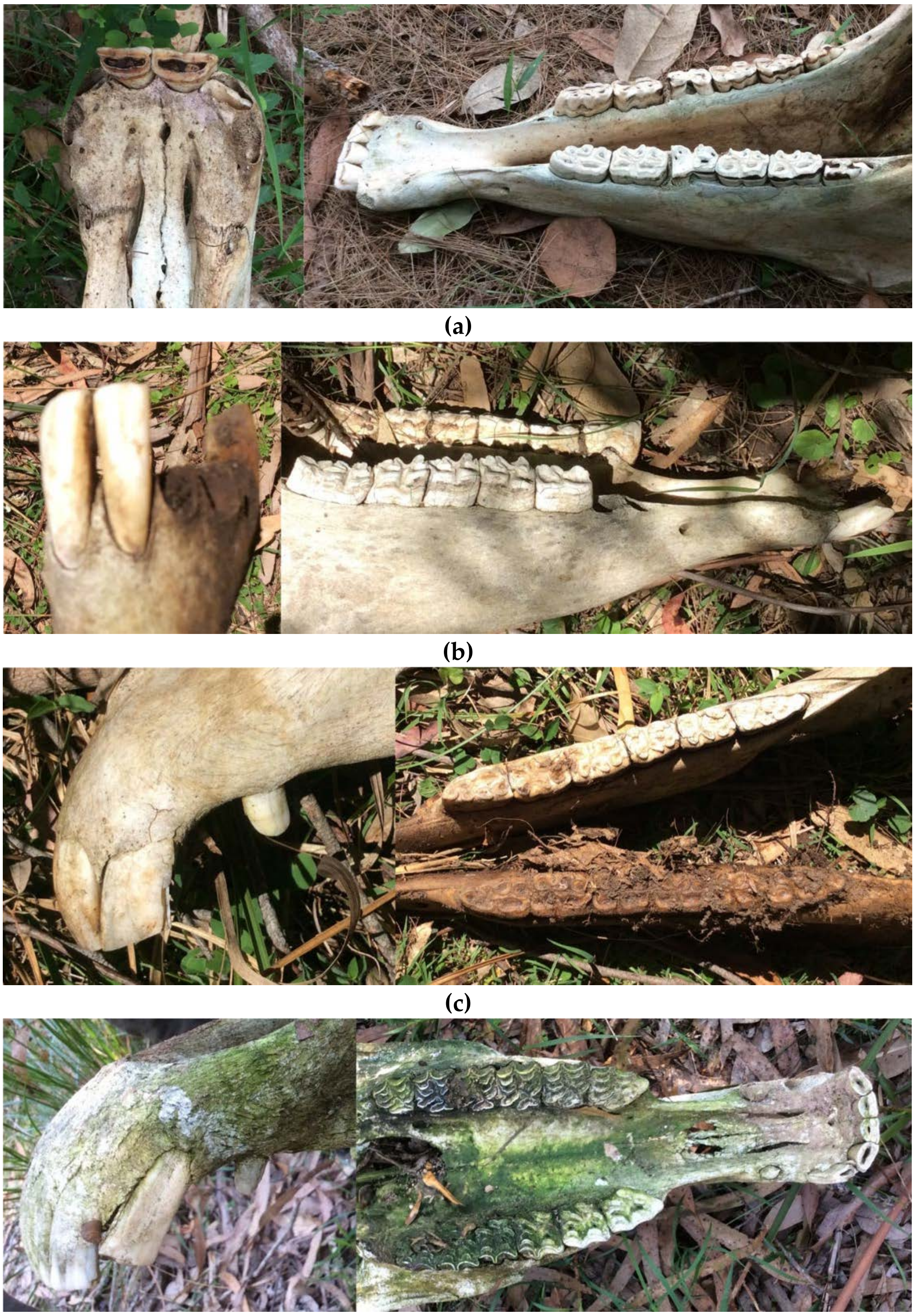
Estimated ages at death

For the study population, one foal was estimated to be approximately six weeks of age at death, and the other two approximately one year of age. For the mature horses, age at death was unknown, other than for one horse (1H) whose carcass was found. This mare was evaluated to be approximately 8 years old at death, based on close evaluation of dentition (Figure 4).

Historical information regarding age at death of horses previously in the population was obtained by evaluating the dentition of any skulls that were opportunistically found during ground surveys (Figure 5). Four skulls were found with age at deaths estimated to be 3.5 years, 10-12 years, 12-18 years and > 20 years (Figure 5).

(a) **(b)**

Figure 4. Dentition from mare 1H. **(a)** Mandible, occlusal aspect of incisors. The occlusal surfaces are smooth and all cups are gone. The dental star is visible on the central and middle incisors, but not yet on the lateral incisors, indicating the horse is 7 – 8 years of age. **(b)** Maxilla and mandible, labial aspect. Viewed in profile the upper right corner incisor (103) is slightly wider than it is tall (this tooth appears square at approximately 10 years of age), consistent with an approximate age of 8 years.



(a)

(b)

(c)

(d)

Figure 5. Dentition of skulls found from horses previously in the population that had died prior to the study. **(a)** Age at death approximately 3.5 years old. Left: Maxilla (occlusal aspect). Permanent central incisors (triadan 101 and 201) have erupted, indicating the horse is at least 2.5 years of age. The permanent left middle incisor (202) can be seen inside the alveolus (eruption occurs at 3.5 years of age). The deciduous teeth are missing from the skull. Right: Mandible (occlusal aspect). Permanent second and third premolars have erupted (erupt at 2.5 and 3 years, respectively). The fourth premolar is deciduous. Permanent first and second molars are fully erupted (erupt at 9-12 months, and 2 years). Third molars are erupting, which occurs at 3.5 – 4 years of age. **(b)** > 20 year old mare. Left:

Mandible (labial aspect). Permanent right middle and lateral incisors are present in the skull. The crowns are tall, indicating this is an aged horse. Right: Mandible. The acute incisor angle is further evidence that this is an aged horse. There is no evidence of canine teeth, indicating this skull belongs to a mare. There are sharp enamel points on the lingual aspect of the mandibular cheek teeth, and a ramp on the lower left second premolar (triadan 306). **(c)** 10-12 year old stallion. Left Maxilla (labial aspect). Viewed in profile the upper left corner incisor (203) is slightly taller than it is wide (this tooth appears square at approximately 10 years of age), indicating the horse is likely to be over (but close to) 10 years of age. Well-developed maxillary canine teeth are present. Right 3 Mandible (occlusal aspect). All permanent mandibular cheek teeth are present and in good condition. **(d)** Middle-aged stallion (approximately 12 – 18 years of age). Left: Maxilla (labial aspect). Permanent incisors are present in the skull. The crowns are tall indicating this is a middle aged to older horse. Note the moderate angle of the incisor arcade, indicating the horse is middle aged. Right: Maxilla (occlusal aspect). All permanent maxillary cheek teeth are present and in good condition. Well-developed maxillary canine teeth are present.

3.2 Social organisation

In total, 3,326 horse-days were used to assess social organisation; 82 horse-days where the horse was sighted by direct observations were excluded from social organisation analyses since close companions were not recorded.

Analysis of close companions

Frequency of horses sighted 'only alone'

For each horse sighted in each period, the percentage of the days when it was sighted where it was sighted only alone, was calculated (Figure 6). For example, four horses in Period 1 were only alone for ≤10% of the days when they were sighted. These percentages would be expected to be widely distributed when the number of days when the horse was sighted was extremely low simply because of the limited number of possibilities under the binomial distribution. However few horses had extremely low numbers of days and there was no obvious association between these percentages and numbers of days.

Figure 6 shows that during Period 1 (Summer) horses were sighted only alone on a lower percentage of days than during other periods. Higher proportions were apparent for Periods 2 (Autumn), 3 (Winter) and 4 (Spring), with the greatest number of horses being sighted only alone, for the highest proportion of days, being in period 3 (winter). A higher number of horses were sighted only alone for a greater proportion of days in Summer 2016-17 (Period 5) compared to Summer 2015-16 (Period 1).

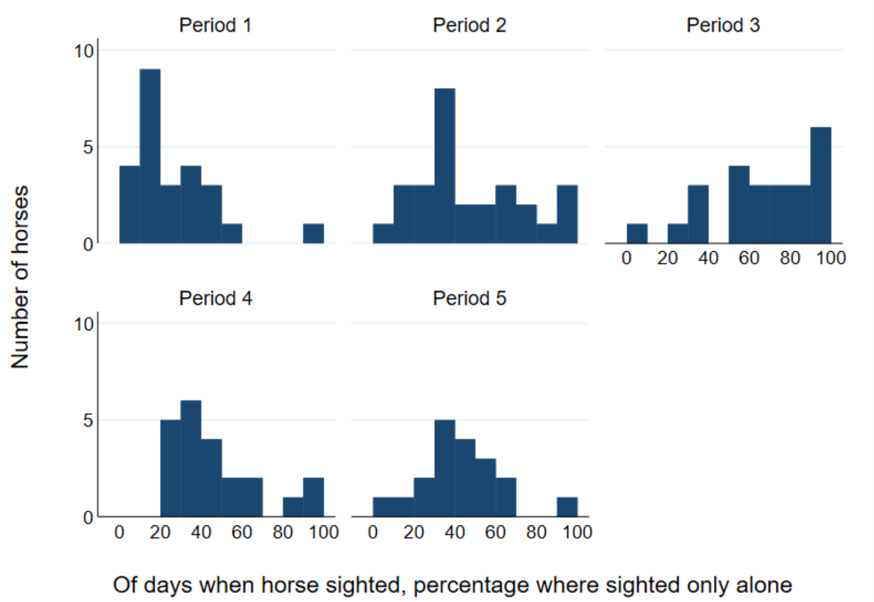


Figure 6. Number of horses sighted only alone, presented as a percentage of the total days they were sighted during Periods 1 to 5, where n=25, 28, 24, 22 and 19 horses, respectively. Bar widths are 10%.

The odds of a horse being sighted only alone (rather than being sighted with close companions) on any particular day were greater in each of Periods 2 to 5 compared to Period 1, and increases in odds were greater for horses in initial subpopulation B (the

3 smaller initial herds combined) compared to increases by Period for initial subpopulation A (P for interaction 0.027). Estimated odds ratios for Periods 2 to 5 compared to Period 1 in initial subpopulation A were, respectively, 2.4 (95% CI 1.8 to 3.0), 6.1 (3.8 to 9.8), 2.7 (2.1 to 3.6) and 2.7 (2.1 to 3.5; for all, P<0.001). Corresponding estimates for initial subpopulation B were 4.1 (2.8 to 6.1), 12.5 (6.7 to 23.2), 5.3 (3.4 to 8.3) and 2.8 (1.7 to 4.6; for all, P<0.001).

Number of close companions on horse-days

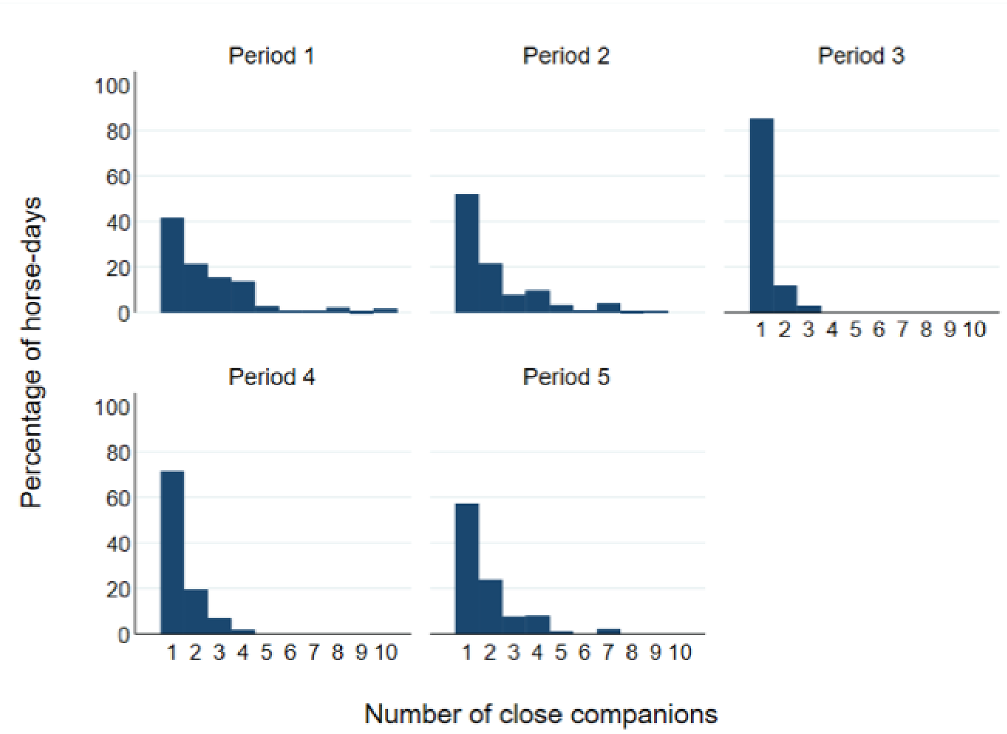
When a horse was sighted with one or more close companions, the number of close companions that a horse had on any given horse days was calculated (Table 4). For example, if horse A was seen on one camera in close spatial proximity to horse B, and on another camera in close spatial proximity to horse C, that constituted two close companions on one horse-day.

Table 4 shows that in Periods 1 (Summer) and 2 (Autumn) some horses had days with up to 9 or 10 close companions, and that up to four close companions was common. Whereas, in Periods 3 (Winter) and 4 (Spring) horses were never observed to have more than four close companions, and only one or two close companions was most common. Figure 7 shows the percentage of horse-days that different numbers of close companions were observed in different seasons for subpopulations A and B. Similar seasonal trends (Periods 1 to 5) are apparent, but horses in subpopulation A generally had more close companions than horses in subpopulation B.

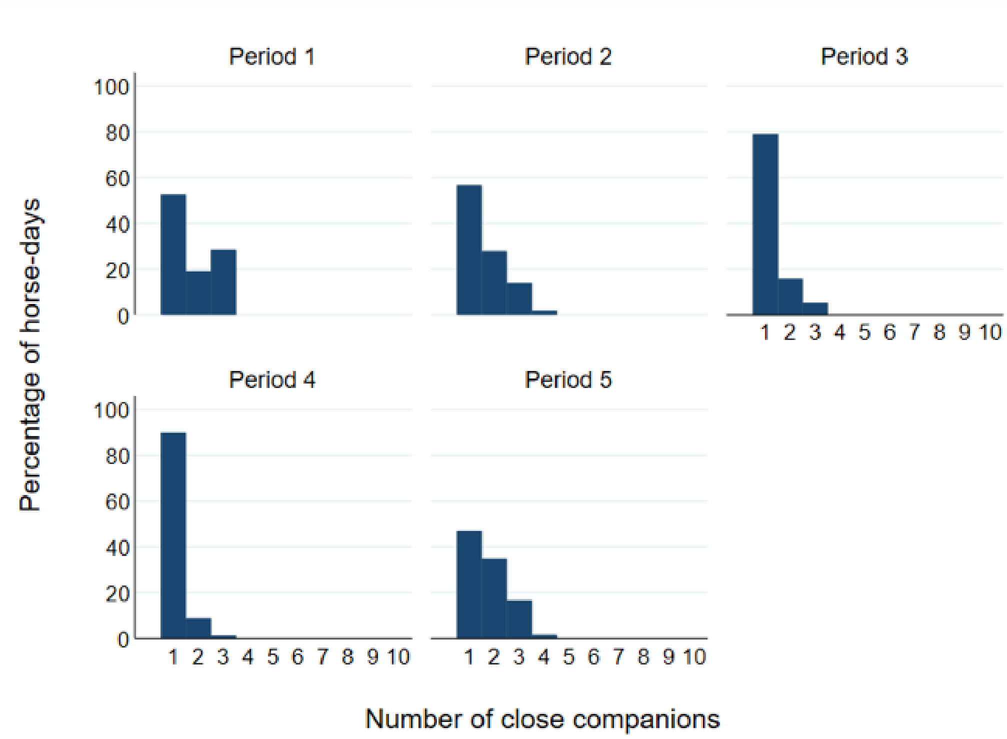
Table 4. Number of horse-days when horses were seen with between 1 and 10 close companions.

Number of close companions	Number of horse - days Period 1 (Summer)	Period 2 (Autumn)	Period 3 (Winter)	Period 4 (Spring)	Period 5 (Summer)	Total number of horse-days
1	375	209	44	228	248	1104
2	177	91	7	50	113	438
3	157	37	2	16	40	252
4	88	29	0	4	31	152
5	17	9	0	0	4	30
6	6	3	0	0	0	9
7	6	11	0	0	8	25
8	13	1	0	0	0	14
9	1	2	0	0	0	3
10	11	0	0	0	0	11

For statistical comparisons of the number of close companions a horse had on any particular day when the horse was sighted with close companions, there was no evidence that the negative binomial model was superior to the Poisson model (P=0.994) so Poisson models were used. The p-value for interaction between initial subpopulation and period was 0.143 and so no interaction was assumed. The mean number of close companions when the horse was seen with one or more of them differed by period, with means in Periods 2 to 5 estimated as being 89% (95% CI 82% to 97% P=0.006), 55% (95% CI 43% to 71% P<0.001), 58% (95% CI 52% to 65% P<0.001) and 77% (95% CI 71% to 84% P<0.001) of the mean for Period 1. The mean for the initial subpopulation B was estimated to be 76% of the mean for the initial subpopulation A (95% CI 69% to 84%; P<0.001).



(a)



(b)

Figure 7. Seasonal changes in the percentage of horse-days where horses were observed to have different numbers of close companions for **(a)** the initial subpopulation A and **(b)** the initial subpopulation B. Only horse-days where the horse had one or more close companions were included.

For comparisons of number of close companions on any particular day including days when the horse was seen only alone, the negative binomial model was superior to the Poisson model (P<0.001) so negative binomial models were used. The p-value for interaction between the initial subpopulation and period was 0.056 and no interaction was assumed. The mean number of companions differed by period, with means in Periods 2 to 5 in initial subpopulation A estimated to be 65% (95% CI 59% to 72%), 23% (18% to 31%), 41% (36% to 46%) and 58% (52% to 64%) of the mean for Period 1 (for all, P<0.001). The mean number of companions in initial subpopulation B was estimated to be 57% (95% CI 47% to 69%; P,0.001) of that for initial subpopulation A.

Total number of different close companions within periods

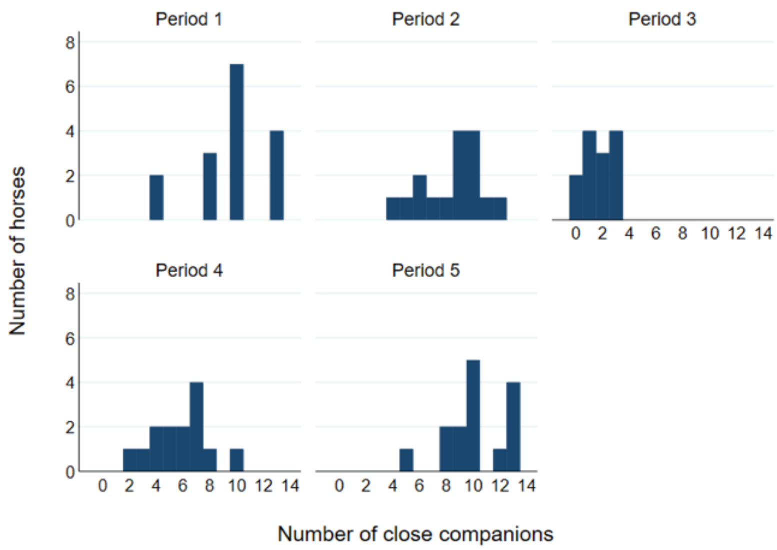
The total numbers of different close companions that horses had over the 450-day study period varied between horses from 0 to 18 (median 12, mean 10.1, SD 5.6). Table 5 shows that horses had more different close companions in Period 1 (Summer), 2 (Autumn) and 5 (Summer), than they did in Periods 3 (Winter) and 4 (Spring).

Table 5. Total number of different close companions in each period.

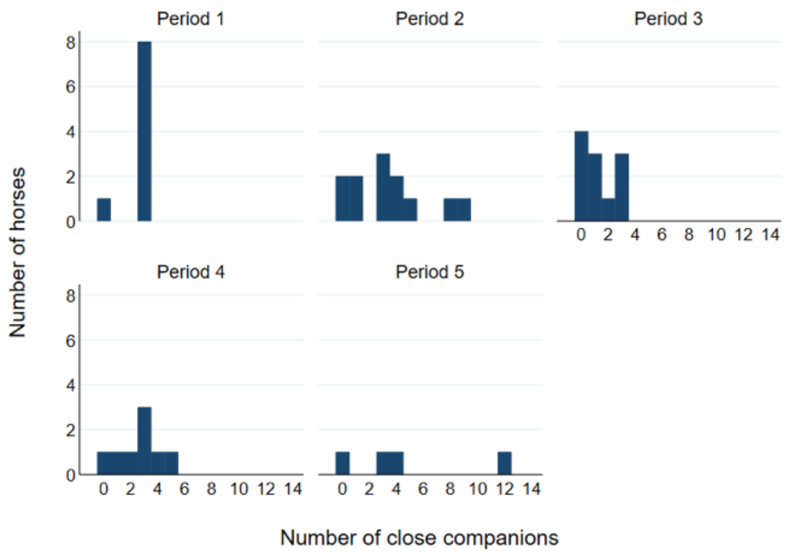
Period	Number of horses sighted	Number of close companions within period[1]			
		Maximum	Mean	Median	SD
1 (Summer)	25	13	7.1	8	4.1
2 (Autumn)	28	12	6.3	6.5	3.6
3 (Winter)	24	3	1.5	1	1.2
4 (Spring)	22	10	4.6	4.5	2.5
5 (Summer)	19	13	9.1	10	3.7

[1]For each period, the minimum was 0 close companions, i.e., within each period, at least one horse was sighted on at least one day, but never with close companions

Figure 8 shows the total number of close companions for horses in subpopulations A and B, illustrating similar trends, but with horses in initial subpopulation A having greater numbers of close companions than horses in subpopulation B.



(a)



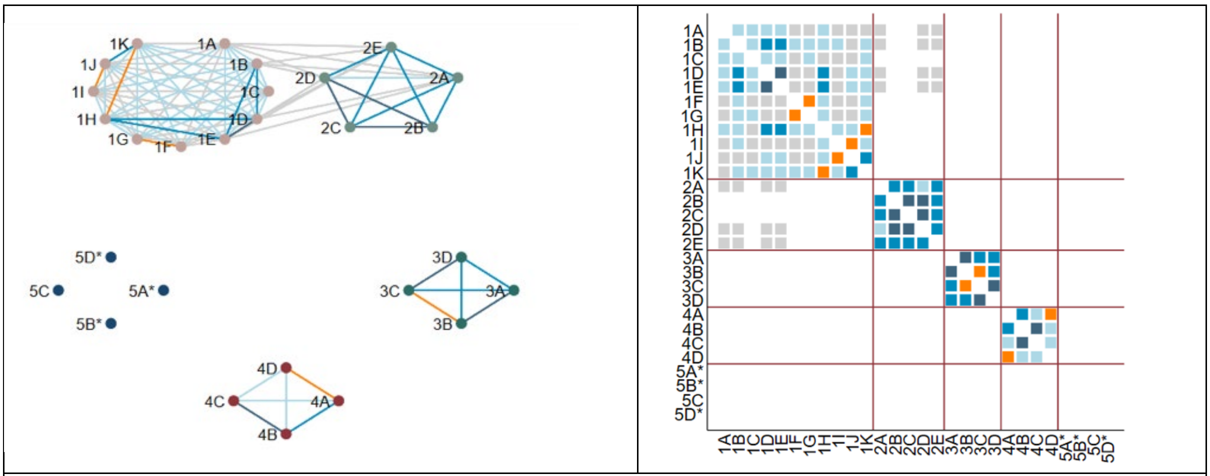
(b)

Figure 8. Seasonal distributions of the number of horses having different total numbers of close companions in **(a)** initial subpopulation A and **(b)** initial subpopulation B. Only horses sighted at least once in the period were included.

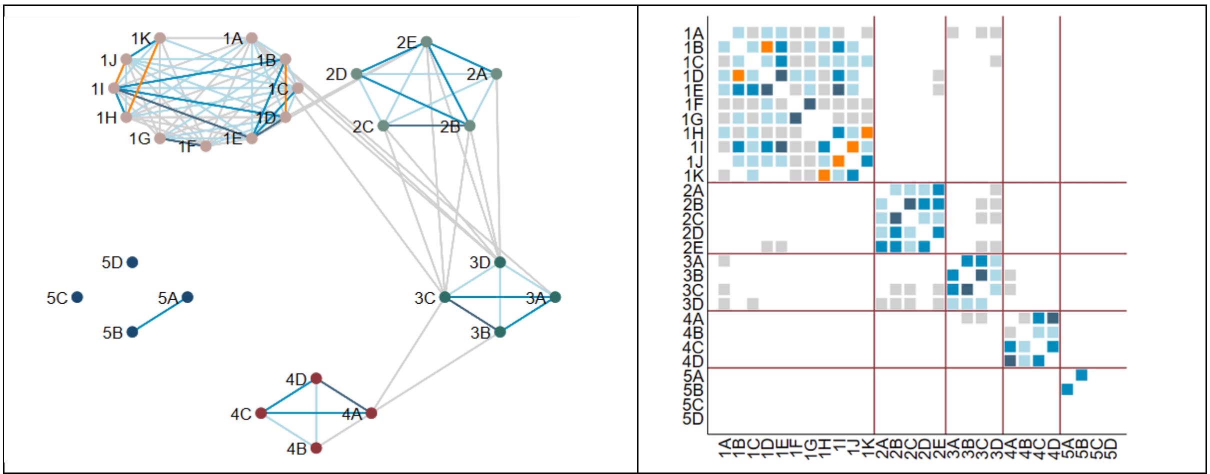
For the horse's total number of close companions for the period, there was evidence of interaction between initial subpopulation and period (P for interaction <0.001). In initial subpopulation A, the number of close companions differed by period, with means in periods 2 to 4 in initial subpopulation A, estimated as being 1.2 (95% CI 0.0 to 2.4; P=0.049), 8.1 (6.7 to 9.5; P<0.001), and 3.9 (2.5 to 5.4; P<0.001) less than the mean for Period 1, respectively, and 0.5 more for period 5 (95% CI 0.9 less to 1.9 more; P=0.485). In contrast, estimated changes by period were less marked for initial subpopulation B; the corresponding estimates were 1.1 more (95% CI 0.4 less to 2.6 more; P=0.159), 1.2 less (95% CI 2.9 less to 0.5 more; P=0.177), 0.0 difference (95% CI 1.9 less to 1.9 more; P=0.971), and 1.9 more (95% CI 0.5 less to 4.3 more; P=0.120) companions compared to Period 1.

Social network analysis

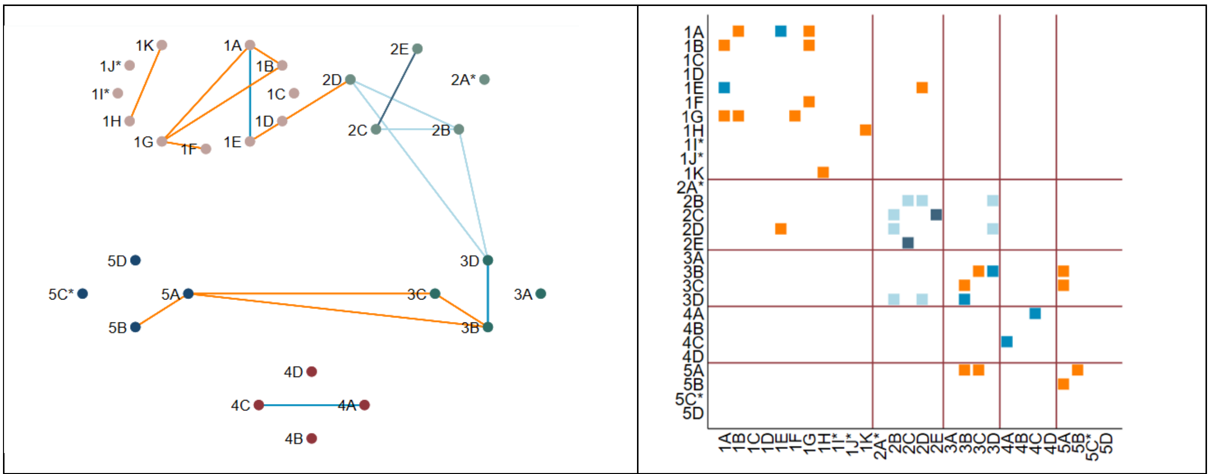
Analysis of social networks demonstrates substantial changes in social organisation throughout the study period, illustrating changes not only in the numbers of close companions, but also who those close companions were (Figure 9). In Period 1 (Summer) there were clearly five distinct bands, with some limited interactions between bands 1 and 2. In Period 2 (Autumn) there still appeared to be distinct bands but more interactions between bands 1, 2 and 3, and between bands 3 and 4. By Period 3 (Winter) there were no distinct bands, and horses exhibited close spatial proximity with much fewer horses, with many having no close companions at all. In Period 4 (Spring) there were more interactions between individual horses but still no distinct bands, and in Period 5 (Summer) interactions occurred between most of the remaining horses in the population.



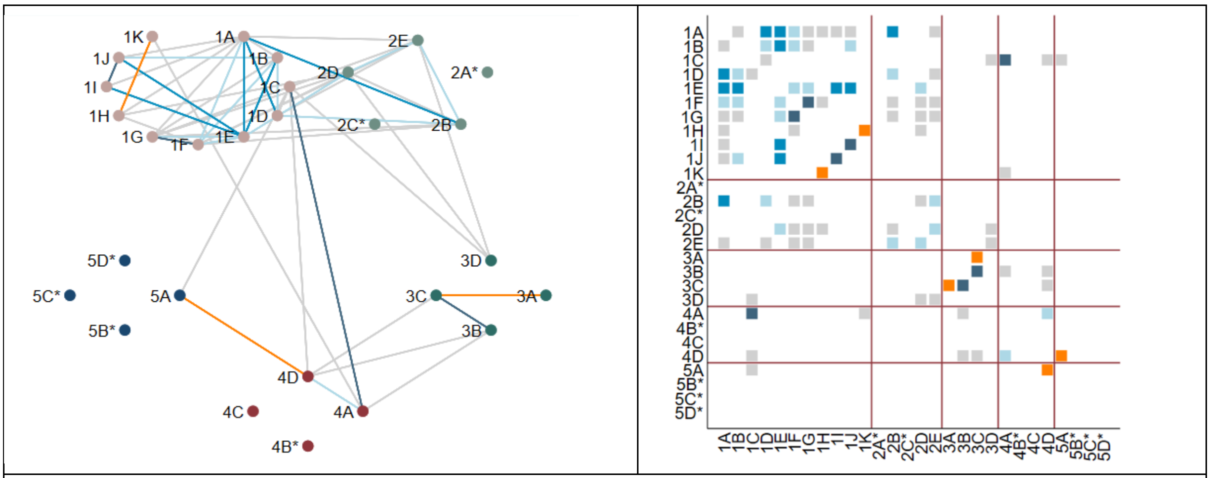
(a) Period 1 (Summer)



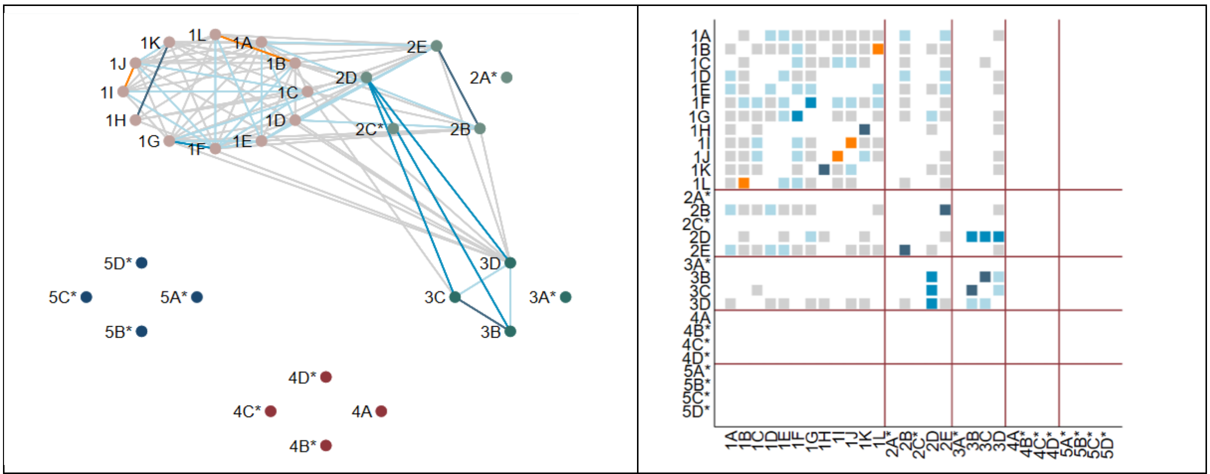
(b) Period 2 (Autumn)



(c) Period 3 (Winter)

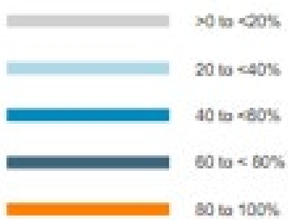


(d) Period 4 (Spring)



(e) Period 5 (Summer)

Figure legend



Percentages are of the days when both horses in the pair were each sighted at least once in the period, and on what percentage of those days they were sighted as close companions.

Figure 9. Social network (left hand side) and matrix (right hand side) analyses for **(a)** Period 1 (Summer 2015/16); **(b)** Period 2 (Autumn 2016); **(c)** Period 3 (Winter 2016); **(d)** Period 4 (Spring 2016); **(e)** Period 5 (Summer 2016/17). The asterisked horse ID codes indicate that the horse was not sighted in the period. In Period 1, there were three horses known to be present (because they were sighted in Period 2 and could not have entered the population during the study period) but not sighted in Period 1. All 28 study horses were sighted in Period 2. In Period 3, four horses sighted in Period 2 were not sighted in Period 3. In Period 4, six horses sighted in Period 2 were not sighted in Period 4. In Period 5, 10 horses sighted in Period 2 were not sighted in Period 5. Some of these horses were assumed to have died (see Table 3).

Percentages for social network analyses would be expected to be widely distributed when the number of days that both the horses were sighted was extremely low, simply because of the limited number of possibilities under the binomial distribution. However, very few pairs of horses had very low numbers of days when both were sighted, and there was no obvious association between these low percentages and numbers of days.

3.3 Habitat use

Geographical regions utilised

The different habitat types and geographical regions were defined in Chapter 5 and are illustrated in this Chapter in Figure 2. When all horse-days were pooled, a horse was seen in only one region on 3085 horse-days, in two regions on the same day for 124 horse-days, in three regions on the same day on 14 horse-days and only one horse was seen in four regions on the same day.

Tables 6a-e illustrate substantial seasonal changes in habitat use. In Period 1 (Summer), band 1 predominantly resided on the larger open grassland areas 1 and 2, band 2 on open grassland area 3, band 3 on open grassland area 4, whereas band 4 moved between the disturbed open woodland area and woodland area 2, and horses in band 5 where only observed in woodland area 3. A relatively similar pattern of habitat use was observed in Period 2 (Autumn) but with horses from most bands spending more time outside the main areas they occupied during from Period 1. However, by Periods 3 (Winter) and 4 (Spring), horses from all bands were spending substantially more time across more geographical regions and habitat types. It should be noted that these results do not represent horses moving from one habitat to the next, nor do they represent the habitat use of all horses in each band. Interpreted together with the social networks, the results show wide dispersal of the bands with members spread out across wider geographical regions at the same points in time.

Table 6a-e. The percentage of horse-days (number of days) that horses from the initial bands to 5 were observed in the different habitat types and geographical regions described in detail in Chapter 5 and illustrated in Figure 2. Each band is represented by a different colour with the darker shade used for proportions > 30% of horse-days, and the lighter shade for proportions < 30% of horse-days.

Key: G1: Open grassland 1; G2: Open grassland 2, G3: Open grassland 3, G4: Open grassland 4, DOW: Disturbed open woodland, R1: Riperian 1, R2: Riperian 2, W1: Woodland 1; W2: Woodland 2; W3: Woodland 3, W4: Woodland 4

Table 6a. Period 1 (Summer 2015/16)

Initial band	Percentage of horse-days (number of days) observed in each habitat type/geographical region											Total number horse-days
	G1	G2	G3	G4	DOW	R1	R2	W1	W2	W3	W4	
1	59% (418)	37% (258)	2% (14)	1% (8)		<1% 2		<1% 5				705
2	2% (6)	12% (35)	79% (226)	5% (13)		2% (6)						286
3		2% (3)		94% (198)					1% (2)	3% (7)		210
4					63% (59)	1% (1)			33% (31)	3% (3)		94
5										1 (100%)		1

Table 6b. Period 2 (Autumn 2016)

Initial band	Percentage of horse-days (number of days) observed in each habitat type/geographical region											Total number horse-days
	G1	G2	G3	G4	DOW	R1	R2	W1	W2	W3	W4	
1	33% (116)	45% (159)	5% (18)	5% (16)		8% (29)	2% (8)	2% (8)				354
2	1% (3)	2% (5)	69% (150)	19% (41)		9% (19)						218
3		<1% (1)		67% (88)			30% (40)			<1% (1)	2% (2)	132
4				1% (1)	78% (83)				11% (12)	10% (10)		106
5					3% (1)					57% (17)	40% (12)	30

Table 6c. Period 3 (Winter 2016)

Initial band	Percentage of horse-days (number of days) observed in each habitat type/geographical region											Total number horse-days
	G1	G2	G3	G4	DOW	R1	R2	W1	W2	W3	W4	
1	17% (6)	11% (4)				19% (7)	31% (11)	19% (7)			3% (1)	36
2	3% (2)		23% (14)	7% (4)		49% (30)	18% (11)					61
3			9% (3)	3% (1)		3% (1)	13% (4)			34% (11)	38% (12)	32
4					17% (7)					83% (34)		41
5					13% (2)					67% (10)	20% (3)	15

Table 6d. Period 4 (Spring 2016)

Initial band	Percentage of horse-days (number of days) observed in each habitat type/geographical region											Total number horse-days
	G1	G2	G3	G4	DOW	R1	R2	W1	W2	W3	W4	
1	33% (94)	12% (33)	6% (16)	<1% (1)	8% (22)	17% (49)	3% (9)	15% (42)	1% (4)	5% (14)		284
2	11% (16)	7% (10)	39% (55)	15% (21)		24% (34)	3% (4)	1% (2)				142
3			3% (3)	54% (56)		3% (3)		1% (1)		13% (14)	26% (27)	104
4			1% (1)		23% (17)		4% (3)		8% (6)	64% (48)		75
5					19% (5)		4% (1)		4% (1)	65% (17)	8% (2)	26

Table 6e. Period 5 (Summer 2016/17)

Initial band	Percentage of horse-days (number of days) observed in each habitat type/geographical region											Total number horse-days
	G1	G2	G3	G4	DOW	R1	R2	W1	W2	W3	W4	
1	47% (228)	15% (72)	19% (94)	10% (49)		5% (26)	1% (3)	2% (12)		1% (4)		488
2	10% (19)	5% (10)	41% (76)	36% 66		7% (12)	1% (2)					185
3	8% (11)		7% (9)	80% (106)		2.5% (3)	2.5% (3)					132
4					67% (2)					33% (1)		3
5												0

Estimated Area Utilised

The estimated areas utilised were substantially larger in Period 4 (Spring) compared to the other periods (Table 7). This is consistent with horses being sighted across wider geographical areas in that period (Table 6d). There was no obvious relationship between estimated area utilised and number of days in which the horse was sighted for the period, suggesting that estimated areas were not markedly biased downwards for horse-period combinations where the horse was sighted on fewer occasions.

Table 7. Estimated areas utilised by period for the whole population of horses pooled.

Period (season)	Number of horses observed on ≥ 3 cameras	Estimated area utilised (km²)				
		Minimum	Maximum	Mean	Median	SD
1 (Summer)	24	0.44	13.86	2.43	1.17	3.23
2 (Autumn)	27	0.31	9.93	2.51	1.72	2.73
3 (Winter)	21	0.0003	7.77	1.84	1.41	1.77
4 (Spring)	21	0.029	14.58	7.83	8.60	5.12
5 (Summer)	19	0.35	14.77	3.61	1.90	4.11

For the horse's estimated area utilised for the period, the P value for interaction between initial subpopulation and period was 0.057 and no interaction was assumed. The mean estimated area utilised in Period 4 (Spring) was estimated as being 5.4 km^2 greater than the mean for Period 1 (Summer) (95% CI 3.4 to 7.4 km^2 greater; $P<0.001$). Means between each of Periods 2, 3 and 5 were all estimated as being similar to that for Period 1 (estimated differences varied from 0.7 km^2 less to 1.2 km^2 greater; $P\geq0.252$), and the mean area for initial subpopulation B was also estimated as being similar to that for initial subpopulation A (estimated difference 0.3 km^2 greater; $P=0.634$).

Separately analysing data from subpopulations A and B (Tables 8a and 8b) show that the estimated area utilised in Period 1 was substantially larger for sub-population B than it was for sub-population A. In Periods 2 and 3, areas were more similar between the subpopulations, having increased for subpopulation A, and decreased for subpopulation B. The greatest estimated areas utilised were in Period 4 for both subpopulations, with areas substantially reducing again in Period 5.

Table 8a-b. Estimated areas utilised for each period, analysed separately by subpopulation.

Table 8a. Estimated areas utilised by period for subpopulation A.

Period (season)	Number of horses observed on ≥ 3 cameras	Estimated area utilised (km²)				
		Minimum	Maximum	Mean	Median	SD
1 (Summer)	16	0.98	2.64	1.05	0.99	0.71
2 (Autumn)	16	0.28	8.40	2.32	1.83	1.99
3 (Winter)	11	0.42	7.77	2.51	2.45	2.04
4 (Spring)	14	0.28	14.58	8.22	10.33	5.34
5 (Summer)	15	0.35	14.77	3.91	2.52	4.54

Table 8b. Estimated areas utilised by period for subpopulation B.

Period (season)	Number of horses observed on ≥ 3 cameras	Estimated area utilised (km²)				
		Minimum	Maximum	Mean	Median	SD
1 (Summer)	8	0.44	13.86	5.21	3.29	4.47
2 (Autumn)	11	0.31	9.9	2.78	1.28	3.64
3 (Winter)	10	< 0.01	3.12	1.10	1.39	1.08
4 (Spring)	7	0.03	13.56	7.05	8.25	4.95
5 (Summer)	4	1.32	5.10	2.46	1.70	1.78

Tables 9a to 9e show the breakdown of estimated areas utilised for all initial bands for each period. This demonstrates the trends described above, noting however that the number of horses observed in bands 4 and 5 were often very small.

Table 9a-e. Estimated areas utilised for all initial bands for each period.

Table 9a. Estimated areas utilised for all initial bands during Period 1 (Summer).

Initial band	Number of horses observed on ≥ 3 cameras	Estimated area utilised (km^2)				
		Minimum	Maximum	Mean	Median	SD
1	11	0.98	2.64	1.03	0.95	0.87
2	5	0.93	1.24	1.08	1.07	0.11
3	4	0.04	9.70	4.54	4.20	4.14
4	4	3.09	13.86	5.89	3.29	5.32
5	0	-	-	-	-	-

Table 9b. Estimated areas utilised for all initial bands during Period 2 (Autumn).

Initial band	Number of horses observed on ≥ 3 cameras	Estimated area utilised (km^2)				
		Minimum	Maximum	Mean	Median	SD
1	11	0.28	8.40	2.60	2.13	2.38
2	5	1.41	1.04	1.70	1.72	0.25
3	4	0.70	9.93	3.30	1.28	4.43
4	4	0.28	9.87	3.87	2.66	4.18
5	3	0.03	1.87	0.66	0.09	1.04

Table 9c. Estimated areas utilised for all initial bands during Period 3 (Winter).

Initial band	Number of horses observed on ≥ 3 cameras	Estimated area utilised (km²)				
		Minimum	Maximum	Mean	Median	SD
1	7	0.43	7.77	2.94	2.45	2.35
2	4	0.42	3.38	1.76	1.62	1.25
3	4	1.41	3.12	1.84	1.41	0.86
4	4	0.0003	1.37	0.35	0.01	0.68
5	2	0.04	2.26	1.15	1.15	1.57

Table 9d. Estimated areas utilised for all initial bands during Period 4 (Spring).

Initial band	Number of horses observed on ≥ 3 cameras	Estimated area utilised (km²)				
		Minimum	Maximum	Mean	Median	SD
1	11	0.28	14.58	9.60	11.35	5.17
2	3	1.78	4.88	3.17	2.84	1.57
3	4	0.029	8.60	4.70	5.08	4.37
4	2	5.27	11.73	8.50	8.50	4.57
5	1	13.56	13.56	13.56	13.56	N/A

Table 9e. Estimated areas utilised for all initial bands during Period 5 (Summer).

Initial band	Number of horses observed on ≥ 3 cameras	Estimated area utilised (km²)				
		Minimum	Maximum	Mean	Median	SD
1	12	0.35	14.77	4.17	1.75	5.07
2	3	2.52	3.51	2.85	2.52	0.57
3	3	1.32	5.10	2.77	1.90	2.04
4	1	1.51	1.50	1.50	1.50	N/A
5	0	-	-	-	-	-

4. Discussion

This chapter describes, for the first time, the use of camera traps to evaluate the population dynamics of a wild horse population across a range of habitats, and temporal changes in social networks and habitat use. Importantly, the use of camera traps also enabled these data to be collected from horses in woodland habitats that could not be seen directly.

Although the age distribution in this population was similar to that reported in other studies (Chapter 3), foaling rates were much lower, and both foal and adult mortality much higher. Previously reported foaling rates in free-roaming horse populations mostly ranged from 0.7 to 0.9 (Berger 1986; Keiper 1986; Duncan 1992), with the lowest reported rate being 0.27 (Scorelli et al. 2010), compared to 0.07 – 0.28 in this study. Furthermore, all foals were born to bands that initially resided in grassland habitats, whilst horses residing solely in woodland habitats had no foals. Mortality rates were high in all age groups and sexes, but 75% (6/8) of the adults that died resided solely in woodland habitats.

It is hard to draw conclusions about the mean age at death of adult horses in this study. The age of only one was known; it was estimated at post-mortem to be 8 years old. However, from the small number of skulls found from previously deceased horses, it is evident that at least some adult horses died at relatively young ages, which is consistent with reports on some other populations (Chapter 3).

Close spatial proximity (close companions) has been reported to be a good measure of social bonds (Chapter 5). Using this index, drone technology has recently been used to evaluate social bonds in free-roaming horses (Mendonca et al. 2021), but this is the first study to use camera traps. Smaller band sizes were present in the woodland habitats and the smaller grassland areas, where the horses had fewer close companions, and fewer days observed with close companions. Moreover, they were more frequently sighted alone than were horses in larger bands on open grassland habitats. There were also seasonal variations in the percentage of days with close companions and in the number of close companions, both of which were lower in Winter and Spring (Periods 4 and 5).

Social network analysis has been suggested as an important tool for assessing aspects of the welfare of wild animals (Beisner & McCowan 2015; Kleinhappel et al. 2016; Brakes 2019), and for evaluating processes and their consequences on populations (Snijders et al. 2017). To date, few reports have been found on the dynamics of changes in social structures in wild free-roaming species. However, social network analysis was recently used to evaluate social stability in semi-feral ponies, finding temporal social stability, but with some seasonal flexibility (Stanley et. al 2017). In this study, I demonstrated marked seasonal changes in social structures during the study, with complete dispersal of bands during Winter and Spring.

Seasonal changes in close companions and social structures also coincided with changes in habitat use, demonstrated through changes in the geographical locations and habitat types that the horses were observed in, along with changes in the estimated areas utilised. Horses roamed larger areas and spent more time in the different geographical locations and habitat types in Winter and Spring, compared to the first Summer and Autumn in the study where they appeared to reside in much smaller distinct areas.

It should be noted that the estimated area utilised was a fairly crude measure because the camera traps had not been set up with the initial intention of attempting to assess home range sizes. Furthermore, the method of calculation will have included areas that the horse did not enter, and equally could miss areas they did enter. Movement detected on one day only could also significantly impact the area calculated for the period. Within these limits, however, the spatial data gained was informative because my purpose was to interpret trends rather than absolute areas utilised. Indeed, this may be a simpler way of providing meaningful data, than making more complex accurate measurements of home range size. Of interest here is a recent detailed discussion of how most research into home ranges has focused on technological advances for more accurately measuring the areas, as opposed to exploring the biological processes and behaviours that affect movement patterns and habitat use (Powell & Mitchell 2012). The latter orientation was precisely the broader aim of this part of my research. Population demographics and habitat use may be important *welfare alerting indices*, whilst social interactions are an important *welfare status indicator*. What the drivers for these changes are, and how these changes affect the welfare status of these horses is important information which, to date, has not apparently been explored in any free-roaming wild species.

The limitations of camera trap observations have been previously discussed (Harvey et. al 2021, Chapter 5), but another limitation of this study is the small population size. However, this enabled intensive monitoring, producing a large data set of 3408 horse-days, which lends weight to the interpretations. Nevertheless, being a single small population, further caution needs to be exercised when extrapolating results to other populations, and further studies are also required to evaluate whether similar results would be found in larger populations and throughout different geographical regions (Chapter 9).

In conclusion, camera traps enabled informative data to be obtained from horses that could otherwise not be seen. I found spatial differences across bands residing in different habitats. Horses in woodland habitats had smaller band sizes, lower foaling rates, higher mortality, roamed larger areas and had fewer social interactions. I also found substantial seasonal changes in social organisation and habitat use, with bands dispersing during Winter and Spring with horses having fewer social interactions, and utilising larger areas and a wider range of habitats. This is the first study that has detailed such changes using camera traps. The next stage (Chapter 8) is to assess the drivers for these changes, and to assess the welfare status of the horses during this period, evaluating the relationships between welfare status, population demographics, habitat use and social organisation.

5. Supplementary material

Methods for calculation of the area of the second rectangle used for Estimated Area Utilised

The second rectangle was oriented with the sides (2 parallel lines with positive slopes) running 45^0 from the South-North line, from South-West to North-East, and top and bottom (2 parallel lines with negative slopes) running perpendicular to the sides, 45^0 from the West-East line, from North-West to South-East. For each horse, all lines with 45^0 positive slopes through each camera location were assessed and the cameras with the most North-West line and the most South-East line were selected; these lines were the sides of the rectangle. Similarly, all lines with 45^0 negative slopes through each camera location were assessed and the cameras with the most North-East line and the most South-West line were selected; these lines were, respectively, the top and bottom of the rectangle.

The width of that rectangle was the distance between sides along a line parallel to top and bottom, and the height was the distance between top and bottom lines along a line parallel to the sides.

Figure 1 shows distances used for calculating rectangle width for the second rectangle. X- and y-axes were constructed with y-axis oriented North-South and the origin co-ordinates set, respectively, as the latitude midpoint of the Northern-most and Southern-most cameras, and the longitude midpoint of the Western-most and Eastern-most cameras.

Using the co-ordinates of the camera with the most North-West line (camera 1; $cam1_{lat}$,$cam1_{long}$), the x-and y co-ordinates of that camera relative to the origin ($cam1_x$, $cam1_y$) were calculated using Pythagoras' theorem on an equirectangular projection as described for the first rectangle. As the most North-West line had a slope of 45^0, the x-intercept if that line ('x-intercept') was calculated as $cam1_x$ minus $cam1_y$.

The shortest distance from the most North-West line to the origin ($rect_{widtha}$) was calculated as the side of an isoceles right-angled triangle using Pythagoras' theorem:

$(rect_{widtha})^2 + (rect_{widtha})^2 = (\text{x-intercept})^2$

Thus,

$rect_{widtha}=\sqrt{((\text{x-intercept})^2/2)}$

The same method was used to calculate $rect_{widthb}$, using the co-ordinates of the camera with the most South-East line (camera 2).

The rectangle width was then calculated as $rect_{widtha} + rect_{widthb}$.

The corresponding method was used to calculate rectangle height.

Area of that rectangle in km^2 was then calculated as width*height (both in km).

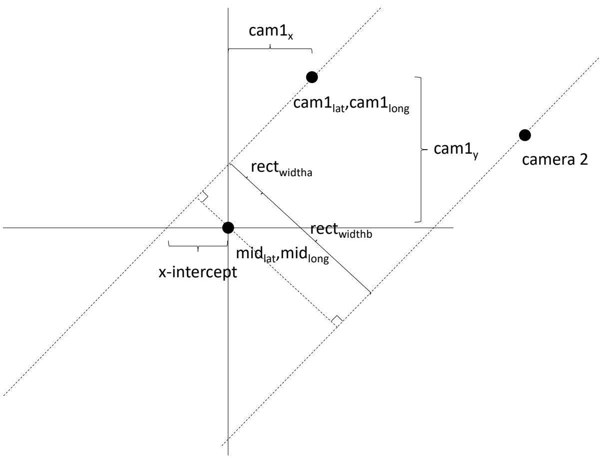


Figure S1. Distances used for calculating rectangle width for the second rectangle.

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Thesis outline summarizing chapter titles and contents		
Chapter	**Chapter title**	**Brief description**
1	Introduction: The wild horse controversy & the importance of incorporating animal welfare science in decision making.	Summary of the history of wild horses, their cultural significance, environmental impacts and controversies in wild horse management. Introduces the importance of animal welfare science in wild animal controversies.
2 Published	A Ten-Stage Protocol for assessing the welfare of individual non-captive wild animals: free-roaming horses as an example.	Presentation of a novel conceptual framework that I designed in order to guide a systematic and scientific approach to assessing the welfare of free-roaming wild animals, using the Five Domains Model. Summarizes the principles of interpreting indicators of biological function and behaviour in terms of the mental experiences that those indicators reflect (Stage 1 of my Protocol), and how the Five Domains Model is used for assessing welfare (Stage 2 of my Protocol).
3	Literature review: A review of the species-specific information required to enable assessment of wild horse welfare.	The current status of wild horse knowledge is summarized in a novel holistic and multidisciplinary framework drawing together the relevant literature on horses across each of the four physical/functional domains of the Five Domains Model. It addresses Stage 3 of my Ten-Stage Protocol.
4 Published	Addressing the knowledge gap of gastrointestinal parasitology in free-roaming wild horses in south-east Australia.	An example of the type of detailed original research required for addressing any knowledge gaps identified in Stage 3. This chapter describes a detailed parasitological investigation of 293 faecal samples collected from 6 wild horse populations. It describes results of faecal egg counts, larval cultures and molecular diagnostics.
5 Published	Use of remote camera traps to evaluate animal-based welfare indicators in individual free-roaming wild horses.	A large body of original research investigating for the first time, both the use of remote cameras for identifying individual horses across a range of habitats, and for acquiring data on an extensive range of animal-based welfare indicators. It addresses Stages 4-6 of my Ten-Stage Protocol.
6	Scientific validation of welfare indicators for using the Five Domains Model to grade the welfare status of individual free-roaming wild horses.	Addressing Stage 7 of my Ten-Stage Protocol, this chapter evaluates the scientific evidence linking the described objective measurable/observable welfare indicators to physical/functional impacts in Domains 1-4, and the relationship between those impacts and the mental experiences that are inferred in Domain 5. This concludes with the formulation of a Five Domains Model wild horse specific welfare grading scheme.
7	Dynamic changes in wild horse social organisation and habitat use revealed with remote camera trap monitoring.	With the aim of evaluating traditional ecological metrics alongside welfare status, this chapter describes original research using the remote camera trapping methodology described in chapter 5, to evaluate population dynamics, and temporal and spatial changes in social organization and habitat use of a wild horse population over a 15 month period.
8	**The cascading influence of resource availability on the welfare status of wild horses, and association with population demographics, social organization and habitat use.**	**This original research applied the methodology from all preceding chapters to assess welfare status and changes in welfare status in individual wild horses over a 15 month period, addressing stages 8 -10 of my Ten-Stage Protocol. It further evaluates drivers of change in welfare status and correlations between welfare status, and the population dynamics, social organization and habitat use described in chapter 7.**
9	Conclusions, applications and future directions.	Summarizes overall conclusions, and contributions to the fields of wild animal welfare, wild horse ecology and welfare, wild horse management, and general horse welfare. Highlights ongoing work, some of which I have already partially completed that was not included in the main body of the thesis.

Chapter 8. The cascading influence of resource availability on the welfare status of wild horses, and association with population demographics, social organisation and habitat use

Abstract

This Chapter reports for the first time the systematic and scientific assessment of the welfare status and welfare risks in a free-roaming wild species. In this Chapter I draw together all the information presented in Chapters 3 to 7 and relate it to stages 8 to 10 of my Ten Stage Protocol, i.e., using the adjusted version of the Five Domains Model to grade welfare compromise, enhancement and future risk. I describe the assessment of the welfare status of each horse and evaluate how their welfare changed over a 15-month period. In addition, I evaluate the drivers for the identified changes, and the associations between population demographics, dynamics, social organisation, habitat use and welfare status.

Methods: The study site and population was as described in Chapters 5 and 7. Resource-based indices were recorded comprising monthly cumulative rainfall, daily ambient temperatures, details of shade, shelter and underfoot substrate, and forage and availability across the different habitat and locations, for each period (season). The incidence of individual animal-based welfare indicators captured with camera traps and the quantitative behavioural assessment across the population, were analysed by period (season) using the random sample of camera-day combinations as described in Chapter 5. For rarely observed indicators, the all-occurrence method was

used. For overall assessments and grading welfare status and risk for each horse, the full data set of 42,925 camera trap image/video files was used. Every image and video file for each horse was collated and assessed for each period (season) in addition to data obtained from direct observations. Body condition score was recorded monthly. Data on close companions, social networks, and estimated areas utilized, reported in Chapter 7, were also incorporated into welfare assessments. Using all resource-based and animal-based indicators welfare status and risk was then graded in each individual horse, in each period (season) as detailed in Chapter 6.

Results: Significant results related to resource-based indicators were as follows: (i) rainfall was well below average during Autumn, Winter and Spring, (ii) forage availability was poorer in woodland than grassland habitats, (iii) forage availability reduced throughout the study in all habitats.

Animal-based indicators of welfare compromise and risk regularly detected by camera traps were: poor body condition, head-lower-than-withers body posture, poor coat condition, facial grimace, skin lesions, weakness, dull demeanor and apathy. All-occurrence analysis identified additional indicators of welfare compromise (increased respiratory rate, reduced vigilance, a weak gait and skin excoriation), and enhancement (alertness, trotting, cantering and galloping, herding behaviour and affiliative social interactions). Body condition scores reduced in all horses throughout the study, such that by the end of the study all were very thin or emaciated, and 12 of the 29 horses had died, attributed to starvation. Most of these were emaciated for at least 2- 8 months before death. Presence of other indicators of welfare compromise were correlated with poor body condition.

For grading welfare status & risk I chose one mare as an example to illustrate in detail how the welfare grading scheme (detailed in Chapter 6) was applied to each individual horse, before analysing the welfare grades for all individuals. Overall welfare status was worse for horses residing in woodland habitats than grassland habitats. Overall welfare status reduced for all horses throughout the study. Undernutrition was the most common cause of overall welfare compromise. Forage availability was the main determinant of welfare status and risk, which in turn was influenced by the combined effects of rainfall and ambient temperature.

Combined with results presented in Chapter 7, horses in predominantly woodland habitats roamed larger areas, had smaller band sizes, lower reproductive rates, higher mortality, fewer social interactions, and poorer overall welfare. Horses in grassland habitats also experienced declining welfare throughout the study. During this time, initial bands dispersed into smaller groups or single horses, with fewer social interactions, roaming larger areas, and moving into woodland habitats in addition to grasslands.

Conclusion: I was able to document the welfare of horses across a range of habitats, and demonstrated how their welfare changed during successive seasons. Welfare compromise in all domains was linked to forage availability and subsequent undernutrition. Horses residing in woodland habitats had the worst welfare compromises even during periods of good rainfall when forage availability was good in other habitats. Welfare status was closely linked to demographic parameters, habitat use and social organisation.

1. Introduction

In Chapter 2 (Harvey et al. 2020) I described the Ten-Stage Protocol that I developed for assessing the welfare of free-roaming wild animals. Chapter 3 addressed stage 3 of the Protocol, acquiring species-specific knowledge relevant to each Domain of the Model, representing the body of knowledge that is essential for conducting Model-based welfare assessments. In Chapter 4 I addressed one specific knowledge gap in wild horse health, and with my detailed parasitological investigations I found that gastrointestinal parasites were a significant welfare risk. In Chapter 5 (Harvey et. al 2021), I addressed stages 4 to 7 of the Protocol, by developing (i) lists of potential welfare status and alerting indices that could be measured or observed, (ii) methods for identifying individual animals, and (iii) methods for assessing welfare indicators across a range of habitats where horses frequently could not be seen directly. In Chapter 6 I applied a process for scientifically validating those indices that could be measured or observed, and I inserted them into the Five Domains Model to create a welfare grading system for wild horses. In Chapter 7 I described the population demographics, social organisation and habitat use for all horses in this same population.

This Chapter now draws all of this knowledge together and relates it to stages 8 to 10 of the Ten Stage Protocol, i.e., using the adjusted version of the Five Domains Model to grade welfare compromise and enhancement. More specifically, I have used the Model to assess the welfare status of each horse in my small study population to assess how their welfare changed over a 15-month period. In addition, I have evaluated the drivers for these changes identified by these parameters, and the associations between population demographics, social organisation, habitat use and welfare status. This is the first reported systematic and scientific assessment of the welfare status and welfare risks in a free-roaming wild species.

The aims of this chapter were to research the previously described small, geographically constrained population of wild free-roaming horses, by (i) using camera traps and direct observations to assess an extensive range of scientifically validated resource-based and animal-based welfare indicators in all individuals, (ii) applying the grading scheme described in Chapter 6 to separately grade welfare compromise, enhancement and risk in each horse during each season, (iii) evaluating the main determinants of welfare compromise, and of the changes in social organisation and habitat use described in Chapter 7, and (iv) evaluating associations between welfare status and the population dynamics, social organisation and habitat use that was described in Chapter 7.

2. Materials and Methods

2.1 Study Overview

I made camera trap (still images and video) and direct observations of a small and geographically constrained population of free-roaming wild horses in Kedumba Valley of the Blue Mountains National Park, NSW, Australia over a 15-month period. The study area, habitats, geographical regions, and methodology for direct observations of horses, and camera trapping, have already been described in detail in Chapter 5 (Harvey et al. 2021). Also, the horse population, demographics, social organization and habitat use have been outlined in Chapter 7.

The study extended over the same 450-day period, from 18th December 2015 to 11th March 2017, inclusive, as described in Chapter 7. Data on animal-based and resource-based indicators of welfare status and risk were collected via direct observations and camera traps (as detailed in Chapters 5 and 7). Initially, these data were assessed on a month-by-month basis. However, no significant changes in key variables were apparent between consecutive months, but there were important differences between seasons. Most data were therefore collapsed into five consecutive 90-day periods for further analyses, as detailed in Chapter 7. Exceptions were rainfall and horse body condition scores which were reported and analysed by month.

2.2. Assessment of resource-based indicators

Rainfall

Monthly rainfalls for the nearest weather station (Katoomba, NSW) were obtained from the Australian Government Bureau of Meteorology website (BOM 2012). Monthly rainfall during the study period was compared with the previous monthly rainfalls on record since 1886.

Temperature

All remote camera traps recorded the ambient temperature each time the camera recorded an image or video clip. Cursory evaluation revealed little difference in temperatures between different habitats except for differences between exposed and shaded areas. Accordingly, the temperatures recorded from a single camera were used for the analysis of ambient temperatures. This camera was in an open grassland area out of the shade in order to best represent overall maximum temperatures. For each period (i.e., season), the minimum and maximum temperatures recorded by this camera were obtained. Based on the literature on thermoneutral zones and lower and upper critical temperatures for horses (see Chapters 3 and 6), temperature categories were defined as 'very cold' being below lower critical temperature (< 5°C), 'cold' being the cool zone of the thermoneutral zone (5-10°C), 'warm' being the warm zone of the thermoneutral zone (20-25°C), and 'hot' being above the upper critical temperature (> 25°C). For each of these categories, the number of days in each period when a temperature was recorded in that category was calculated. The number of days temperature reached > 35°C was also recorded. Note that on a single day, temperatures in more than one category could be recorded.

Other features of the physical environment

Other features of the physical environment across the different regions were assessed during ground surveys (described in Chapter 5, Harvey et al. 2021) including terrain, presence of shelter and shade, and the nature of underfoot substrate. Any extreme weather events were also recorded.

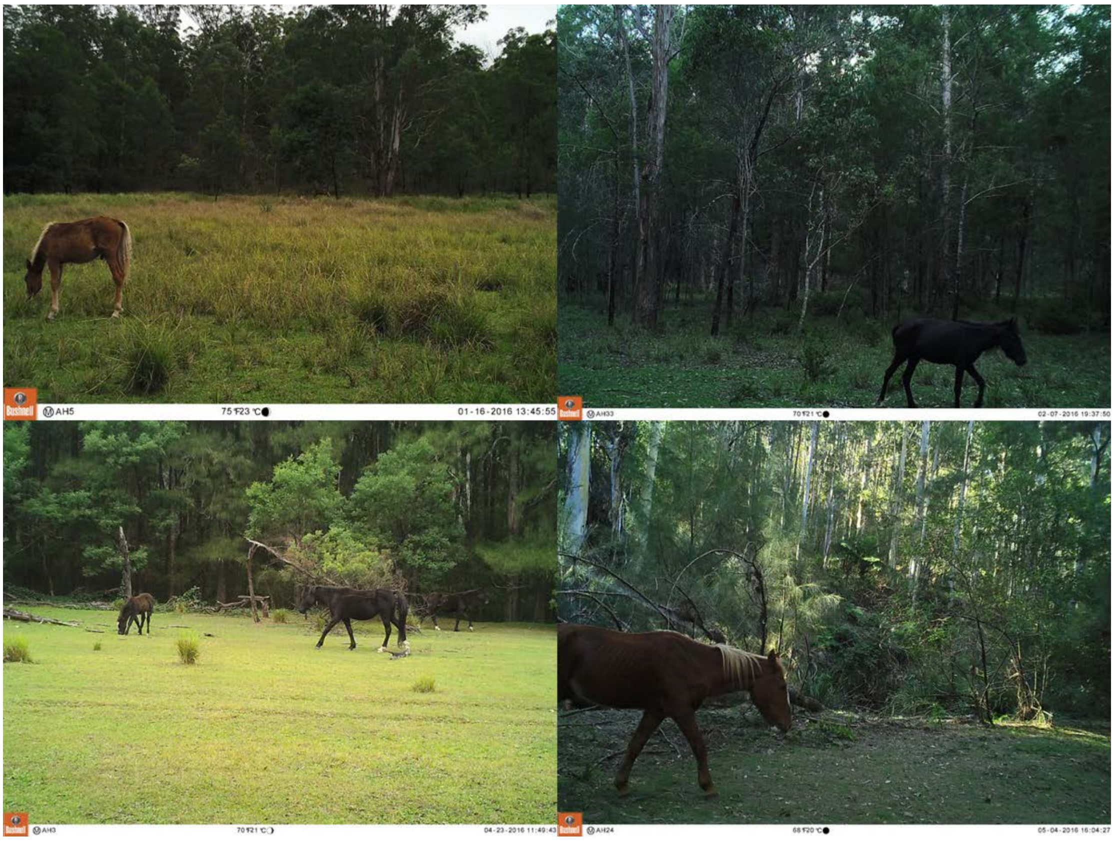
Forage availability

For each of the eleven geographical regions (Chapter 5, Harvey et al. 2021), a forage availability grade was assigned for each period. Forage availability was graded from A (very good) to E (very poor) based on the collective assessment of grass height coverage, density and subjective quality (Table 1, Figure 1). With uneven grass coverage the average length of high-quality grass was used.

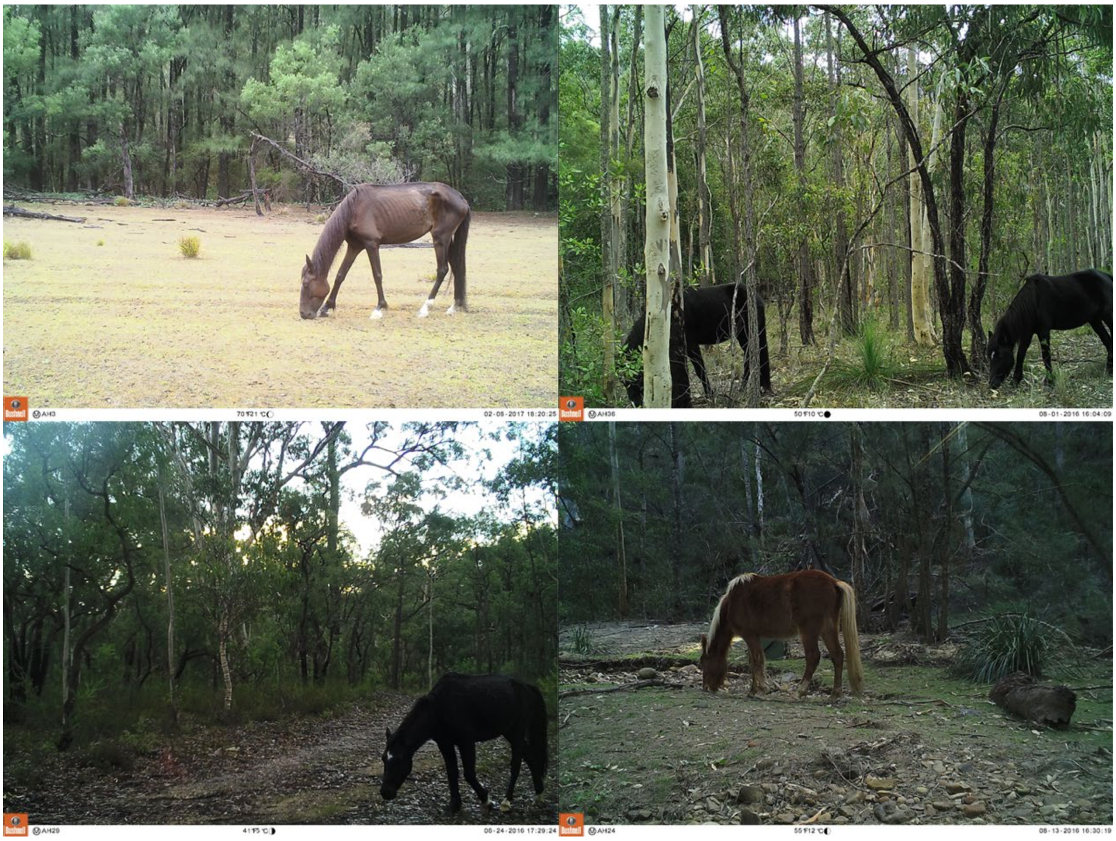
Table 1. Forage availability grades.

Forage availability grade	Grass height (cm)	Grass coverage & density	Vegetation quality
A (Very good)	> 10	> 80% coverage of dense grass	Good quality green grass
B (Good)	> 5 - 10	> 80% coverage moderately dense grass	Moderate quality green grass
C (Moderate)	> 2 - 5	50-80% grass coverage with poor density	Low quality dried grasses
D (Poor)	0.5 - 2	20-50% grass coverage	Grass mostly dry, predominantly fibrous tussocks
E (Very poor)	< 0.5	< 20 % grass coverage	Little to no good quality grass, only fibrous tussocks & shrubs

(a)



(b)



(c)

(d)

Figure 1. Examples of assigned forage availability grades based on grass length, density and quality. **(a)** Grade B (Good); left to right: Open grassland 1 in period 1; Open grassland 2 in period 1. **(b)** Grade C (moderate); top left to bottom right: Open grassland 3, period 1; Woodland 2, period 1; Open grassland 1, period 2; Riparian 1; period 2. **(c)** Grade D (Poor); top left to bottom right: Open grassland 1, period 5; Woodland 2, period 3; Disturbed open woodland, period 2; Riparian 1, period 3. **(d)** Grade E (Very poor); top left to bottom right: Riparian 1, period 5; Woodland 3, period 2; Disturbed open woodland, period 3; Woodland, period 4. No grade A forage availabilities were found.

Water availability

Extensive ground surveys (as described in Chapter 5, Harvey et al. 2021) were performed to identify the locations of water sources. Although rivers and creeks ran throughout the valley, the topography of the region made most of them inaccessible to horses, as illustrated in Chapter 5. Accessible sections of rivers and creeks, and other water sources such as water holes, were mapped. Water sources were monitored throughout the study period to distinguish between permanent water sources, and those that dried out during periods of low rainfall. Distances from locations of horse sightings to nearest water sources were calculated.

2.3. Assessment of animal-based indicators

The incidence of individual animal-based welfare indicators that were captured with camera traps (Chapter 5) and the quantitative behavioural assessment across the population, were analysed using the random sample of camera-day combinations as described in Chapter 5 (Harvey et al. 2021). The methodology was as described in detail in Chapter 5. Analysis was performed by period (season).

For rarely observed indicators, the all-occurrence method was used, where every camera trap image and video file for every horse was assessed from the full data set; if an indicator was present it was recorded.

For overall assessments of welfare status and risk for each horse (as described below in section 2.4), the full data set of 42,925 camera trap image/video files was used. Results of horse sightings have been reported in Chapter 7. Every image and video file for each horse was collated and assessed for each period (season). Further, for those horses that could be observed directly (Harvey et al. 2021), additional assessments were made both in real-time, and using photographs and videos taken during direct observations in addition to camera trap images/videos. All available data for each horse were assessed in each period for all of the animal-based indicators detailed in Table 2.

For body condition score, the full data set was used to assign a monthly average body condition score for each horse when observed within any month. The 9-point body condition scoring system was used, as detailed in Chapter 3. Body condition scores were treated as continuous data as, over the range of scores observed in study horses (1 to 6), each incremental increase in score reflects approximately the same absolute change in body fat percentage (Dugdale et al. 2012). Scores were assessed statistically, testing the following null hypotheses: 'In horse populations such as the study population with forage availability as experienced by the study population and initial subpopulations such as those in the study population, BCS does not change over time and BCS does not differ between initial subpopulations.' Scores were predicted by month for each of initial subpopulations A and B using linear mixed models fitted using the -mixed- command in Stata (version 16; StataCorp, College Station, Texas, USA). Horse was fitted as a random effect and a first order autoregressive correlation structure was used to model correlations in residuals between months within horses. Based on fractional polynomial analyses, linear and quadratic effects of month were appropriate. The joint p-value for the two interaction terms for interaction between initial subpopulation and each of linear and quadratic terms for month number was calculated using the likelihood ratio test.

Faecal egg counts and *S. vulgaris* PCR were also performed as described in Chapter 4.

Data on close companions, social networks, and estimated areas utilized, reported in Chapter 7, were also incorporated into welfare assessments. Mortality was reported in Chapter 7, and here causes of mortality and the time to death from the earliest observation of compromised health have been evaluated.

2.4 Associations between body condition score and other welfare indices

Correlations between body condition score and each of the binary variables of abnormal body posture, poor coat condition, grimace, weakness, and apathy were assessed using point biserial correlation coefficients disregarding clustering of observations within horse. These were calculated using the -pbis- command in Stata. All other statistical analyses were also performed using Stata (version 16).

2.5 Grading welfare status and risk

All resource-based and animal-based indicators (Table 2) that were possible to assess in each individual horse in each period (season), were incorporated into welfare status and risk assessments. For resource-based indicators, where resources varied throughout regions, such as water and forage availability, the assessment was made for individual horses based on the regions where they were sighted during that period, as detailed in Chapter 7. For forage availability, when horses were sighted in more than one region, the availability score for the individual was based on the best score for those regions. For evaluating possible thermal challenges in Domain 2, temperatures for the period were interpreted in combination with other individual animal-based factors that may impact thermoregulatory ability, such as thin body condition, reduced food intake and/or being wet from rain, and ability to walk to shelter, shade or water.

Welfare status was assessed using only those indices validated as welfare status indicators (as outlined in Chapter 6). These data were all collated to assign grades for welfare compromise and enhancement, for each horse, for each domain and for each period (season). Grading of welfare status was done according to the detailed scheme described in Chapter 6. Welfare compromise was graded on a 3-tier scale where A/B was 'no to low' level compromise, C was 'moderate' compromise, and D/E was severe compromise. Welfare enhancement was also graded on a 3-tier scale where 0 was 'none', + was 'low to medium' enhancement and ++ was 'high' enhancement.

Table 2. Welfare status and welfare alerting indices that were assessed.

Domain	Animal-based indices	Resource-based indices
1. Nutrition	Body condition score* Foraging activity	Forage availability* Distance to water*
2. Physical Environment	Sweating Shivering Wet from rain* Huddling together with other horses	Temperature* Rainfall* Extreme weather events* Presence of shade/shelter*
3. Health	Body posture* Coat condition* Wounds or other injuries* Growth rate of young* Reproductive status* Faecal egg counts & S. *vulgaris* PCR* Faecal consistency & colour* Skin lesions * Hoof condition Nasal discharge Ocular discharge Blepharospasm Quidding Food pouching Facial grimace Gait at walk* Gait at trot Gait at canter Weakness* Respiratory rate & effort	
4. Behavioural Interactions	Specific quantifiable behaviours (feeding, resting, maintenance, locomotory & social behaviours)* Social networks, close companions* Qualitative assessment of behaviour (relaxed, alert, dull/depressed, apathetic)*	

* Indices that were most easily assessed

A confidence score of 'low', 'medium' or 'high' was also assigned for each horse, for each domain and for each period (season). This score was based jointly on the following parameters: the number of sightings of the horse during the period; whether sightings were only still images, or included video and/or direct observations; and the range of welfare status indicators able to be assessed at those sightings.

Future welfare risk was then graded for each horse, for each domain and for each period as 'low', 'medium' or 'high' using welfare alerting indicators as described in Chapter 6.

One horse (mare 1B) has been chosen as an example to explain in detail the process of grading welfare compromise, enhancement and risk.

2.6 Confirming the main determinants for welfare compromise and risk

Associations between forage availability, and welfare enhancement, compromise, and welfare risk grades for each domain were assessed using Spearman's correlation coefficients, calculated using Stata's -spearman- command. The unit of analysis was the horse-period combination. These analyses did not account for clustering by horse.

The relationship between forage availability and horse's mean body condition score for the period was assessed using a linear mixed model, with the forage availability grade fitted as a categorical variable, horse fitted as a random effect, and first order autoregressive residual correlation structure to account for serial correlations in mean body condition score within horses between periods. Stata's -mixed- command was used. The relationships between forage availability score and each of the horse's estimated areas utilised for the period, and total number of close companions for the period were assessed in the same way.

Possible determinants of future welfare risk in Domain 5 were assessed using ordered logit models. The proportional odds assumption was that the odds ratios for welfare risk being medium or high (as opposed to low) and for welfare risk being high (as opposed to medium or low) were the same. This assumption was assessed by comparing log likelihood from constrained and unconstrained models with the likelihood ratio test, using the -gologit2- command in Stata. As the p-value was 0.360, proportional odds were assumed, and an ordered logit model was fitted using the -ologit- command in Stata, with robust standard errors that accounted for clustering of period within horse.

3. Results

3.1 Resource-based indices

Rainfall

Cumulative rainfall was recorded for each calendar month and plotted on a graph overlaying distributions of monthly rainfall totals from the previous 130 years since (Figure 2). During Period 1 (Summer; Dec '15 – Feb '16) rainfall was on average higher than the median average rainfall for that period. However in Period 2 (Autumn; Mar-May '16), a critical time period for grass growth prior to winter, rainfall was well below average. During Period 3 (Winter; Jun – Aug '16) there was above average rainfall, however temperatures were below that required for grass growth (see below). During Period 4 (Spring; Sept – Nov '16) when temperatures rose, rainfall was below average. In Period 5 (Summer; Dec '16 – Mar '17) rainfall for the period rose to above average again.

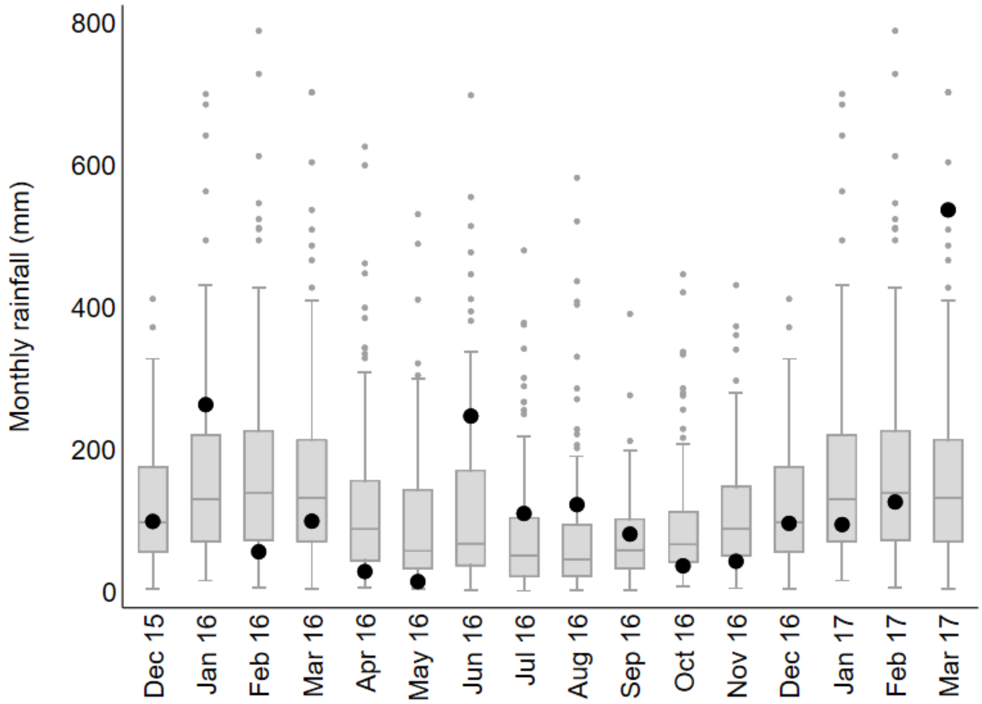


Figure 2. Cumulative monthly rainfall during the study period (black dots). The box and whisker plot shows distributions of monthly rainfall totals from the previous 130 years. The middle band is the median, and the box extends from the 25th to 75th percentile. The whiskers extend upwards to the largest observed value that is less than the 75th percentile plus 1.5 times the inter-quartile range, and extend downwards to the lowest observed value that is more than the 25th percentile minus 1.5 times the inter-quartile range.

Temperature

Data on ambient temperatures is reported in Table 3. The climate in the region is generally temperate, however, some very cold days were recorded in Winter (Period 3), and particularly in the 2nd Summer (Period 5), very hot days were recorded.

Table 3. Ambient temperatures ranges in each period, and number of days ambient temperature was recorded in the cool and warm zones of the thermoneutral zone, and below lower or above upper critical temperatures.[1]

90-day period	Minimum temperature (°C)	Maximum temperature (°C)	Number of days very cold, < 5°C (%)	Number of days cold, 5-10°C (%)	Number of days warm, 20-25°C (%)	Number of days hot, > 25°C (%)
1 (Summer)	8	36	0	1 (2)	18 (20)	47 (52)
2 (Autumn)	-7	31	23 (26)	8 (9)	12 (13)	16 (18)
3 (Winter)	-6	31	47 (52)	15 (17)	19 (21)	3 (3)
4 (Spring)	1	43	17 (19)	29 (32)	23 (26)	39 (43)
5 (Summer)	3	48	1 (2)	10 (11)	13 (14)	65 (87)*

*32 days in this period (36%) the temperature was > 35°C
[1]Note that different temperature zones could be present on the same day so percentages do not add up to 100. The number of days only means that the temperature was within one of those zones for at least some of the day.

Other features of the physical environment

The terrain throughout the study area has been illustrated in Chapter 5. All open grassland regions were moderately flat, separated by undulating woodlands. For all woodland and the riparian regions, terrain was variable, being extremely steep in places. However, horses were able to access resources without having to negotiate extremely steep terrain. Throughout all regions, shelter and shade were plentiful and were provided by woodland and cliff edges. Underfoot substrate varied from grass, soil often covered with fallen leaves and branches, plus rocks and dirt tracks. There were no recordings of hail, snow, lightning strikes, or bushfires during the study period. There were some periods of heavy rainfall, particularly in June 2016 (Period 3, Winter).

Forage availability

Forage availability grades for the eleven geographical regions, for each period, are reported in Table 4. In the period when forage availability was most optimal during the study (Period 1), its availability varied by habitat type, being best in larger open grasslands, and worst in woodlands. Forage availability reduced in all habitats between Periods 1 and 2, and between Periods 2 and 3, and then remained poor or very poor throughout the remainder of the study.

Table 4. Forage availability grades assigned to the different locations and habitats over each 90 day time period.

Region/habitat	Period 1 (Summer)	Period 2 (Autumn)	Period 3 (Winter)	Period 4 (Spring)	Period 5 (Summer)
Open grassland 1	B	C	D	D	D
Open grassland 2	B	C	D	D	D
Open grassland 3	C	D	D	D	D
Open grassland 4	C	D	D	D	D
Disturbed open woodland	C	D	E	E	E
Riparian 1	B	C	D	E	E
Riparian 2	C	D	E	E	E
Woodland 1	D	D	D	D	D
Woodland 2	C	D	D	D	D
Woodland 3	D	E	E	E	E
Woodland 4	D	E	E	E	E

A = Very good (never observed), B: Good; C: Moderate, D: Poor, E: Very poor

In the open grassland and riparian habitats, forage availability was positively associated with the combination of above average rainfall at a time of warm temperatures (Period 1, Summer), and was negatively associated with below average rainfall (Period 2, Autumn and Period 4, Spring) and above average rainfall at a time of cool temperatures (Period 3, Winter). However, as the best forage availability grades for open grassland habitats were grades B, and C, overgrazing may have been an additional factor, which if so, suggests that the population was at, or close to, the regions' carrying capacity. In disturbed open woodland and woodland, there was a similar correlation but as the forage availability grades were never above C or D, this seems more likely to be due to the habitat type itself not being conducive for growth of forage types preferred by horses.

Water availability

Locations of permanent and temporary water sources during the study, from rivers, creeks, waterholes, and the lake are shown in Figure 3. The temporary water sources were mostly unavailable during Periods 4 and 5 when rainfall was below average for most of that time. The maximum distance from horse sightings to the nearest water source was approximately 3.5km, which was from parts of 'Woodland 2', especially when water was not available in Reedy Creek. For most horse sightings, however, distance to water was no greater than 2km. For all open grassland regions, woodland 1, and riparian regions, water was always readily available.

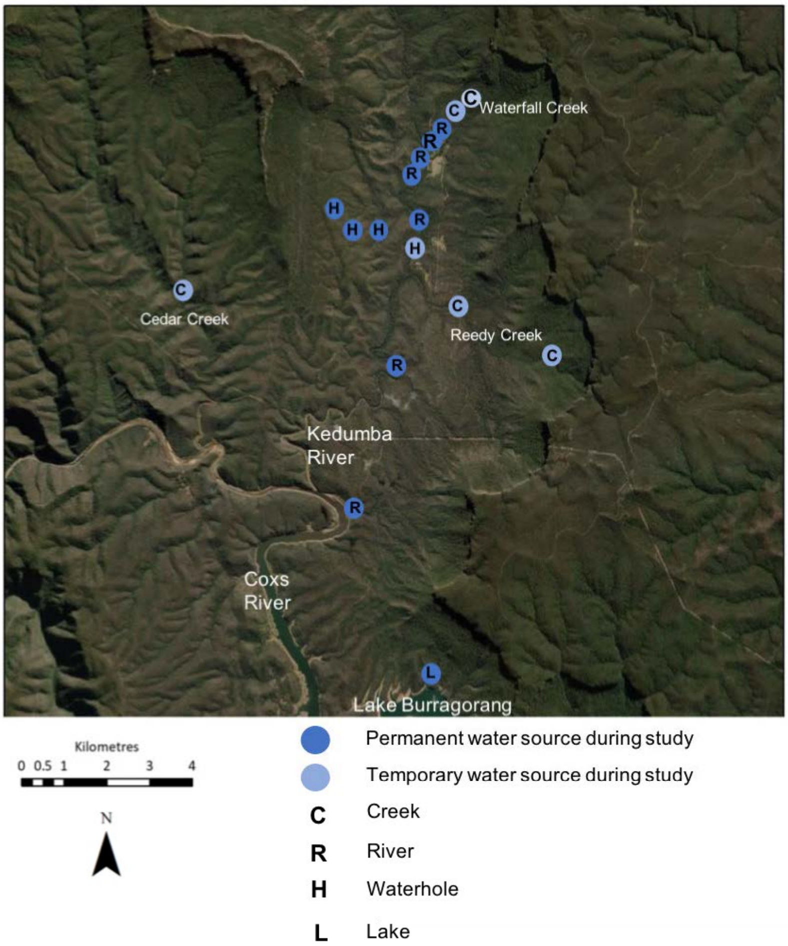


Figure 3. Map illustrating locations of accessible water sources during the study.

3.2 Animal-based indices

Animal-based welfare indicators detected from the random sample of camera-days (still images and videos)

Table 5 shows the proportion of observation events (as defined in Chapter 5) where different welfare status or risk indicators were present during each period (season). The proportion of horses that were thin (BCS 3) or emaciated (BCS ≤ 2) was high in all periods, and particularly from Period 3 (Winter) where the proportion rose progressively to 94% of the population in Period 5 (Summer) being thin or emaciated. The proportion of horses with a head-lower-than-withers posture was highest in

Periods 3 (Winter) and 4 (Spring), whilst poor coat condition was common from Period 3 (Winter) onwards. Weakness, being dull/depressed or apathetic was most common in Period 4 (Spring), whereas being alert or relaxed was least common in this period.

Table 5. Proportion of observation events where welfare indices were present in each period.

Welfare indicator [1]	Proportion of observation events where indicator was present[2]				
	Period 1 (Summer)	**Period 2 (Autumn)**	**Period 3 (Winter)**	**Period 4 (Spring)**	**Period 5 (Summer)**
BCS ≤3/9	34/76 (45%)	50/148 (34%)	44/59 (75%)	74/94 (79%)	101/108 (94%)
Head-lower-than-withers body posture	1/81 (1%)	10/155 (7%)	19/60 (32%)	26/95 (27%)	13/111 (12%)
Poor coat condition	0	28/85 (33%)	25/37 (68%)	61/66 (92%)	37/50 (74%)
Facial grimace[3]	0	5/48 (10%)	5/16 (31%)	1/27 (4%)	3/14 (21%)
Skin lesions - left	1/27 (4%)	2/50 (4%)	0	7/34 (21%)	2/28 (7%)
Skin lesions - right	0	1/33 (3%)	0	2/27 (7%)	7/30 (23%)
Weakness	0	1/32 (3%)	1/16 (6%)	5/14 (36%)	1/16 (6%)
Dull/depressed	0	3/28 (11%)	1/12 (8%)	3/9 (33%)	1/14 (7%)
Alert or relaxed	0	26/29 (90%)	13/16 (81%)	6/11 (55%)	13/15 (87%)
Apathetic	0	3/29 (10%)	3/16 (19%)	5/11 (46%)	2/15 (13%)

[1]Any indices from Table 2 not listed here, are because no abnormalities were detected
[2] Only observation events where the indicator was able to be assessed were included
[3] Note that facial grimace was not scored but was considered present when any features were obviously present (Dalla Costa et al. 2014) and persistent

Table 6 shows the proportion of observation events (as defined in Chapter 5) where different welfare status or risk indices were present, in each band. Higher proportions of horses that were thin or emaciated, had a head-lower-than-withers body posture, poor coat condition, weakness and apathy, were in bands 4 and 5, compared to bands 1 – 3.

Table 6. Proportion of observation events where welfare indices were present in each band.

Welfare indicator[1]	Proportion of observation events where abnormality was present[2]				
	Band 1	Band 2	Band 3	Band 4	Band 5
BCS ≤3/9	83/160 (52%)	73/114 (64%)	42/71(59%)	39/51 (77%)	17/19 (90%)
Head-lower-than-withers body posture	20/159 (13%)	13/177 (7%)	12/73 (16%)	12/48 (25%)	7/21(33%)
Poor coat condition	40/74 (54%)	37/76 (49%)	32/54 (59%)	13/34 (38%)	15/16 (94%)
Facial grimace[3]	1/35 (3%)	4/34 (12%)	1/22 (5%)	5/19 (26%)	3/12 (25%)
Skin lesions - left	3/52 (6%)	1/43 (2%)	2/28 (7%)	3/17 (18%)	1/7 (14%)
Skin lesions - right	2/33 (6%)	1/28 (4%)	3/26 (12%)	0	1/10 (10%)
Weakness	0	1/25 (4%)	3/21 (14%)	2/4 (50%)	1/8 (13%)
Dull/depressed	1/14 (7%)	2/20 (10%)	2/17 (12%)	1/2 (50%)	2/6 (33%)
Alert or relaxed	0	17/20 (85%)	17/20 (85%)	2/3 (67%)	5/8 (63%)
Apathetic	0	3/20 (15%)	3/20 (15%)	1/3 (33%)	3/8 (38%)

[1] Any indices from Table 2 not listed here, are because no abnormalities were detected
[2] Only observation events where the indicator was able to be assessed were included
[3] Note that facial grimace was not scored but was considered present when any features were obviously present (Dalla Costa et al. 2014) and persistent

Quantitative behavioural assessments of horses captured on still images and video, reported as the proportion of images, and the duration of time (as described in Chapter 5), are summarised separately for each period (Tables 7a to 7e). Challenges in the interpretation of each methodology are summarised in Chapter 5, but when combined these results suggest that in Period 1 (Summer) horses spent the most time grazing, followed by walking, and not much time resting. In Period 2 (Autumn) they spent less time grazing and more time resting than in Period 1. In Period 3 (Winter) they spent less time walking and more time resting. In Period 4 (Spring) they spent more time walking and resting, and less time grazing, and in Period 5 (Summer) less time resting, and more time walking and grazing.

Table 7a-e. Quantitative behavioural assessments of horses captured on still images and video, reported as the proportion of images, and the duration of time for Periods 1 to 5.

Table 7a. Quantitative behavioural assessments for Period 1 (Summer).

Behaviour	Number of behaviour events from still images (n = 114)	Number of behaviour events from video *	Number of still images (n = 425[1]) with this behaviour (%)	Duration of time from still images (n = 3537 secs[1]) with this behaviour (%)	Duration of time from video with this behaviour (%) *
Locomotion total	71	N/A	185 (44)	289 (8)	N/A
Walking	63	N/A	166 (39)	283 (8)	N/A
Trotting	3	N/A	6 (1)	3 (< 1)	N/A
Cantering	5	N/A	13 (3)	3 (< 1)	N/A
Standing resting	6	N/A	13 (3)	5 (< 1)	N/A
Grazing	35	N/A	225 (53)	3254 (92)	N/A
Maintenance behaviours	2	N/A	2 (< 1)	3 (< 1)	N/A
Social behaviours	0	N/A	0	0	N/A
[1]Total numbers are for pooled behaviour events where the behaviour was identified					

* no videos were recorded during this period

Table 7b. Quantitative behavioural assessments for Period 2 (Autumn).

Behaviour	Number of behaviour events from still images (n = 167)	Number of behaviour events from video (n = 63)	Number of still images (n = 718[1]) with this behaviour (%)	Duration of time from still images (n = 5797 secs[1]) with this behaviour (%)	Duration of time from video (n = 832 secs[1]) with this behaviour (%)
Locomotion total	94	32	254 (35)	860 (15)	203 (24)
Walking	92	32	250 (35)	858 (15)	203 (24)
Trotting	2	0	4 (1)	2 (0)	0
Cantering	0	0	0	0	0
Standing resting	23	12	129 (18)	863 (15)	161 (19)
Grazing	43	17	303 (42)	4008 (69)	433 (52)
Maintenance behaviours	5	2	28 (4)	64 (1)	35 (4)
Social behaviours	2	0	4 (1)	2 (0)	0
[1]Total numbers are for pooled behaviour events where the behaviour was identified					

Table 7c. Quantitative behavioural assessments for Period 3 (Winter).

Behaviour	Number of behaviour events from still images (n = 50)	Number of behaviour events from video (n = 87)	Number of still images (n = 189[1]) with this behaviour (%)	Duration of time from still images (n = 5034 secs[1]) with this behaviour (%)	Duration of time from video (n = 294 secs[1]) with this behaviour (%)
Locomotion total	31	24	57 (3)	352 (7)	138 (47)
Walking	31	24	57 (3)	352 (7)	138 (47)
Trotting	0	0	0	0	0
Cantering	0	0	0	0	0
Standing resting	10	7	62 (33)	906 (18)	82 (28)
Grazing	7	3	68 (36)	3776 (75)	56 (19)
Maintenance behaviours	2	2	2 (1)	1 (< 1)	15 (5)
Social behaviours	0	1	0	0	3 (1)
[1]Total numbers are for pooled behaviour events where the behaviour was identified					

Table 7d. Quantitative behavioural assessments for Period 4 (Spring).

Behaviour	Number of behaviour events from still images (n = 109)	Number of behaviour events from video (n = 38)	Number of still images (n = 408[1]) with this behaviour (%)	Duratio of time from still images (n = 12,458 secs[1]) with this behaviour (%)	Duration of time from video (n = 181 secs[1]) with this behaviour (%)
Locomotion total	76	14	230 (56)	3488 (28)	60 (33)
Walking	70	14	224 (55)	3488 (28)	60 (33)
Trotting	3	0	3 (< 1)	< 1	0
Cantering	3	0	3 (< 1)	< 1	0
Standing resting	7	11	57 (14)	5855 (47)	51 (28)
Grazing	25	12	122 (30)	3115 (25)	65 (36)
Maintenance behaviours	1	1	2 (< 1)	< 1	5 (3)
Social behaviours	0	0	0	0	0
[1]Total numbers are for pooled behaviour events where the behaviour was identified					

Table 7e. Quantitative behavioural assessments for Period 5 (Summer).

Behaviour	Number of behaviour events from still images (n = 120)	Number of behaviour events from video (n = 40)	Number of still images (n = 419[1]) with this behaviour (%)	Duration of time from still images (n = 9885 secs[1]) with this behaviour (%)	Duration of time from video (n = 376 secs[1]) with this behaviour (%)
Locomotion total	69	13	176 (42)	1937 (20)	64 (17)
Walking	69	13	176 (42)	1937 (20)	64 (17)
Trotting	0	0	0	0	0
Cantering	0	0	0	0	0
Standing resting	10	1	17 (4)	20 (< 1)	4 (1)
Grazing	38	24	222 (53)	7908 (80)	297 (79)
Maintenance behaviours	3	2	4 (1)	20 (< 1)	11 (3)
Social behaviours	0	0	0	0	0
[1]Total numbers are for pooled behaviour events where the behaviour was identified					

All-occurrence analysis

The all-occurrence method was used to evaluate the number of horse-camera-days from the full data set by which horses were identified with less commonly observed indicators of welfare compromise, or of positive welfare.

Increased respiratory effort was identified in four horse-camera-days, a weak gait in nine, markedly reduced vigilance in three, and skin excoriation in 13. All of these occurrences were in emaciated horses (BCS ≤ 2). Conversely, trotting was identified on 23 horse-camera-days, and only two (9%) of these were emaciated horses (BCS ≤ 2), cantering or galloping was observed on 55 horse-camera days, and only four (7%) of these were in emaciated horses, allogrooming or nuzzling another horse was identified on 12 horse-camera-days, only three (25%) of these were emaciated horses. Herding behaviour was observed on four horse-camera-days and none of these were in emaciated horses.

Monthly body condition scores

Figure 4 shows the distributions of monthly body condition scores (BCS) for all the horses in the population for the whole study duration. There was a broader distribution of BCS between February and May than for the other months. Median BCS was stable at 4 (on 9-point scale) for the first eight months of the study, with more horses having higher body condition scores between February to May 2016 (Summer/Autumn). Median BCS dropped to 3 towards the end of Winter (August), and remained at 3 through Spring and Summer, with no horses having scores above

4. By January 2017 all horses had BCS less than 3, and the median BCS had reduced to 2 in February 2017. Figure 5 illustrates the same trend, with mean body condition scores reducing in each period.

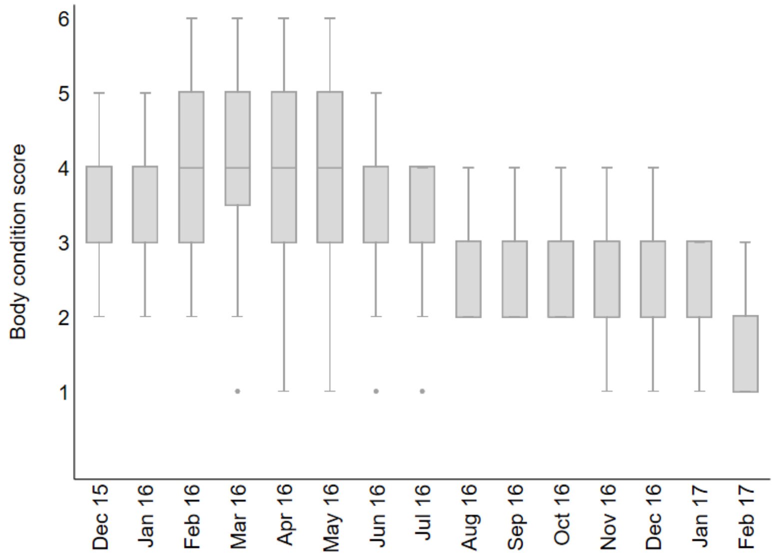


Figure 4. The box and whisker plot shows distributions of monthly body condition scores for all the horses in the population for the whole study duration. The middle band is the median, and the box extends from the 25th to 75th percentile. The whiskers extend upwards to the largest observed value that is less than the 75th percentile plus 1.5 times the inter-quartile range, and extend downwards to the lowest observed value that is more than the 25th percentile minus 1.5 times the inter-quartile range.

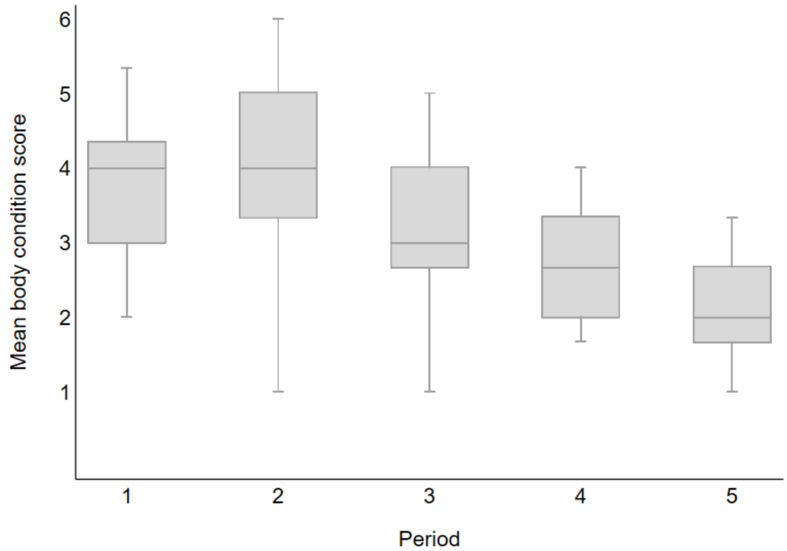
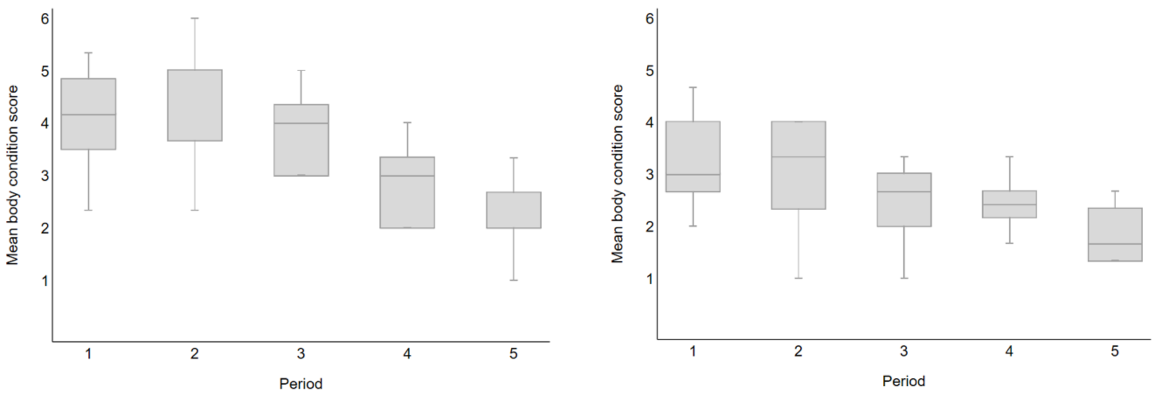


Figure 5. The box and whisker plot shows distributions of mean monthly body condition scores for each period.

Figure 6 shows the distribution of mean body condition scores by period, divided into subpopulation A and B. This illustrates that body condition scores decreased during the last three periods of the study in both subpopulations, but that mean body condition scores were lower for subpopulation B than A.



(a) **(b)**

Figure 6. The box and whisker plot shows distributions of mean monthly body condition scores for each period for **(a)** Subpopulation A and **(b)** Subpopulation B.

Figure 7 shows all body condition scores for all horses over the study period. This demonstrates a similar trend for lactating and non-lactating mares, stallions and foals/weanlings/juveniles, with body condition scores generally increasing slightly at the beginning of the study period, and then gradually declining to ≤ 3 for all horses by the end of the study period, unless they died prior to that. When lines are seen to stop before the end of the study period this indicates that the horse was no longer sighted and is assumed to have died. Foals/yearlings/juveniles generally had higher body condition scores and maintained their body condition for longer, but likely because undernutrition was reflected more by way of poor growth.

The joint p-value for interaction between initial subpopulation and month number was 0.273 and no interaction was assumed. Predicted mean body condition score for initial subpopulation A in month 1 was 4.0 (95% CI 3.5 to 5.5). Predicted means in months 2 to 5 were similar to this, before declining in a curvilinear fashion, with more rapid decreases with each additional month. Predicted means in months 7, 11-and 15 were, respectively, 3.8 (95% CI 3.4 to 4.2), 3.0 (95% CI 2.6 to 3.3), and 1.6 (95% CI 1.1 to 2.1). Mean body condition score was 0.8 units less for initial subpopulation B relative to initial subpopulation A (95% CI 0.3 to 1.4 units less; P=0.002).

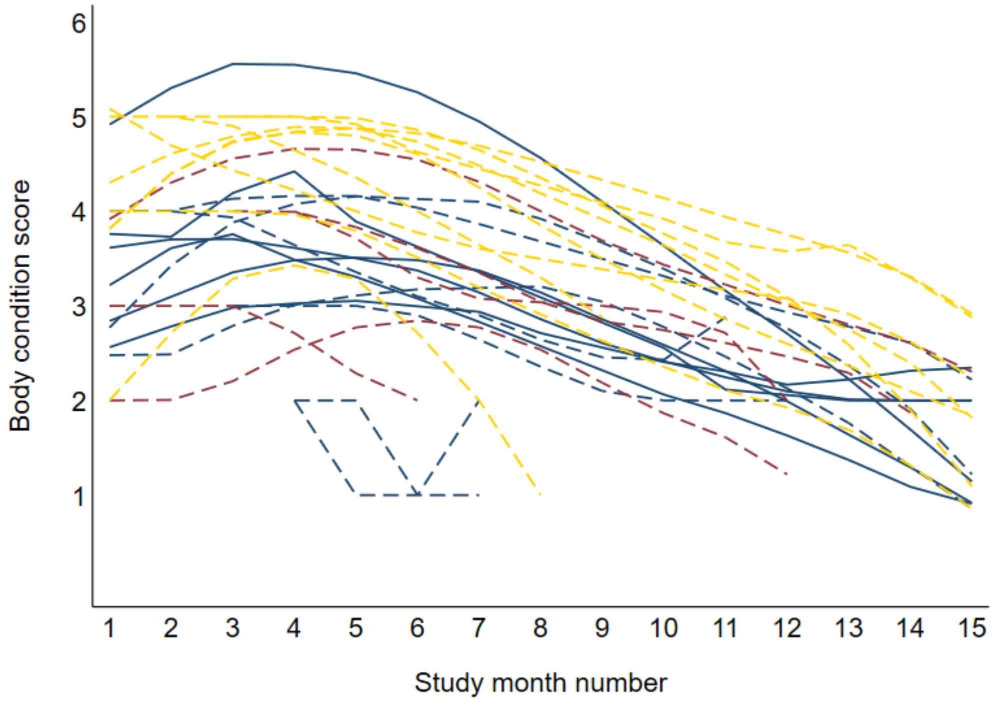


Figure 7. Locally weighted regression (lowess) plots of horse body condition scores by study month number. Navy solid lines: mares with foal or yearling at foot at some stage during study period; navy dashed lines: mares with no foal or yearling at foot at any stage during study period; maroon dashed lines: mature stallions; gold dashed lines: foals, yearlings and juveniles.

3.3 Mortalities – cause and time to death

Mortalities were reported in Chapter 7. Foal 1L that was born in Period 5 and survived for only about six weeks, was thought most likely to have died from under-feeding because the mare was emaciated at foaling and therefore probably did not produce enough milk. However, predation, injury or drowning are other possibilities. Ten of the other 11 horses known or assumed to have died, as reported in Chapter 7, were emaciated (BCS≤ 2) when last sighted and for some time before that. Cause of death in these cases was therefore attributed to starvation. One yearling (2C) had a BCS of 3 when last sighted at the end of winter, so his thick winter coat may have led to overestimation of BCS.

Although it was not possible with most horses to know precisely when they died, it was possible to record the minimum time for which they survived from when they were first detected as emaciated (BCS ≤ 2), until their last sighting. One yearling (3C) was stunted and emaciated for 8 months prior to last sighting. Of the remaining nine horses, they had been emaciated for at least two months (4 horses; 2A, 4A, 4B, 4C), 4 months (2 horses; 5B, 5D), 5 months (2 horses; 3A, 5C) and 6 months (1H). Horses 5B, 5C and 5D were emaciated at first observation so are likely to have been emaciated for much longer than that prior to death. Others had previous periods of emaciation before regaining weight, and then losing weight again.

3.4 Associations between body condition and other welfare indicators

There were negative correlations between body condition score and each of head-lower-than-withers body posture (correlation coefficient -0.40), poor coat condition (correlation coefficient -0.57), facial grimace (correlation coefficient -0.33), weakness (correlation coefficient -0.52) and apathy (correlation coefficient -0.48). Thus, all of these indicators were more common when body condition score was low ($P < 0.001$ for all).

3.5 Grading welfare status and risk

A detailed explanation using a case example

A mature mare from Band 1 (1B) was chosen as an example to illustrate in detail how the welfare grading scheme (detailed in Chapter 6) was applied to each individual horse. This mare exhibited the full range of welfare grades available; detailed in Table 8. Mare 1B was sighted on 171/450 days (38%) of the whole study period, with 212 different camera-day sightings (some days she was sighted on more than one camera).

For reference during the detailed description which follows, a season-by-season summary of the graded welfare status and risks for mare 1B is presented in Table 8. A detailed description of the basis for assigning each grade in this mare is then presented.

Table 8. Mare 1B: Welfare enhancement, compromise, confidence and risk grades for each domain during each period.

Domain grades*		1 (Summer)	2 (Autumn)	3 (Winter)	4 (Spring)	5 (Summer)
Domain 1 Nutrition	Welfare compromise	A/B	A/B	C	D/E	D/E
	Welfare enhancement	++	+	0	0	0
	Confidence	H	M	M	H	H
	Welfare risk	L	M	M	H	H
Domain 2 Physical environment	Welfare compromise	A/B	A/B	A/B	A/B	C
	Welfare enhancement	++	++	++	++	+
	Confidence	M	M	M	M	M
	Welfare risk	L	L	L	L	M
Domain 3 Health	Welfare compromise	A/B	A/B	A/B	C	D/E
	Welfare enhancement	++	++	+	0	0
	Confidence	M	M	L	M	H
	Welfare risk	M	M	M	H	H
Domain 4 Behavioural interactions	Welfare compromise	A/B	A/B	A/B	C	D/E
	Welfare enhancement	++	++	++	+	0
	Confidence	H	M	L	M	M
	Welfare risk	L	L	M	H	H
Domain 5 Mental status (Overall welfare status)	Welfare compromise	A/B	A/B	C	D/E	D/E
	Welfare enhancement	++	++	+	0	0
	Confidence	H	H	L	H	H
	Welfare risk	L	L	M	H	H

* Welfare compromise: grade A/B = 'no to low' level compromise, C = 'moderate', D/E = severe compromise
Welfare enhancement: grade 0 = 'none', + = 'low to medium, ++ = high enhancement
Confidence score: L = low, M = medium, H = high level of confidence
Welfare risk: L = low, M = medium, H = high chance of future welfare risk

Period 1 (Summer 2015/16)

Mare 1B was sighted on 77/90 days in Period 1; 72 days on camera trap still images, and 6 days by direct observations (Figure 8). She is the chestnut mare with bilateral hindlimb white socks and a star with a connected stripe.

Figure 8. Camera trap images and photos from direct observations of mare 1B during Period 1 (Summer 2015/16) demonstrating good body condition and forage availability, ability to seek shade and shelter in the trees, no externally observable indicators of compromised health, relaxed body posture at walk, time spent cantering, and close social proximity with a range of band members.

Domain 1: Nutrition

Mare 1B was sighted mostly in open grassland regions 1 and 2 during Period 1 (Figure 9). Based on both camera trap and direct observations, she appeared to stay mostly in these regions, with an estimated area utilised of 1.23km^2 (Chapter 7). There was a wide range of quality grasses and shrubs, with a forage availability score of B. Her body condition score was 5 to 6 (out of 9). Water was readily available from the river and creek running next to these grasslands.

I therefore assigned grade A/B (none to mild) for welfare compromise, and ++ (high) for welfare enhancement. I gave a high confidence score given the frequency at which I had sighted mare 1B, both with camera traps and direct observations. Welfare risk was graded as L (low) due to the good food and water availability, and not being heavily in foal nor having a foal at foot.

Domain 2: Physical environment

Ambient temperature was mostly within the thermoneutral zone (see Chapter 3), ranging from 8- 36°C. There were no days where it was below 5°C, and only 1 day where it was below 10°C. There were no extreme weather events such as heavy rain, hail or snow. Temperatures rose to over 25°C on 52% of days during this period, but plenty of shade was available from trees, and water in the river and waterholes in the immediate vicinity allowed for drinking and immersion in water if required for cooling. Sweating was never observed. Terrain and underfoot substrate was variable and posed no particular risks in this region.

I therefore assigned grade A/B (none to mild) for welfare compromise, and ++ (high) for welfare enhancement. I gave a moderate confidence score since this grading was predominantly based on resource-based measures. Sweating is usually a short term indicator which was not possible to evaluate frequently, so could have been missed. I graded welfare risk as L (low) based on the forecast for ongoing ambient temperature and weather conditions.

Domain 3: Health

No indicators of comprised health were detected. Mare 1B was observed to be either alert and vigilant or relaxed, had good coat condition, good hoof condition, no evidence of ocular, respiratory, skin or limb pathology, was strong and active, with a normal gait at walk, trot and canter. She was regularly observed trotting, cantering and galloping.

I therefore graded welfare compromise as A/B (none to mild) and enhancement ++ (high). I gave a moderate confidence score since this was based only on external observable indices. Short periods of health compromise could have occurred on days that mare 1B was not observed. A high faecal egg count was present and *S. vulgaris PCR* was positive (see Chapter 4), both being welfare alerting indices, so I graded welfare risk, as M (moderate).

Domain 4: Behavioural interactions

Mare 1B was in a band of 11 horses. Social networks (Chapter 7) demonstrated that she was close companions with all these other horses for variable proportions of time, in addition to some close contact with horses from band 2. She had a total of 13 different close companions in the period, and a mean of 2.63 close companions on days sighted. She had close companions on 68% of days sighted, observed only alone on 32% of the days. She was not nursing any foals, and other positive affiliative interactions were observed infrequently. However, as this is commonly the case (see Chapters 3 and 5), the number of close companions, and proportion of days with close companions were used as the main measure of affiliative interactions. I therefore assigned grade A/B (none or mild) for welfare compromise, and ++ (high) for enhancement, with a confidence score of H (high), given the high proportion of days she was sighted during this period. Welfare risk was graded as L (low) as no reasons were apparent for behavioural interactions to change in the foreseeable future.

Domain 5: Mental status (overall welfare status)

Mental status was cautiously inferred based on the information used to grade welfare compromise and enhancement in Domains 1-4, as described above, together with the scientific evidence for the link between these physical/functional indicators and mental experiences (detailed in Chapter 6). This information suggested no to mild negative mental experiences, and a high degree of positive mental experiences consisting of the following: rewarding engagement with exploration and foraging, pleasures of variations in tastes and textures, satiety and gastro-intestinal comfort, hydration, thermal and physical comfort, vitality of fitness, affectionately sociable, calm, content and exercising choice and a sense of agency. A grade A/B (no or low) for welfare compromise, ++ (high) enhancement, with high confidence (H), and a low welfare risk (L) was assigned.

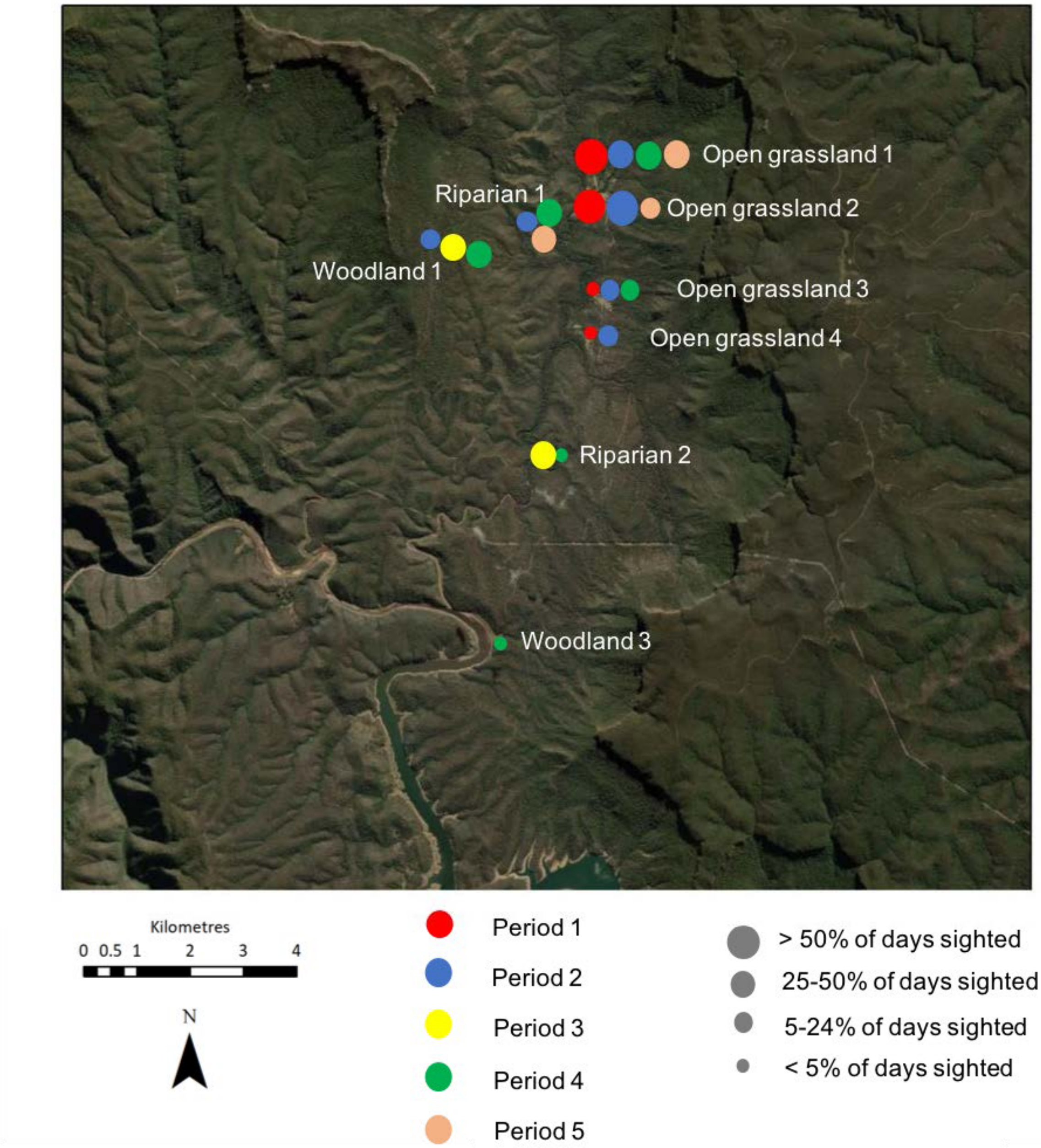


Figure 9. The locations that Mare 1B was sighted during different periods (coloured dots). The size of the dots shows the proportion of days the mare was sighted (combining camera trap and direct sightings) in the different geographical regions and habitats during each period.

Period 2 (Autumn 2016)

Mare 1B was sighted on 25/90 days in Period 2, 22 days on camera trap still images, 3 days on camera trap videos, and 2 days by direct observations (Figure 10).

The same grades were assigned in Period 2 for all domains except Domain 1 (nutrition) where only + (moderate) for enhancement was assigned, with only moderate (M) confidence this time (Table 8). This was because the forage availability score had reduced to C, and she was observed in a larger number of different locations (see Figure 9) with an increase in estimated area utilised to 4.08km^2. The reduction in forage availability combined with the increased number of locations, and estimated area utilised are suggestive of a need for more wide-ranging foraging. Body condition score remained at 6, so I still assigned an A/B (no to low) for welfare compromise, but with moderate (M) confidence because changes in BCS lag-behind decreases or increases in nutritional status. Welfare risk was now graded at moderate (M) given the reduction in forage availability.

There was no evidence of significant changes in the other domains. The physical environmental conditions were similar but with fewer hot days. Indicators of health remained unchanged. She had a total of 9 close companions during the period, with a mean of 2.69 close companions. She had close companions on 64% of days sighted, only alone on 35% of the days. Although the welfare compromise and enhancement grades remained the same in Domain 4 (behavioural interactions), the confidence score was reduced to moderate (M), given that mare 1B was observed on much fewer days during this period (25 days compared to 77 days in Period 1).

Figure 10. Camera trap images of mare 1B during Period 2 (Autumn 2016) demonstrating observations in a wider range of habitats and geographical regions and lower forage availability, but still a good body condition score, no externally observable indicators of compromised health, and mostly observed with close companions.

Period 3 (Winter 2016)

Mare 1B was only sighted on 2/90 days in Period 3, two days on camera trap still images, two days on camera trap videos, and no direct observations (Figure 11). For this reason confidence scores were low (L) or moderate (M) in all domains. These sightings were in completely different geographical areas to sightings in previous periods (Figure 9).

Domain 1: Nutrition

Forage availability score had further reduced to D (on the A to E scale). Two body condition scores were obtained from camera trap videos, scoring a 4 and 5, suggesting weight loss since Period 2 sightings. For these reasons she was graded C (moderate) for welfare compromise and 0 for enhancement, with moderate confidence. Water was readily available in the areas sighted, with a waterhole in one area, and river in the other. Although body condition score was still good, given that both body condition and forage availability had decreased since Period 2, welfare risk was graded at moderate (M). Estimated area utilised was less than in Period 2, at 2.45km^2, but this figure is not likely to be accurate given the very limited number of sightings during this period.

Domain 2: Physical environment

Assigned grades were all the same as for Periods 1 and 2 in this domain (Table 8). It was a mild winter, and although 52% of days had temperatures below 5°C, there were no extreme weather conditions, no reason for poor thermoregulation (good body condition, ambulatory) and good shelter was available in all regions.

Domain 3: Health

Poor coat condition was noted from a video clip, although this can be challenging to interpret with a winter coat. Indicators of health otherwise remained unchanged on the limited number of times she was observed. Grade A/B (no to low) was assigned for welfare compromise, and + (mild to moderate) for enhancement given there were no observations enabling physical fitness to be assessed. However, a low (L) confidence score was assigned for this grading. Welfare risk was graded as moderate (M) given the trend in weight loss and further reduction in forage availability.

Domain 4: Behavioural interactions

Mare 1B was observed with two close companions in this period, on one of the days observed, and on her own on the other day. With the very few sightings, it is hard to draw any conclusions, so the same compromise and enhancement grades were assigned as for Period 2, but this time with low (L) confidence. Welfare risk was graded as moderate (M) given the suggestion that close companionship may be reducing.

Domain 5: Mental status (overall welfare status)

Based on the information used to grade welfare compromise and enhancement in Domains 1-4, as described above, together with the scientific evidence for the link between these physical/functional indicators and mental experiences (detailed in Chapter 7), I cautiously inferred the mental experience of moderate hunger. There was inadequate information from other domains to infer any other mental experiences for this period. Grade C for welfare compromise and + for enhancement was assigned but with low confidence given the lack of information upon which to infer any other mental experiences. Future welfare risk was graded as moderate given the concerns outlined above.

Figure 11. Camera trap images of mare 1B during Period 3 (Winter 2016) demonstrating the limited information able to be obtained from still images during this period. Body condition and coat condition were able to be assessed twice on video clips.

Period 4 (Spring 2016)

Mare 1B was sighted on 24/90 days in Period 4, 24 days on camera trap still images, and one day by direct observation (Figure 12). These sightings were in many different geographical regions compared to sightings in previous periods, with regions more distant from each other, resulting in a substantial increase in estimated area utilised of 13.32km^2 (Figure 9).

Domain 1: Nutrition

Forage availability was now scored at D (on the A to E scale), and body condition score reduced from 4 to 3 during this period. The increase in estimated area utilised is consistent with increased food searching behaviour. Welfare compromise was therefore graded D/E and enhancement 0, with a high confidence score, and high ongoing welfare risk. Water was readily available in the regions sighted.

Domain 2: Physical environment

This was graded the same as for Periods 1 to 3, for the same reasons previously stated.

Domain 3: Health

When body condition score decreases to 3 and below, undernutrition related health consequences start to arise, as detailed in Chapter 3. Some other indices of declining health also became apparent in this period with frequently observed head-lower-than-withers body posture, poor coat condition despite being Spring, and only ever sighted walking and not trotting, cantering or galloping. Welfare compromise was therefore graded C (moderate), and enhancement 0 (none), with moderate confidence (M), and a high (H) future welfare risk given the continuing trend of declining body condition and forage availability.

Domain 4: Behavioural interactions

Mare 1B still had a total of six close companions during this period, with a mean of 1.75. The proportion of days that she was observed with close companions had reduced to 33%, whilst on 67% of days she was only observed alone. Welfare compromise was therefore graded as C (moderate) and enhancement as 0 (none). Moderate (M) confidence score was assigned as although she was observed relatively frequently, with camera trap limitations it is hard to be certain that there were not more days with close companionship, and interpreting the significance of a reduction from 13 to six close companions is problematic. However as companionship appeared to be declining, a high (H) future welfare risk was assigned.

Domain 5: Mental status (overall welfare status)

Based on the information used to grade welfare compromise and enhancement in Domains 1-4, as described above, together with the scientific evidence for the link between these physical/functional indicators and mental experiences (detailed in Chapter 7), I cautiously inferred the mental experiences of severe hunger, and moderate loneliness and malaise/exhaustion. Grade D/E (severe) for welfare compromise and 0 (none) for enhancement was assigned with high (H) confidence given the more frequent sightings in this period and larger amount of data obtained on a range of welfare indicators. Future welfare risk was graded as high (H) given the ongoing declines in forage availability, body condition score, and consequent declines in health and behavioural interactions.

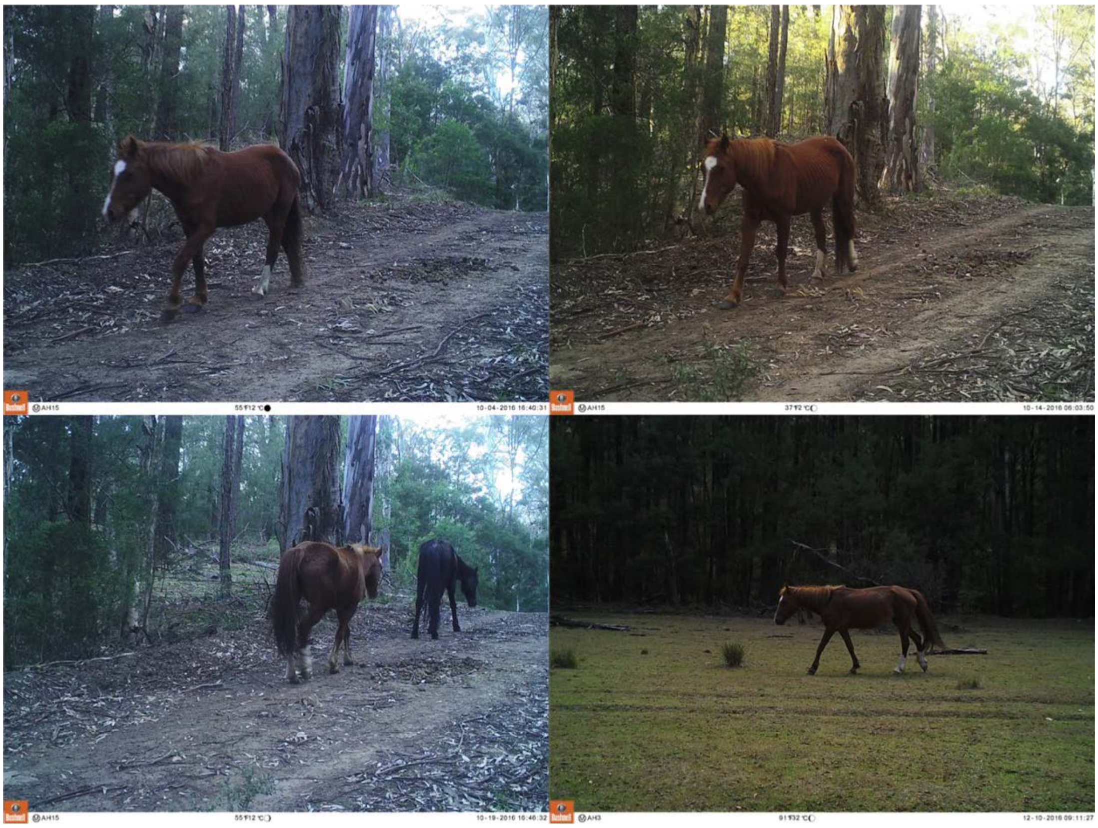


Figure 12. Camera trap images of mare 1B during Period 4 (Spring 2016) demonstrating the reduced body condition, poor coat condition and more frequent observations without any companions.

Period 5 (Summer 2016/17)

Mare 1B was sighted on 43/90 days in Period 5, 41 days on camera trap still images, and five days by direct observations (Figure 13). These sightings were in fewer geographical regions again, resulting in a reduced estimated area utilised of 1.75km^2 (Figure 9).

Domain 1: Nutrition

Forage availability remained at score D (on the A to E scale), and body condition score initially reduced to 2, and then later to 1. She also foaled during this period, consequently having the additional nutritional demands of lactation. The reduction in estimated area utilised may be a combination of the following reasons: (i) reduced roaming due to having a newborn foal, (ii) reduced activity due to declining health and weakness, since chronic starvation is known to result in decreased activity levels (Chapter 3), and (iii) choosing to remain close to water sources, due to increased water requirements from the combination of higher temperatures (Table 3), and increased lactational demands for water. Welfare compromise was therefore graded D/E (severe) and enhancement 0 (none), with a high (H) confidence score, and high (H) ongoing welfare risk.

Domain 2: Physical environment

In this period, welfare compromise was graded as C (moderate), and enhancement + (mild), since 65% of days in this period were > 25°C, and 36% of these days were > 35°C, with maximum temperatures of 48°C, giving rise to a much higher likelihood of heat challenge. Being coupled with declining health (see below), thermoregulatory mechanisms and ability to seek shade or immersion in cool water may be impacted. No sweating was observed. I graded confidence as moderate (M), given its reliance on resource-based measurements only. Welfare risk was graded as moderate (M) because of the potential for significant heat challenges if there were any restriction on the mare's access to water.

Domain 3: Health

As body condition score had dropped to 1, serious metabolic consequences of starvation were likely to be present, as detailed in Chapter 3. Additional indicators of declining health continued to be apparent in this period with ongoing frequently observed head-lower-than-withers body posture, poor coat condition, reduced alertness and vigilance, a weak gait, suspected melaena suggestive of reduced gut mucosa integrity, and development of skin excoriations over prominent bony protuberances (Figure 13). Cessation of sightings of her foal also suggested that it had died, almost certainly due to inadequate milk production. Welfare compromise was therefore graded D/E (severe), and enhancement 0 (none), with high (H) confidence despite assessment being based only on externally observable indices, because of the mare's extreme emaciation. Welfare risk was graded as high (H) given the continuing trend of declining trend in indicators of health.

Domain 4: Behavioural interactions

Mare 1B had 13 total close companions again in this period, with a mean of 1.86. She was observed with close companions on 65% of days and only alone on 35%. However, given her extreme emaciation, starvation, and weakness, it was not considered likely that opportunities for positive social interactions were being utilised, and more likely that all the group of horses, all in similarly poor welfare states, had gathered near a readily accessible water source. Her foal, unsighted, was assumed dead. Here welfare compromise was therefore graded as D/E (severe) and enhancement as 0 (none), but with only a moderate (M) confidence score to reflect the challenge in interpreting companionship in this setting. Welfare risk was graded as high (H), as mare 1B was at great risk of dying, as were her equally stricken close companions.

Domain 5: Mental status (overall welfare status)

Based on the information used to grade welfare compromise and enhancement in Domains 1-4, as described above, together with the scientific evidence for the link between these physical/functional indicators and mental experiences (detailed in Chapter 7), I cautiously inferred that this mare would have had the mental experiences of extreme hunger, moderate heat discomfort, and severe weakness and exhaustion. Grades D/E (severe) for welfare compromise and 0 (none) for enhancement were assigned with high (H) confidence given the more frequent sightings in this period and larger body of data obtained on a range of welfare indices. Future welfare risk was graded as high (H) given the high risk of starvation-induced death.

Figure 13. Camera trap images of mare 1B during Period 5 (Summer 2016/17) demonstrating the further decline in body condition to the point of severe emaciation. She also foaled, with the foal dying within about 6 weeks of birth. There were frequent observations with head-lower-than-wither body posture, a weak gait, reduced alertness, and skin excoriations can be seen over bony prominences.

Grading welfare status and risk of all horses in the population

The same process of grading as detailed above for mare 1B was applied to every horse individually for each period (season). The overall welfare (Domain 5) compromise and enhancement grades for each horse, for each period, are shown in Table 9. This demonstrates that horses in bands 3 to 5 (subpopulation B), that resided in woodland habitats (Chapters 5 & 7) had more severe welfare compromise than horses in bands 1 and 2 (subpopulation A) during Period 1, with welfare compromise becoming more severe in Periods 2 and 3, with deaths increasing thereafter. Horses in band 1, the largest band residing on the largest open grassland area in Period 1 had little welfare compromise in Periods 1 and 2, but developed severe welfare compromise by Periods 4 and 5.

Table 9. Overall welfare compromise/enhancement grades for all individual horses.

Unique Identifier[1]	Domain 5 Mental experience/overall welfare compromise/enhancement grade				
	Period 1 (Summer)	Period 2 (Autumn)	Period 3 (Winter)	Period 4 (Spring)	Period 5 (Summer)
1A	A/B/++	A/B /+	C/0	D/E/0	D/E/0
1B	A/B/++	A/B/++	C/1	D/E/0	D/E/0
1C	D/E/0	C/1	D/E/0	D/E/0	D/E/0
1D	A/B/++	A/B/++	C/1	D/E/0	D/E/0
1E	C/1	A/B/++	C/1	D/E/0	D/E/0
1F	C/1	C/1	D/E/0	D/E/0	D/E/0
1G	A/B/++	A/B/++	C/1	C/1	D/E/0
1H	A/B/++	C/1	D/E/0	D/E/0	D/E/0[2]
1I	A/B/++	A/B/++	Not observed	D/E/0	D/E/0
1J	A/B/++	A/B/++	Not observed	D/E/0	D/E/0
1K	A/B/++	A/B/++	D/E/0	D/E/0	D/E/0
2A	C/0	D/E/0	Not observed	Not observed	Not observed[3]
2B	C/1	C/1	D/E/0	D/E/0	D/E/0
2C	C/1	D/E/0	D/E/0	Not observed	Not observed[3]
2D	A/B/++	A/B/++	D/E/0	C/1	D/E/0
2E	C/1	C/1	D/E/0	D/E/0	D/E/0
3A	D/E/0	D/E/0	D/E/0	D/E/0	Not observed[3]
3B	D/E/0	C/1	D/E/0	D/E/0	D/E/0
3C	C/1	C/1	D/E/0	D/E/0	D/E/0[3]
3D	A/B/++	C/1	D/E/0	D/E/0	D/E/0
4A	C/1	C/1	D/E/0	D/E/0	D/E/0[3]
4B	D/E/0	D/E/0	D/E/0	Not observed	Not observed[3]
4C	C/1	D/E/0	D/E/0	D/E/0	Not observed[3]
4D	D/E/0	D/E/0	D/E/0	D/E/0	Not observed[4]
5A	Not observed	D/E/0	D/E/0	D/E/0	Not observed[4]
5B	Not observed	D/E/0	D/E/0	Not observed	Not observed[3]
5C	D/E/0	D/E/0	Not observed	Not observed	Not observed[3]
5D	Not observed	D/E/0	D/E/0	Not observed	Not observed[3]

[1] Number = band; Letter = sequential letter within band

[2] Known to have died at the end of the study

[3] Never sighted again, assumed to have died

[4] Observed alive after the end of the study

The number of horses assigned each grade, for each domain in each period are detailed in Tables 10a to 10e. Some examples of welfare compromise and enhancement are illustrated in Figures 14 and 15.

For welfare compromise, grades were moderately to closely correlated between domains (r = 0.56 to 0.92), illustrating how the domains interact. Domain 5 (Mental status) was, not surprisingly, most closely correlated with Domain 1 (Nutrition) (r = 0.92) and Domain 3 (Health) (r = 0.81). This demonstrates that hunger, weakness, malaise/exhaustion were the predominant inferred mental experiences. On univariable analysis, Domain 1 accounted for 81% of the deviance in Domain 5, with Domains 2 to 4 accounting for 21%, 52%, and 39% of the deviance in Domain 5. This demonstrates that undernutrition was the main driver of overall mental experiences (Domain 5), and also had subsequent impacts on environmental challenges (Domain 2), health (Domain 3) and behavioural interactions (Domain 4).

For enhancement, grades were also moderately to closely correlated between domains (r = 0.48 to 0.87). Domain 5 was most closely correlated with Domain 1 (r = 0.85) and Domain 3 (r = 0.87). On univariable analysis, Domain 1 accounted for 49% of the deviance in Domain 5, with Domains 2 to 4 accounting for 20%, 54%, and 35% of the deviance in Domain 5. This shows the same trend as welfare compromise grades, illustrating that compromise and enhancement are linked, with enhancement reducing as welfare compromise increases.

For welfare risk, grades were moderately to closely correlated between domains (r = 0.45 to 0.88). Domain 5 was most closely correlated with Domain 1 (r = 0.88), Domain 3 (r = 0.80) and Domain 4 (r = 0.82). On univariable analysis, Domain 1 accounted for 56% of the deviance in domain 5, with Domains 2 to 4 accounting for 20%, 40%, and 53% of the deviance in domain 5. Again this demonstrates how the domains interact, and that nutrition was a main determinant of welfare risk in other domains.

Confidence scores were generally highest for Domain 1 (Nutrition) since body condition score is straightforward to assess, and usually can be assessed on very few observations. This is a situation where the number of days the horse was observed did not influence the confidence of the welfare compromise grade since one sighting demonstrating emaciation is sufficient to be confident that welfare compromise is severe. Conversely in Domain 4 (Behavioural interactions) frequent observations of the horse were required to be confident in grading. Thus Period 3 has the highest proportion of low confidence scores in this domain as horses were sighted less frequently. Domain 3 (Health) had the highest proportion of low confidence scores overall, reflecting the reliance on externally observable indicators of health.

Table 10a-e. The number of horses in the whole population assigned each grade for welfare compromise and enhancement, confidence and risk, for each domain in each period.*

* Note that as highlighted in Chapter 7, some horses were not observed in every period and will not have been assigned compromise/enhancement grades except for Domain 2 since that was largely based on resource-based indicators. Risk grades were also assigned in this situation for Domains 1 and 2.

Table 10a. Period 1 (Summer) grading of welfare status and risk in each Domain.

	Grade	Number of horses				
		Domain 1	**Domain 2**	**Domain 3**	**Domain 4**	**Domain 5**
Welfare compromise	A/B	11	24	16	22	10
	C	8	1	7	2	9
	D/E	6	0	2	1	6
Welfare enhancement	0	7	1	5	1	7
	+	9	0	6	2	8
	++	9	24	14	22	10
Confidence Score	L	0	0	4	0	0
	M	10	25	20	8	10
	H	15	0	1	17	15
Welfare risk	L	8	23	0	23	12
	M	13	2	24	1	9
	H	4	0	1	1	4

Table 10b. Period 2 (Autumn) grading of welfare status and risk in each Domain.

	Grade	Number of horses				
		Domain 1	**Domain 2**	**Domain 3**	**Domain 4**	**Domain 5**
Welfare compromise	A/B	9	25	17	17	9
	C	11	3	7	5	9
	D/E	8	0	4	6	10
Welfare enhancement	0	16	3	10	6	10
	+	10	2	8	5	10
	++	2	23	10	17	8
Confidence Score	L	0	1	5	0	0
	M	16	27	20	16	12
	H	12	0	3	12	16
Welfare risk	L	3	24	0	14	7
	M	7	4	18	11	9
	H	18	0	10	3	12

Table 10c. Period 3 (Winter) grading of welfare status and risk in each Domain.

	Grade	Number of horses				
		Domain 1	**Domain 2**	**Domain 3**	**Domain 4**	**Domain 5**
Welfare compromise	A/B	0	8	7	2	0
	C	9	16	10	12	5
	D/E	15	2	7	9	19
Welfare enhancement	0	23	2	17	9	20
	+	1	16	6	12	4
	++	0	8	1	2	0
Confidence Score	L	0	4	15	13	1
	M	14	22	7	9	12
	H	12	0	2	2	12
Welfare risk	L	0	8	0	0	0
	M	5	18	3	14	8
	H	21	0	23	12	18

Table 10d. Period 4 (Spring) grading of welfare status and risk in each Domain.

	Grade	Number of horses				
		Domain 1	**Domain 2**	**Domain 3**	**Domain 4**	**Domain 5**
Welfare compromise	A/B	0	14	1	2	0
	C	3	8	9	14	2
	D/E	19	0	12	6	20
Welfare enhancement	0	22	0	21	6	20
	+	0	10	1	13	2
	++	0	12	0	3	0
Confidence Score	L	0	0	2	2	0
	M	0	22	18	19	3
	H	22	0	2	1	19
Welfare risk	L	0	13	0	0	0
	M	0	9	0	3	0
	H	22	0	22	19	22

Table 10e. Period 5 (Summer) grading of welfare status and risk in each Domain.

	Grade	Number of horses				
		Domain 1	**Domain 2**	**Domain 3**	**Domain 4**	**Domain 5**
Welfare compromise	A/B	0	6	0	2	0
	C	0	15	5	11	0
	D/E	19	0	14	6	19
Welfare enhancement	0	19	0	19	6	19
	+	0	16	0	9	0
	++	0	5	0	3	0
Confidence Score	L	0	1	0	0	0
	M	0	19	15	17	2
	H	19	0	4	2	17
Welfare risk	L	0	2	0	0	0
	M	0	18	0	2	0
	H	20	0	19	17	19

A selection of images below show a range of examples of different features of welfare compromise (Figure 14a-p).

(a)

(b)

(c)

Figure 14a-p. Images illustrate different features of welfare compromise. **(a)** Mare 5B emaciated. Also observed weak and with increased respiratory effort on video clips. Died in Period 3 (winter). **(b)** Mare 1H and yearling 1K. Emaciated and severely stunted growth of the yearling. 1H observed to have reduced vigilance, progressing to apathy and weakness. She died in Period 5 (Summer) at 8 years of age. **(c)** Stallion 3A. Observed on video clips with increased respiratory effort. Died in Period 5 following a long period of emaciation.

(d)

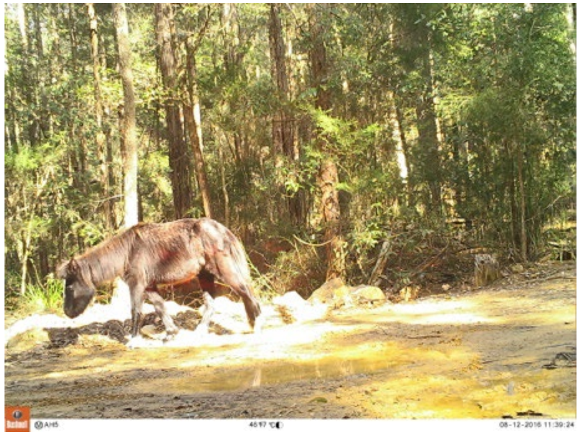
(e)

(f)

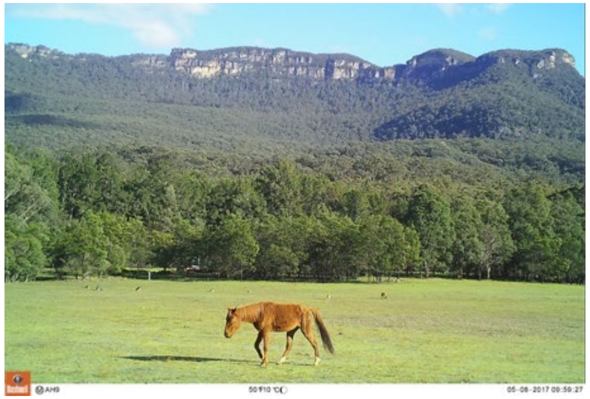
(g)

Figure 14a-p (cont'd). **(d)** Mare 1F, yearling 1G, and colt 2D. Thin body condition and dull rough coat condition. **(e)** Mare 3B Right mandibular wound that could be an abscess. **(f)** Mare 1B in Period 5 when she was emaciated, had recently lost her foal and was observed to be weak. Four out of six features of the facial grimace are evident; ears held stiffly and turned backwards, orbital tightening with close eyelids, tension above the eye area and strained nostrils. However, note caution needs to be exercised to not overinterpret features from a single image. **(g)** Mare 2B recently lost her yearling foal, thin body condition, ears held stiffly and turned backwards.

(h)



(i)



(j)

(k)

Figure 14a-p (cont'd). (h-k) Examples of head-lower- than-withers body posture, which was frequently observed in thin and emaciated horses.

(l)

(m)

(n)

(o)

Figure 14a-p (cont'd). (l-m) Emaciated mare 1I. Skin excoriations can be seen over the ribs. **(m)** Area of alopecia behind the wither and evidence of scurfy coat. **(n-o)** Thin young horses with head-lower-than-withers body posture, and coats wet from rain increasing the thermoregulatory challenge of cold exposure**.**

(p)

Figure 14a-p (cont'd). (p) Some behaviours may be normal maintenance behaviours but when performed excessively can be indicators of health compromise. This can be difficult to distinguish when only capturing snap shots of the behaviour with camera trap images. This series of images is a good example illustrating how it was determined that the behaviour of 'rubbing' was being performed excessively, indicative of pruritus. Horse 3D was frequently observed rubbing on this tree at night. Subsequent photographs during daylight revealed mild skin excoriations suggestive of self-trauma from excessive rubbing. A common cause of this would be pediculosis, particularly in young or immunocompromised horses (see Chapter 3).

A selection of images below show a range of examples of different features of positive welfare states (welfare enhancement) (Figures 15a-g).

(a)

(b)

(c)

(d)

Figure 15a-g. Images illustrate different features of welfare enhancement. **(a)** Mare 1B and her foal in close spatial proximity, alert, good body condition, well-muscled, shiny coats, good forage availability. **(b)** Band 1 all in close spatial proximity with an encounter with some members of Band 2. **(c)** Mare 1F with a relaxed body posture, nursing her yearling. **(d)** Mare 1B trotting, alert. On 21/23 days when horses were observed trotting they had body condition scores > 3.

(e)

(f)

(g)

(h)

Figure 15a-g (cont'd). (e) Band stallion 1A and a mare grazing whilst a yearling and a foal rest lying down. **(f)** Stallion and mare cantering/galloping together. On 51/55 days when horses were observed cantering they had body condition scores > 3. **(g)** Stallion 2A and yearling colt trotting together. **(h)** Stallion 2A and young colt allogrooming. Of 9/12 days that horses were observed allogrooming, they had body condition scores > 3.

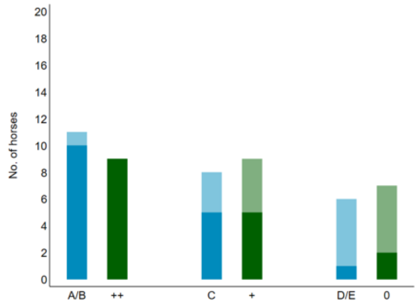
Figure 16a relates to Domain 1 (Nutrition). It shows that in Period 1 (Summer) most horses in sub-population A were graded A/B/++ in Domain 1; i.e., had good nutrition, whereas most horses in sub-population B were graded D/E/0, i.e., had poor nutrition. Moving from Period 2 (Autumn) through to 5 (the following Summer), there is a gradual increase in welfare compromise and decrease in enhancement, for both subpopulations, with almost all remaining horses being graded D/E/0 for nutrition, by Period 5.

Figure 16b relates to Domain 2 (Physical environment). It shows that in Periods 1 (Summer) and 2 (Spring) most horses in both sub-populations were graded A/B/++ in Domain 2; i.e., they experienced no/mild thermal challenges. In Period 3 (Winter) half of the horses in both sub-populations were graded C/+; i.e., moderate thermal challenge. This reflects cold periods during winter, where thin or emaciated horses with inadequate feed are likely to have had less thermoregulatory capacity to compensate for cold temperatures. In Period 5 (Summer) an increased proportion of horses in both sup-populations were graded C/+, this time reflecting thermal challenge from ambient heat, and a difference in the ability of horses to access shade and water.

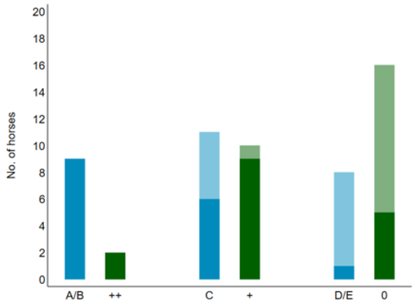
Figure 16c relates to Domain 3 (Health). It shows that that the trends during the whole study in Domain 3 were approximately parallel to those in Domain 1 (Nutrition). This reflects that the development of undernutrition during the study was the main driver of health compromise in this population. More horses in subpopulation B had greater health-related welfare compromise than in subpopulation A, and welfare compromise increased during the study, with most horses in both subpopulations being graded D/E/O in Period 5.

Figure 16d relates to Domain 4 (Behavioural interactions). It shows that in Period 1 (Summer) most horses in both subpopulations had no/mild welfare compromise. Welfare compromise increased (i.e., behavioural interactions reduced) for subpopulation B in Period 2, and for subpopulation A in Period 3, with most horses in both subpopulations having moderate to severe compromise by Periods 4 and 5. This reflects that with worsening undernutrition, the bands dispersed and horses spent more time alone searching for food, so there were fewer opportunities to interact with each other.

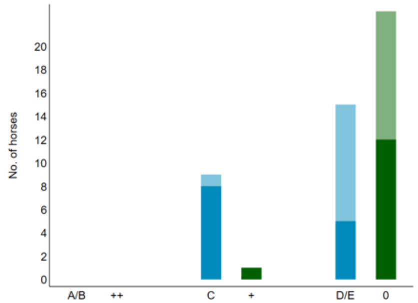
Figure 16e relates to Domain 5 (Mental status/overall welfare status). It shows the grading of the cautiously assigned mental experiences, informed by all the objective information used to grade welfare compromise and enhancement in Domains 1- 4. Also included in the assessment was the scientific evidence of links between the physical/functional indicators in Domains 1-4 and the mental experiences they are likely to reflect (as detailed in Chapter 6). Low/mild welfare compromise (grade A/B) with high enhancement (++) reflects mostly positive mental experiences such as rewarding engagement with exploration and foraging, pleasures of variations in tastes and textures of feed, satiety and gastro-intestinal comfort, hydration, thermal and physical comfort, vitality of fitness, affectionately sociable interactions, being calm and content, and exercising choice expressing agency. Severe welfare compromise (grade D/E) with no enhancement (0) reflects mostly negative mental experiences such as hunger, thermal discomfort, weakness, exhaustion, loneliness. Figure 16e shows that (i) more horses in subpopulation B had more negative experiences (lower overall welfare status) than horses in subpopulation A, and (ii) the number of horses with more negative experiences and less positive experiences increased in both populations during the study, such that by the end of it all horses in the population had mostly negative mental experiences, representing severe welfare compromise.



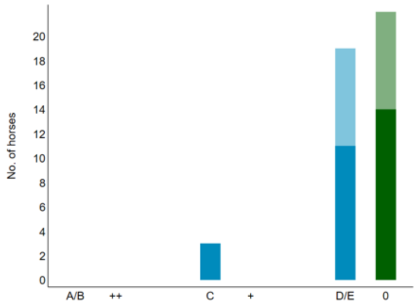
(a) Period 1 (Summer)



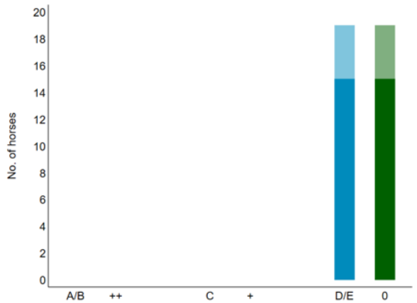
(b) Period 2 (Autumn)



(c) Period 3 (Winter)

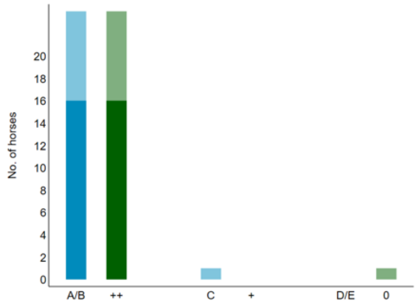


(d) Period 4 (Spring)

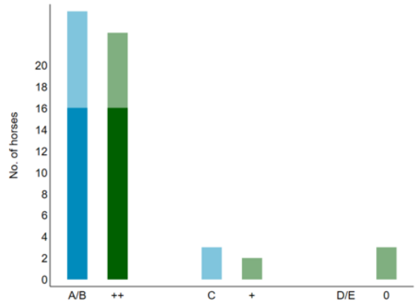


(e) Period 5 (Summer)

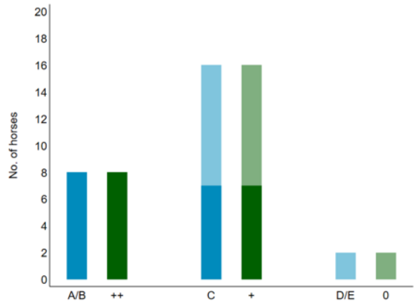
Figure 16. Graphs show the number of horses, differentiated by subpopulation, with each overall welfare compromise and enhancement grade, for each domain, in each period. **(a)** Domain 1 (Nutrition). The number of horses with grades of A/B, C, and D/E for compromise (blue bars) and ++, + and 0 for enhancement (green bars). Darker (i.e. lower) sections of bars are subpopulation A (initial bands 1 and 2) and lighter (i.e., upper) sections of bars are sub population B (initial bands 3, 4 and 5). For example, for Domain 1 in period 1, 10 horses from subpopulation A had a grade of A/B for compromise and 1 horse from subpopulation B had a grade of A/B. And in Period 1, 2 horses from sub population A had a grade of 0 for enhancement and 5 horses from subpopulation B had a grade of 0 for enhancement.



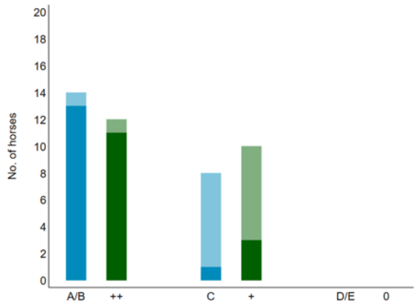
(a) Period 1 (Summer)



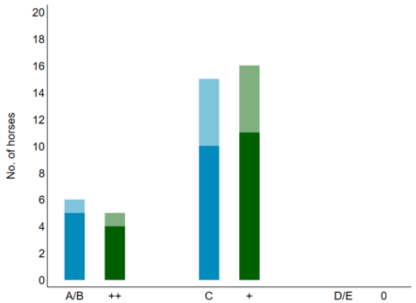
(b) Period 2 (Autumn)



(c) Period 3 (Winter)

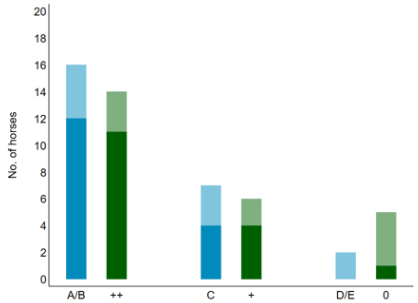


(d) Period 4 (Spring)

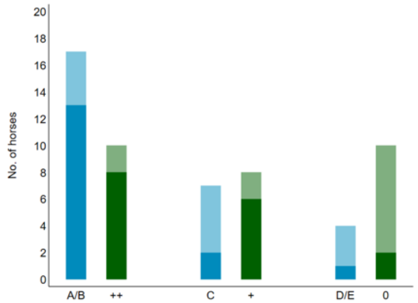


(e) Period 5 (Summer)

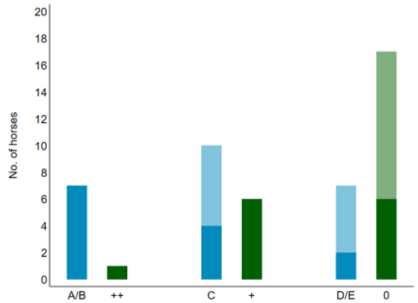
Figure 16 (cont'd). (b) Domain 2 (Physical environment). The number of horses with grades of A/B, C, and D/E for compromise (blue bars) and ++, + and 0 for enhancement (green bars). Darker (i.e. lower) sections of bars are sub population A (initial bands 1 and 2) and lighter (i.e. upper) sections of bars are sub population B (initial bands 3, 4 and 5).



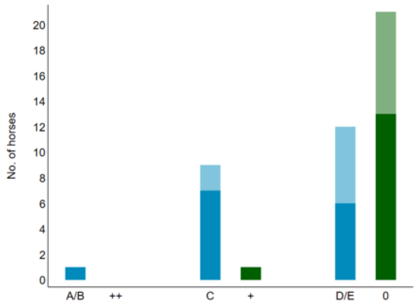
(a) Period 1 (Summer)



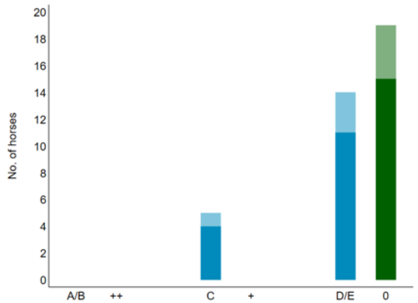
(b) Period 2 (Autumn)



(c) Period 3 (Winter)

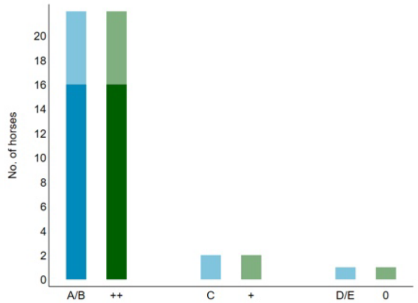


(d) Period 4 (Spring)

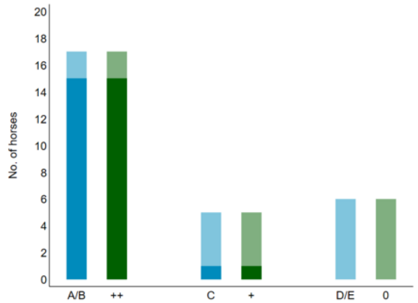


(e) Period 5 (Summer)

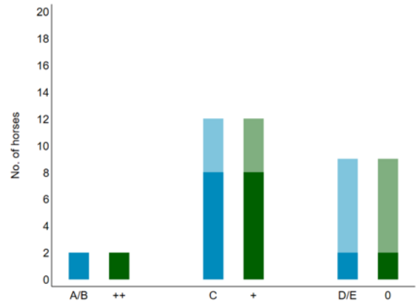
Figure 16 (cont'd). (c) Domain 3 (Health). The number of horses with grades of A/B, C, and D/E for compromise (blue bars) and ++, + and 0 for enhancement (green bars). Darker (i.e., lower) sections of bars are subpopulation A (initial bands 1 and 2) and lighter (i.e., upper) sections of bars are subpopulation B (initial bands 3, 4 and 5).



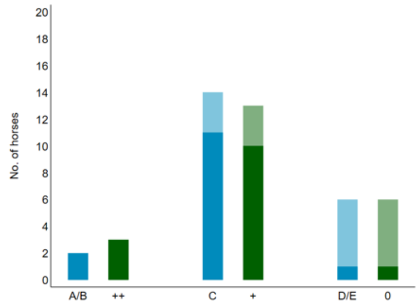
(a) Period 1 (Summer)



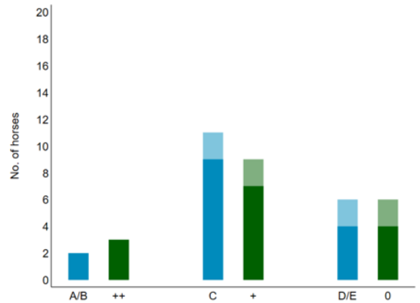
(b) Period 2 (Autumn)



(c) Period 3 (Winter)

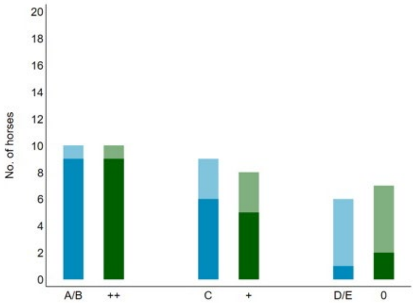


(d) Period 4 (Spring)

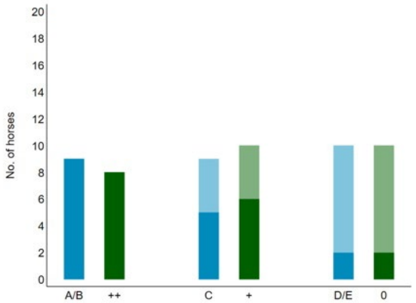


(e) Period 5 (Summer)

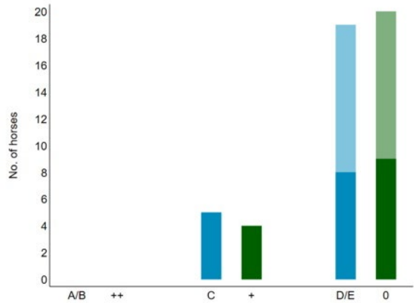
Figure 16 (cont'd). (d) The number of horses with grades of A/B, C, and D/E for compromise (blue bars) and ++, + and 0 for enhancement (green bars) for Domain 4 (Behavioural interactions). Darker (i.e. lower) sections of bars are sub population A (initial bands 1 and 2) and lighter (i.e. upper) sections of bars are sub population B (initial bands 3, 4 and 5).



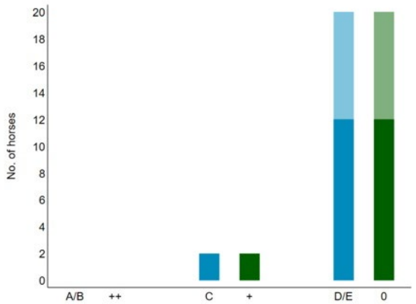
(a) **Period 1 (summer)**



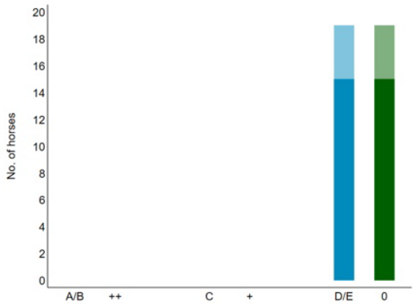
(b) **Period 2 (autumn)**



(c) **Period 3 (winter)**



(d) **Period 4 (spring)**



(e) **Period 5 (summer)**

Figure 16 (cont'd). (e) The number of horses with grades of A/B, C, and D/E for compromise (blue bars) and ++, + and 0 for enhancement (green bars) for Domain 5 (Mental status/overall welfare status). Darker (i.e. lower) sections of bars are sub population A (initial bands 1 and 2) and lighter (i.e. upper) sections of bars are sub population B (initial bands 3, 4 and 5).

3.6 The main determinant of welfare status

Forage availability score was positively correlated with the welfare compromise and welfare risk grades for each domain (r = 0.60 to 0.77) and negatively correlated with the enhancement grades for each domain (r = -0.67 to -0.77), thereby accounting for 36 to 59% and 45 to 59%, respectively of the variation in these welfare indices. Therefore, forage availability was a significant determinant of welfare status of the horses in this study.

Welfare risk in Domain 5 was never classified as high when the allocated forage availability score was B (a total of 13 horse-periods), and it was never classified as low when the allocated forage availability score was D (n = 52 horse-periods). Welfare risk was classified as high for all 29 horse-periods where the allocated forage availability score was E. This demonstrates that forage availability was the main risk factor for future welfare compromise during the study.

Mean body condition score was estimated to be 1 unit lower (95% CI 0.4 to 1.6 units lower; P=0.001) when forage availability was classified as E compared to when it was classified as B. This demonstrates a link between resource-based indicator of forage availability, and the animal-based indicator of body condition score.

The mean estimated area utilised was found to be 2.5 km^2 more (95% CI 0.2 less to 5.1 more; P=0.067), 3.2 km^2 more (95% CI 0.9 to 5.5 more; P=0.006) and 3.6 km^2 more (95% CI 1.1 to 6.1 more; P=0.005) when forage availability was classified as C, D and E, respectively, compared to when it was classified as B. This suggests that forage availability was driving habitat use, and that a significant determinant of the estimated area utilised is a drive to extend the area of foraging activity in the face of feed scarcity.

The horse's total number of close companions for the period was estimated to be 4.4 (95% CI 2.3 to 6.6), 4.7 (95% CI 2.8 to 6.7) and 8.5 (95% CI 6.4 to 10.6) less when forage availability was classified as C, D and E, respectively, compared to when it was classified as B ($P<0.001$ for all). This is consistent with observations that bands dispersed and social interactions were less, when forage availability was poor.

As noted above, overall welfare risk (Domain 5) was classified as high for all 29 horse-periods where the allocated forage availability score was E. Accordingly, possible determinants of overall welfare risk (Domain 5) were explored for horse-periods where food availability was classified as B, C, or D (i.e. not E). Odds ratios for higher risk were increased when the allocated forage availability score was C compared to B (estimated odds ratio 2.6 (95% CI 1.1 to 4.2; $P=0.001$) and were much increased when the allocated forage availability score was D compared to B (estimated odds ratio 6.0 (95% CI 4.2 to 7.9; $P<0.001$). Thus, across forage availability categories of B, C, or D, welfare risk was strongly associated with forage availability.

Additional possible determinants of overall welfare risk (mean body condition score, area traversed and total number of close companions for the period were assessed by comparing distributions of these within food availability categories of C and D, as there was some variation in overall welfare risk within these categories. With forage availability score C, for most of the horse-periods, welfare risk in Domain 5 was low (n = 8) or medium (n = 13) with only four horse-periods with low risk. With forage availability score D, for all horse-periods, welfare risk in Domain 5 was medium (n = 10) or high (n = 41). Body condition scores were negatively associated with welfare risk in Domain 5, for all these forage availability scores, while with forage availability score D, estimated areas utilised was higher when welfare risk in Domain 5 was high. There was no obvious association between total number of close companions for the period and welfare risk in Domain 5 with each of these forage availability grades.

4. Discussion

This chapter describes, for the first time, (i) a systematic and scientific assessment of the welfare of free-roaming wild horses, (ii) an evaluation of differences in welfare status between horses residing in different habitats, and (iii) seasonal changes in their welfare status. Furthermore, it (iv) describes the associations between welfare status, population demographics, social organisation and habitat use, and (v) the main determinant of changes in these parameters.

A wide range of indices of welfare compromise and enhancement were able to be detected using the combination of camera trap still images, video and direct observation. Using camera traps also enabled welfare to be assessed in horses residing in woodland habitats where they could not be observed directly. These horses were found to have the worst welfare status, illustrating the value of this methodology for assessing welfare indices, which I developed in Chapter 5 (Harvey et al. 2021). Whilst

I assessed a wide range of welfare indices, I determined that the key welfare status indicator in this population was body condition score. Other indices of welfare compromise and enhancement were all found to be linked to undernutrition.

In this Chapter I have sought to extend the depth and breadth of my analysis by combining the information on population dynamics from Chapter 5, social organisation and habitat use from Chapter 7, and welfare status from this Chapter. I have found that horses in predominantly woodland habitats (i.e., subpopulation B), roamed larger areas, had smaller band sizes and lower reproductive rates, fewer social interactions, and poorer overall welfare. Horses in grassland habitats (subpopulation A) also experienced increasingly poor welfare through Winter, and into Spring and the following Summer. During this time, initial bands dispersed into smaller groups or single horses, with fewer social interactions, roaming larger areas, and moving into woodland habitats in addition to grasslands.

A major determinant of these changes in welfare status, habitat use and social organisation was apparently low forage availability, which in turn was influenced by the combined effects of rainfall and ambient temperature. Accordingly, these are all valuable *welfare alerting* indices (Harvey et al. 2020).

The following inferences may be drawn cautiously, using the robust methodology aligned with the Five Domains Model (detailed in Chapter 6), to consider the probable affects the horses may have experienced during the progression of the study. Inadequate nutrition would have given rise to the mental experience of hunger, motivating horses to search more for food. As their hunger became more severe, and therefore more dominant in their awareness, it would have demotivated them from engaging in rewarding behaviours such as social interactions, and leading them to disperse from their bands in search of food. In turn, this would have reduced positive mental experiences associated with social behaviours, which may have been replaced with additional negative mental experiences such as loneliness and isolation. The evidence is clear that their chronic and severe undernutrition associated with the marked reductions in body condition, led to health compromises, reduced reproductive success and in some cases death. Moreover, they are likely to have had associated negative mental experiences such as malaise, weakness and exhaustion, as indicated by reduced alertness, reduced vigilance, a slow weak gait, and persistent head-lower-than-withers body posture. Larger areas roamed, smaller band sizes and low reproductive rates are therefore potential early indicators of hunger, as these indices were shown to be observable before horses became emaciated.

As described in detail in Chapter 3, it has long been known that home range, habitat use, and band size are related to resource availability (Klingel 1975; Miller 1979; Salter & Hudson 1979, 1982; Miller 1983; McCort 1984; Berger 1986; Rubenstein 1986; Linklater et al. 2000; Girard et al. 2013), and in particular forage availability and quality (Salter 1978a; Salter & Hudson 1979; Berger 1986; Crane et al. 1997; King & Gurnell 2005; Henley et al. 2007; Girard et al. 2013). Further, it is well documented that food is one of the most important factors limiting wild horse population densities with many populations exhibiting density dependence and being limited by their own impact on food resources (Eberhardt 1987; Fowler 1987; Gaillard et al. 1998; Gaidet & Gaillard 2008; Scorolli & Lopez-Cazorla 2010; Dawson & Hone 2012; Scorolli 2021). Impacts of forage availability on social stability have also been previously evaluated (Ginsberg 1989; Kreuger 2008; King et al. 2016).

However, what has not been well documented to date, is the welfare status of these horses, and how that is associated with other parameters evaluated in population and behavioural ecology. In this study, I have suggested that more specifically, it is the mental experience of hunger that is likely motivating these changes. Hunger motivates the animals to travel further to search for food. When this strength of motivation becomes stronger, i.e. hunger becomes more severe, their motivation for engaging in rewarding behaviours reduces, and they trade off social companionship to disperse from their herd and prioritise searching for food above other activities. This in turn, reduces welfare enhancement and positive affective states that arise from these other activities.

As welfare status further declined as a consequence of undernutrition impacting health, negative mental experiences such as fatigue, malaise and exhaustion, then likely motivated horses to reduce activity and remain closer to water sources instead. Horses that died from starvation were sometimes observed to be weak and have increased respiratory effort at their last sightings. Adult mortality resulting from starvation (Berman 1991; Dorj & Namkhai 2013) and young mean ages of death

(Kirkpatrick & Turner 2007) in free-roaming horses are well documented, but the welfare impacts are not. In this study, I found that death from chronic undernutrition occurred very slowly with many horses having severe welfare compromise for many months before they finally died. I was not able to evaluate any behaviours of conspecifics in response to dying horses, but unusual behaviours towards a dying wild horse have been documented (Mendonça et al. 2020).

If body condition score appears to be a key welfare indicator in other populations, the combination of body condition, herd size, reproductive rate and forage availability provides a simple measure of welfare status and risk that can be applied at a population level, with rainfall and ambient temperature being additional indicators of future risk. The link between rainfall, forage availability, quality and body condition score has been well documented in other populations (Berman 1991; Hampson et al. 2011), and body condition score has been shown to be an indicator of populations reaching the carrying capacity of their range (Scorolli 2021).

The forage availability score I used is somewhat subjective, and it has been suggested that food resource quality cannot be determined only by the physical characteristics of the apparent forage (Hampson et al. 2011). Other measures that have been used include biomass measurement (Dawson & Hone 2012) or relative green channel brightness (greenness) of vegetation from digital photographs, which has been shown to have a high correlation between greenness and biomass (Inoue et al. 2015). However, for routine assessment a simple quick measure that can be performed easily by land managers is important. My food availability score is just that, and appeared to correlate well with body condition scores. Nevertheless, evaluating the correlation with green channel brightness from camera trap photographs may be an additional useful validation.

In conclusion, I was able to document the welfare of horses across a range of habitats, and demonstrated how their welfare changed during successive seasons. Welfare compromise in all domains was linked to forage availability and subsequent undernutrition. Horses residing in woodland habitats had the worst welfare compromises even during periods of good rainfall when forage availability was good in other habitats. Welfare status was closely linked to demographic parameters, habitat use and social organisation. The next phase of this research is to assess welfare in larger populations across different geographical regions to evaluate whether similar findings and associations are present (see Chapter 9).

* Note that there were some follow up observations on this population and interventions guided by the findings from this study, briefly described in Chapter 9.

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Thesis outline summarizing chapter titles and contents		
Chapter	**Chapter title**	**Brief description**
1	Introduction: The wild horse controversy & the importance of incorporating animal welfare science in decision making.	Summary of the history of wild horses, their cultural significance, environmental impacts and controversies in wild horse management. Introduces the importance of animal welfare science in wild animal controversies.
2 Published	A Ten-Stage Protocol for assessing the welfare of individual non-captive wild animals: free-roaming horses as an example.	Presentation of a novel conceptual framework that I designed in order to guide a systematic and scientific approach to assessing the welfare of free-roaming wild animals, using the Five Domains Model. Summarizes the principles of interpreting indicators of biological function and behaviour in terms of the mental experiences that those indicators reflect (Stage 1 of my Protocol), and how the Five Domains Model is used for assessing welfare (Stage 2 of my Protocol).
3	Literature review: A review of the species-specific information required to enable assessment of wild horse welfare.	The current status of wild horse knowledge is summarized in a novel holistic and multidisciplinary framework drawing together the relevant literature on horses across each of the four physical/functional domains of the Five Domains Model. It addresses Stage 3 of my Ten-Stage Protocol.
4 Published	Addressing the knowledge gap of gastrointestinal parasitology in free-roaming wild horses in south-east Australia.	An example of the type of detailed original research required for addressing any knowledge gaps identified in Stage 3. This chapter describes a detailed parasitological investigation of 293 faecal samples collected from 6 wild horse populations. It describes results of faecal egg counts, larval cultures and molecular diagnostics.
5 Published	Use of remote camera traps to evaluate animal-based welfare indicators in individual free-roaming wild horses.	A large body of original research investigating for the first time, both the use of remote cameras for identifying individual horses across a range of habitats, and for acquiring data on an extensive range of animal-based welfare indicators. It addresses Stages 4-6 of my Ten-Stage Protocol.
6	Scientific validation of welfare indicators for using the Five Domains Model to grade the welfare status of individual free-roaming wild horses.	Addressing Stage 7 of my Ten-Stage Protocol, this chapter evaluates the scientific evidence linking the described objective measurable/observable welfare indicators to physical/functional impacts in Domains 1-4, and the relationship between those impacts and the mental experiences that are inferred in Domain 5. This concludes with the formulation of a Five Domains Model wild horse specific welfare grading scheme.
7	Dynamic changes in wild horse social organisation and habitat use revealed with remote camera trap monitoring.	With the aim of evaluating traditional ecological metrics alongside welfare status, this chapter describes original research using the remote camera trapping methodology described in chapter 5, to evaluate population dynamics, and temporal and spatial changes in social organization and habitat use of a wild horse population over a 15 month period.
8	The cascading influence of resource availability on the welfare status of wild horses, and association with population demographics, social organization and habitat use.	This original research applied the methodology from all preceding chapters to assess welfare status and changes in welfare status in individual wild horses over a 15 month period, addressing stages 8 -10 of my Ten-Stage Protocol. It further evaluates drivers of change in welfare status and correlations between welfare status, and the population dynamics, social organization and habitat use described in chapter 7.
9	**Conclusions, applications and future directions.**	**Summarizes overall conclusions, and contributions to the fields of wild animal welfare, wild horse ecology and welfare, wild horse management, and general horse welfare. Highlights ongoing work, some of which I have already partially completed that was not included in the main body of the thesis.**

Chapter 9. Conclusions, applications, and future directions

1. Conclusions

1.1 Overall conclusions

I have developed a framework and methods for systematically and scientifically assessing the welfare of free-roaming wild horses, an approach which can also be applied to a range of other free-roaming wild animal species. I have further demonstrated application of these methods to evaluate differences in the welfare of horses in different bands, and changes in their welfare over time. This is the first time that such a study has been reported.

Chapter 1 outlined the contextual relevance of this work and highlighted the need for animal welfare science to be incorporated into ethical, legal and political debates involving wild horse management, as occurred in the management of the study population following the results of my research (Section 1.2 below). The development of my innovative system and delineation of key features detailed in Chapters 2 to 6 provided the scientifically robust foundations for the detailed animal welfare assessment and grading reported in Chapters 7 and 8.

The small study population and its geographical confinement enabled detailed assessment and monitoring of individual horses over a 15 month period. Furthermore, as no management interventions had been implements for several years, this enabled evaluations of unaltered population dynamics, welfare, social organization and habitat use in this population under natural conditions. The detailed welfare assessments allowed key welfare status and risk indicators to be identified and distinguished reliably. Furthermore, the work provided compelling evidence of differences in demography, population dynamics, social organization and welfare status in horses across different habitats. Such differences within the same geographical region and population have not been reported before. This is likely due to previous wild horse studies relying on direct observations, biasing them towards horses in open landscapes. In turn, this may also result in misleading population modelling for horses residing in different habitats.

The overall conclusions are as follows:

(i) Horses residing in woodland habitats had smaller herd sizes, roamed larger areas, had lower foaling rates and higher mortality.

(ii) The welfare status of individual horses varied markedly both spatially across different habitats and temporally. In this study, horses residing in woodland habitats had lower body condition scores, fewer social interactions, and more health compromises than horses residing in open grassland habitats. The welfare status of horses in grassland habitats deteriorated in each season throughout the 15 month period and was also associated with changes in habitat use and social organisation.

(iii) The main determinant of welfare status in this population was forage availability, which was linked to habitat type and the combined effects of rainfall and ambient temperature on grass growth.

(iv) Social organization and habitat use also varied dynamically during the study and was associated closely with welfare status. When forage availability was low, motivated by the inferred mental experience of hunger of increasing intensity, the horses dispersed from their bands and roamed larger distances in search of food. Together with continuing hunger, persistent undernutrition likely led to compromises in health, which eventually gave rise to reduced vigilance and alertness, fatigue, weakness, exhaustion and eventually death. The combination of these direct effects of undernutrition, together with the indirect effects of fewer social interactions and reduced thermoregulatory competency as they became emaciated, led to severe compromises in all welfare domains.

(v) Key welfare status indicators identified were body condition score, with body posture and close companions also being informative. Key indicators of welfare risk were below average rainfall during Spring, Summer and Autumn, habitat type, low forage availability, small herd sizes, low reproductive rates, and utilization of larger areas.

1.2 Follow-up and interventions with the study population based on my research

The severity of welfare compromise and the number of horses impacted was not evident until mid-January 2017, during Period 5 (2nd Summer). This was both because camera trap images were assessed at approximately three-monthly intervals, and because direct observation did not occur between May 2016 and January 2017 as bands

1 and 2 had dispersed and were roaming wider areas in woodland habitats. Once alerted to this situation, I notified the local National Parks & Wildlife Office, and wrote a detailed report regarding each horse and how their welfare had changed since they were last observed.

The situation evoked lively debate within our research group about related moral, legal and political issues. To me, this highlighted why scientific research into wild animal welfare is important as part of informed decision making in any wildlife issues. For some, who valued the agency and autonomy of wildlife as a priority, their initial reaction was that we should not interfere with Nature. This view is echoed in many wildlife publications, such as the documentation of wild horses' reactions to a dying foal (Mendonça et al. 2020). Whereas others' views were grounded in the welfare of individuals and their care. These disparate views can create tension in decision making, even though each reflect a perspective focussed on compassion for animals. As a veterinarian, I am accustomed to having a moral and professional duty regarding the welfare of animals. The severity of welfare compromise, and number of horses impacted, was the worst welfare situation that I had personally encountered during my 20-year career as a veterinarian. So, my reaction was that intervention was required, comprising hand feeding, and possibly euthanasia of the most severely emaciated horses.

However, as a doctoral researcher I had no power in decision making. Due to the socio-politically contentious nature of wild horses, the situation needed to be discussed at various managerial and political levels before any decisions could be made, and I was not authorised to take any actions that were not within the framework of my defined research activities. This created conflict for me between my moral obligations as a researcher and associated confidentiality agreements, and my moral, professional and legal obligations as a veterinarian. However, further discussions resulted in prompt actions involving the RSPCA, who confirmed that intervention was required. Decisions then led to re-instigating a trapping and removal program, that involved feeding the horses until they were 'fit for transport', a process that involved close oversight by an independent equine veterinarian recommended by the RSPCA. I also continued monitoring the horses during this period. I ended the study period reported in Chapters 5, 6 and 7 at the stage that feeding was initiated, given that they were then no longer an 'unmanaged population', however I continued monitoring the population for a further 13 months after that time. The horses remaining in the population gained body condition relatively rapidly and the majority were then trapped, removed, and relocated into private homes about 4-5 months later. Four of the horses came to live in the sanctuary at my farm.

In Chapter 1, I noted that there had been no intervention with this population for about 8 years, due to the contentious nature of wild horse management. The results of my research resolved this issue, enabling management to be initiated. This clearly illustrates the role of animal welfare data in assisting in debates and resolving controversies. In future work I will report on how this management changed the welfare of those horses I continued monitor.

1.3 Implications of research findings

There are direct implications of my work for both population modelling and wild horse management.

Implications for population modelling

To date, population modelling has generally used data from ecological studies that were performed in open habitats by directly observing horses, usually geographically distant from the populations being modelled. These studies, summarised in Chapter 3, generally show high reproductive rates, low adult mortality, and in the absence of predators, low foal mortality. This has led to suppositions that all wild horse populations grow rapidly. My study results suggest that this may not be the case, as reproductive rate and mortality can vary greatly, even between bands residing in different habitats in the same population. Accordingly, caution needs to be exercised when undertaking projective modelling of wild horse populations with regard to: (i) using data specific to representative bands across a population, and (ii) incorporating forage availability and carrying capacity of a region. In addition, I have shown that in mixed habitats large proportions of horses may reside in woodland areas and not be directly observable. This means that estimating population densities by direct observations from the ground will likely underestimate the number of horses in these habitats.

Implications for wild horse management

Wild horse management programs should incorporate knowledge of population growth rates in different management regions, as highlighted above. I propose that the welfare status of horses should also be considered. This may give rise to important ethical issues that have received little consideration to date. As highlighted by the events described in section 1.2, better policies need to be put in place to guide management agencies about how to respond to such events, to both increase transparency around their decision making, and to ensure prompt responses to minimise further deterioration in horses' welfare.

Some populations may grow more slowly than previously assumed, leading some to conclude that they are likely to be self-regulating and not require intervention. However, horses within those populations may be experiencing severe welfare compromise of varying duration. Without predation, wild horse populations are regulated by resource availability. The notion held by many horse advocates of horses being left 'wild and free', taken overall, is unrealistically idealistic. The reality, as I have demonstrated, is that unmanaged populations can have a high proportion of them experiencing severe welfare compromises, often for long periods of time.

There is debate about the role of dingoes in regulating wild horse populations in Australia, as discussed in Chapter 3. In my study I often observed different dingoes directly, as well as capturing them on camera traps in all the regions where horses resided. I did not witness any evidence of predation pressures, and on occasions observed a dingo walking within a band of horses, and even walking in between a mare and her recumbent sleeping foal, with no reaction from any band members. On another occasion I witnessed play behaviour between a young dingo and a yearling horse, again with no adverse reactions from the dam. These are not behaviours that would be expected if predation from dingoes had been experienced by these horses. This, combined with the long duration of emaciation exhibited by some horses before they died, suggests that it is unlikely that dingoes played any significant role in the regulation of this population. However, the situation in other populations merits investigation.

These findings suggest that if severe welfare compromises in wild horse populations, and slow deaths by starvation, are to be minimised, then human intervention to manage population densities is required. Leaving populations unmanaged or using methods to create natural boundaries to establish self-stabilising populations, will very likely result in severe welfare compromises, eventually impacting large proportions of such populations. Optimal management of wild horse populations is another topic that is touched in sections 2.2 to 2.4.

2. Contributions of this work

My thesis includes a range of novel areas of work that contribute to several fields:

2.1 Contributions to the field of wild animal welfare

It provides contributions to the broader area of wild animal welfare in advancing knowledge of the practical assessment of the welfare of free-roaming wild animals. I have shown that assessing welfare adds a new dimension to the knowledge and understanding of wild animals, providing new insights into the *quality* of their lives, compared to traditional population ecology studies that focus on *quantity* of life metrics such as survival and reproductive success. This thesis has been written as a comprehensive guide on how to apply the Five Domains Model to wildlife, to enable other researchers to assess the welfare of free-roaming wild animals in their natural settings. This will enable welfare to be assessed together with other parameters, thereby providing fresh conceptual frameworks to guide wildlife research, with potential additional applications to wild animal species in captivity.

2.2 Contributions to the field of wild horse ecology and welfare

Outlined here is a step-by-step guide on how to assess the welfare of free-roaming wild horses, opening new avenues for research. As highlighted in Chapter 2, welfare status is an obvious missing part in wild horse studies which may mean that important aspects of horse health, behaviour and ecology are missed, or misinterpreted. For example, in one study of horses in outback Australia (Hampson 2010a), distances travelled were measured using global positioning systems (GPS) attached to collars. Some horses were recorded travelling up to 55km (walking for 12 hours) to reach water. It was concluded, and other authors have made the same suggestions, that these horses had genetically adapted to arid conditions. They further discuss survival of horses and donkeys subjected to water deprivation, and how experimentally donkeys were able to drink 24-30L in 2-5 min to restore their water deficit. They conclude that this must represent genetically driven metabolic adaptation. However, at no point was the welfare status of these equids discussed, nor the severity of thirst that they would have experienced to motivate them to travel such long distances to reach water, nor to drink such large volumes of water at once. Ecological studies tend to emphasise survival and reproductive success but give virtually no attention to the welfare status of animals. The methodology that I have presented allows this deficiency to be corrected.

2.3 Contributions to wild horse management

In Chapter 1 I discussed a range of different types of wild horse populations globally, where substantial welfare problems had been recognised once they had become severe and widespread. The routine monitoring of the welfare of wild horses should be incorporated into any wild horse monitoring program. This would help to provide earlier indicators of such issues arising and could help to inform management decision-making. Use of key welfare status and risk indicators identified (detailed in section 1.1) can provide a simple way for land managers to assess aspects of welfare across populations. For example, with representative sampling, routinely assessing forage availability and the proportion of horses with body condition scores < 3 would be informative. As described in section 1.2 I also identified a need for guidelines and policies regarding actions to be taken when welfare compromises are identified.

Contributions to advancing knowledge related to general horse welfare

Finally, there may be broader implications for the welfare of domestic horses. In attempts to optimise domestic horse welfare, it is commonplace to look to the natural environment, diet and behaviour of wild horses (Veasey et al. 1996). However, as the welfare of wild horses has never been systematically and scientifically studied before, features that are used may be taken from wild horses that are experiencing welfare compromises of various degrees.

For example, already mentioned in section 1.2 was the interpretation of the GPS study of distances walked in arid conditions without any reference to the welfare status of the horses (Hampson et. al 2010a, 2010b). Another example is that of extensive studies on wild horse hooves (discussed in Chapter 3). The conclusion was that pathology was common, and therefore it was not appropriate to judge wild horse hooves as an optimal morphometric model for hoof care of domestic horses (Hampson 2011). However, they were post-mortem studies, so even the most basic of welfare indicators related to foot health, that of lameness, could not be evaluated.

This issue of potentially inappropriate benchmarking from wild to domestic horses has been highlighted before (Waren 1997), with recommendations that there is much to be learnt about domestic horses from the study of wild horse populations. Findings from wild horse welfare studies such as those described here are likely to offer new insights into many aspects of the husbandry, health and behaviour of domestic horses.

3. Future directions

3.1 Extension of wild horse welfare assessments across larger populations

Since the welfare and ecology data presented in this thesis were only from a single small population, to strengthen validity and broaden applicability, this work should be repeated in larger horse populations, and across larger and varied landscape. With this aim, I have already carried out further field research collecting data to investigate the differences in horse populations across different habitats.

I chose five further research sites across different habitats in the Australian Alps in both NSW and Victoria (Figure 1). These sites were chosen to be as representative as possible of many varied habitats found across the Australian Alps. Cooleman Plain is a large open grassland habitat in northern Kosciousko National Park (Figure 2a), Tin Mines is a sub-alpine heathland habitat in southern Kosciousko (Figure 2b), the Lower Snowy is a mountainous eucalyptus forested area either side of the Lower Snowy River running all the way across the border into Victoria (Figure 2c), Cowombat flat is a smaller open grassland area surrounded by eucalyptus forest, bridging the border of NSW and Victoria (Figure 2d), and Bogong High Plains is an open grassland/heathland alpine habitat in Victoria (Figure 2e).

Data were collected at all sites during Spring/Summer 2017/18 from 3 days of direct observations performed 1 – 2 months apart, and from 20 camera traps deployed at each site (except Bogong High Plains) for a 2-month period, collecting a combination of still images and video clips. Data were collected from approximately 600-700 horses. Detailed analysis of the data, using the same methodology described in Chapters 5, 7 & 8 is the next phase of this research.

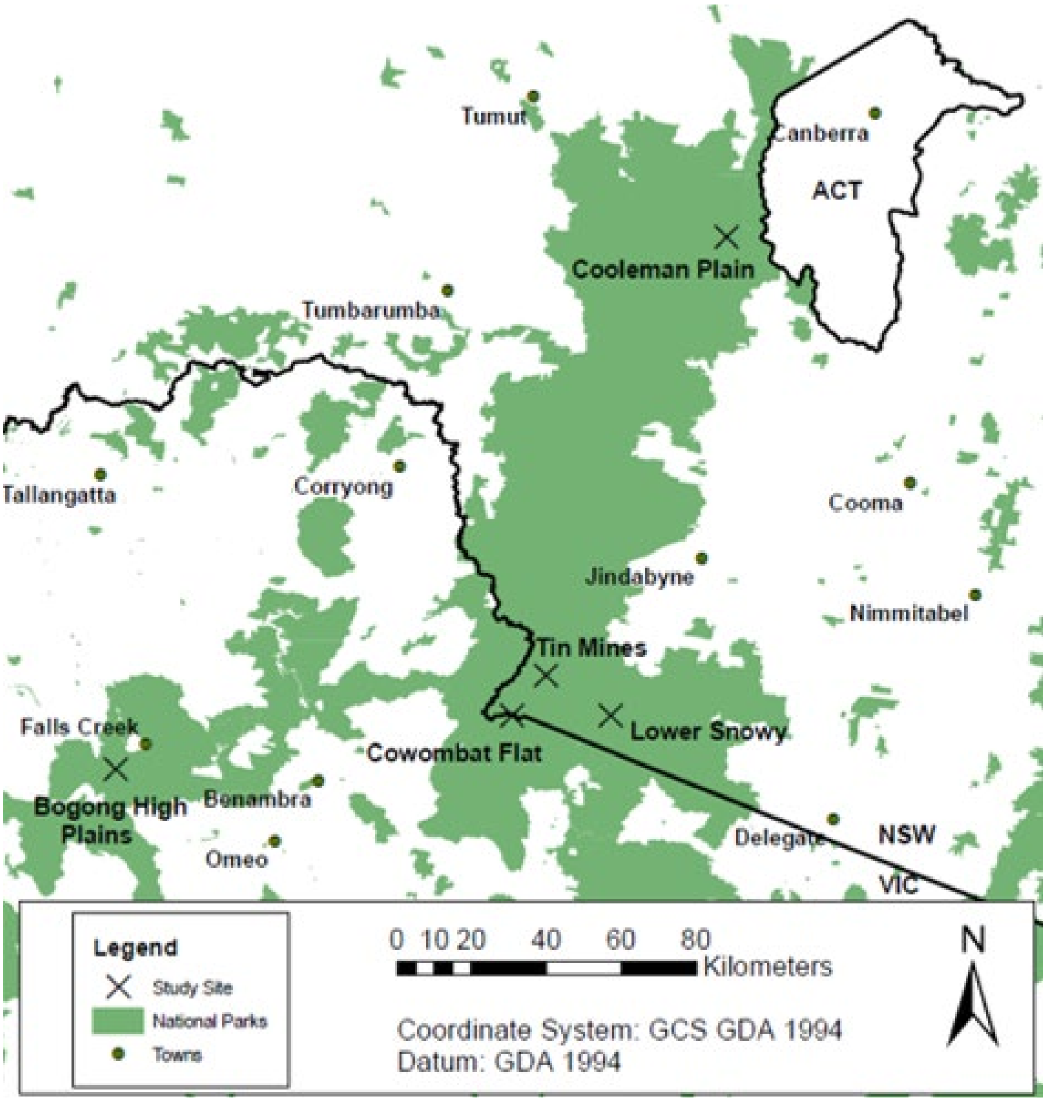

Figure 1. Further research sites from which I have collected data from in the NSW and Victorian Australian Alps, for ongoing analysis. Adapted from Harvey et al. 2019.

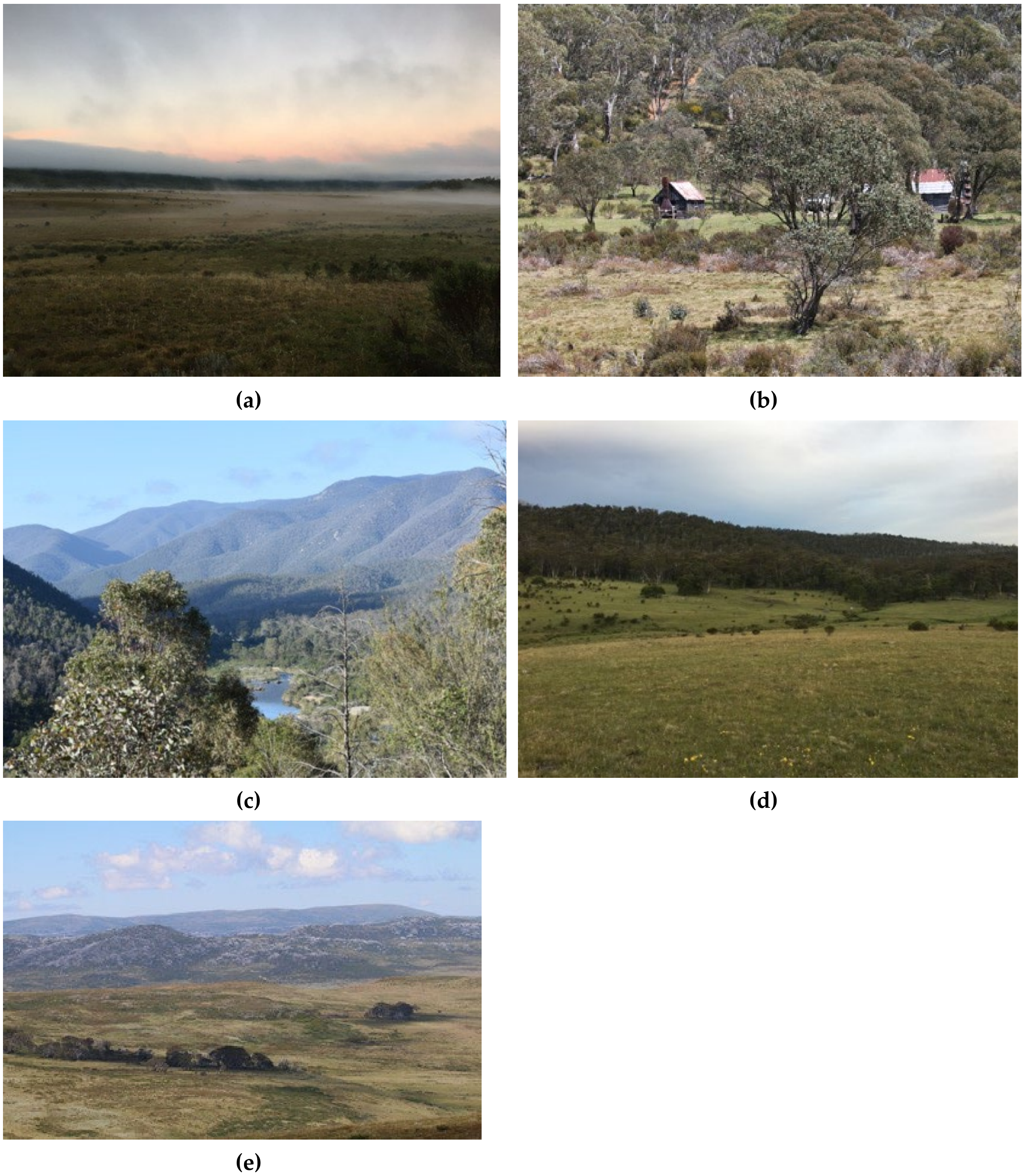

Figure 2. (a) Cooleman Plain, northern Kosciousko National Park, NSW. **(b)** Tin Mines, southern Kosciousko National Park, NSW. **(c)** The Lower Snowy River, southern Kosciousko National Park, NSW. **(d)** Cowombat Flat, Alpine National Park, Victoria. **(e)** Bogong High Plains, Alpine National Park, Victoria.

3.2 Welfare impact assessment of wild horse management operations

If interventions in wild horse populations occur, then the welfare impacts of these interventions also need to be minimized. Despite wild horse management operations occurring worldwide, to date, there are no published reports on welfare assessments and outcomes of such operations. To aid in decision-making regarding management methods, and protocols for carrying out those methods, data on welfare outcomes is needed. In my role on the Scientific Advisory Panel for wild horse management in Kosciousko National Park, this was one of my key recommendations. Details of these can be found in our report in Appendix 7, where I was responsible for all the welfare related recommendations. As part of this work, I have also designed a welfare assessment protocol to facilitate assessing welfare impacts associated with passive trapping and relocation of horses. The newly released management plan mentioned in Chapter 1 incorporates much of this advice, and therefore this is an area that would benefit from ongoing research.

3.3 Exploring methods for reducing population growth rates

There is a need for more research into non-lethal methods of managing wild horse populations. Lethal management is socially unacceptable to many people and controversies about this continue. Whilst there is a wealth of information on the use of immuno-contraceptives in the USA (reviewed by Kirkpatrick 2011; Naz & Saver 2016), they are not practical for managing all populations, have some disadvantages and challenges to their use in most Australian settings (Hobbs & Hinds 2018).

Problematically in Australia, immunocontraceptives reportedly useful in wild horses are not available and potential alternatives are costly. I instigated pilot trials in captive wild horses, using a commercial gonadotropin releasing hormone (GnRH) vaccine, labelled for prevention of boar taint in pigs. It is available in Australia, is inexpensive and has undergone some trials in domestic research horses (Imboden et al. 2006; Botha et al. 2008; Schulman et al. 2013). To date most immunocontraceptives studies have been on mares, but GnRH vaccines can also reduce fertility in stallions (Malmgren et al. 2001; Stout & Colenbrander 2004; Turkstra et al. 2005; Janett et al. 2009). I incorporated mares, stallions and young colts in my trials. In brief, my pilot studies suggested some efficacy of variable duration, but unfortunately not likely to be sufficient for successful use in wild horses. Full details are currently in preparation publication.

More recently, the use of a novel intrauterine device (Gradil et al. 2021) has been proposed for use in wild horses and may be a promising alternative. I am currently collaborating with the authors of this study with the aim of trialling these devices in some wild horses in Australia.

3.4 Improving welfare outcomes in wild horse rehoming

Rehoming is a popular management method amongst the community, although rehoming opportunities are limited. However, there are welfare challenges faced by rehomed brumbies, including issues with husbandry, human interactions during the domestication process, and difficulties with healthcare provision until they are well handled. I have started working in a number of these areas to optimise welfare outcomes of rehomed horses. One such area relates to stallions that present some unique welfare challenges. These include being housed in isolation from other horses, being housed in small areas often for prolonged periods, restrictions on behaviour, and sometimes being without shelter or optimal underfoot substrate. To progress them to a more optimal lifestyle in captivity they need to be gelded, a surgery that carries higher risks in mature stallions. To do this, they first need to be handled well enough to enable anaesthesia to be safely induced. Anaesthesia is also more complex in these minimally handled horses. Many people do not have the expertise, facilities, or finances required for this process. Consequently, older stallions are the demographic that most commonly do not find homes and get slaughtered in knackeries. I have written about the welfare issues associated with this in Horses & People magazine (Appendix 4).

Development of handling, anaesthetic and surgical protocols for optimizing welfare outcomes associated with gelding stallions

With the assistance of colleagues, I have developed strategies for handling/training for intramuscular and intravenous injections to be given, anaesthetizing and gelding wild stallions to achieve optimal welfare outcomes. An additional aim is to minimize the time between removal from the park and gelding, thereby reducing untoward welfare consequences of housing stallions (mentioned above). I have written a preliminary article regarding this work (Appendix 3), but subsequently I have now gelded over 100 stallions and further refined our handling and anaesthetic protocols. More recently I have developed a dedicated facility to do this (Figure 3), where I can also provide educational training in the methodology.

Figure 3. (a-b) The facilities I have developed for housing and training wild stallions. **(c-d)** Preparing a wild stallion for gelding whilst causing minimal anxiety, by using learning theory to habituate him to standing calmly in a crush, and then to human touch, prior to preparing for injections.

I also use a newer technique for gelding, the Henderson method (Appendix 3), which I have found reduces surgical complications such as haemorrhage and swelling. Whilst there are some publications about this technique (Owens 2018; Hinton et al. 2019; Racine et al. 2019), most of them did not include many stallions over four years of age. I have now gelded over 100 brumby stallions and colts using this method, building up a large dataset on mature stallions using this technique. The intention is to publish this work and also to educate more veterinarians on the handling, optimal anaesthetic protocols, and surgical techniques of gelding mature brumby stallions.

4. Concluding comments

In summary, the work I have done on the scientific assessment of the welfare of free-roaming wild horses paves the way for numerous research opportunities that may provide new insights into the lives of free-roaming wild animals and inform their management. It also highlights that welfare status is an important missing piece in many areas of wildlife research, and when included may change some interpretations of previous research. Regarding wild horses, I have also highlighted how animal welfare science needs to be incorporated into all areas from free-roaming horses to management programs, and to those horses that continue their lives in captivity. Finally, the initiation of research into the welfare of free-roaming horses provides the opportunity for new benchmarks by which domestic horse welfare may be assessed.

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Appendix 1

PERSPECTIVE NO. 105

FROM FELINE MEDICINE TO SAVING AUSTRALIA'S BRUMBIES

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Harvey, A. Perspective, No. 105. From Feline Medicine to Saving Australia's Brumbies. CVE *Control & Therapy Series*, 2014, 275, 39-46.

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Appendix 2

A Quest to Research the Welfare and Social Dynamics of Wild Australian Brumbies

Brumby stallion, Cooleman Plain, Ksciusko National Park, New South Wales. Photo by Dr Andrea Harvey.

WORDS BY Dr Andrea Harvey
BVSc DSAM(Fel) DipECVIM-CA MRCVS MANZCVS (Animal Welfare)
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Dr Andrea Harvey is a veterinary specialist and animal welfare scientist, who is currently writing up her PhD research on the welfare and social dynamics of wild brumbies in New South Wales and Victoria.

She grew up on the Island of Guernsey in the Channel Islands, she trained as a veterinarian and became a veterinary specialist in small animal and feline internal medicine.

So, how does a small animal vet from the United Kingdom end up doing a PhD on the welfare of wild Australian brumbies?

Harvey, A. A Quest to Research the Welfare and Social Dynamics of Wild Australian Brumbies. *Horses and People Magazine*, 2019, September-October, 29-35.

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Appendix 3

PERSPECTIVE + MAJOR WINNER!

NO. 138

Castrating mature brumby (wild horse) stallions with the Henderson technique: lessons learnt & 'top tips'

Andrea Harvey

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MAJOR PRIZE awarded to Kim Bensch

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Photos and videos courtesy of Joe and Lauren Hughes, 4BP Horses Australia.

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Harvey, A., & Bensch, K. Perspective, No. 138. Castrating mature brumby (wild horse) stallions with the Henderson technique: lessons learnt & 'top tips'. CVE *Control & Therapy Series*, 2018, 291, 43-52.

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End-of-life Decisions and the Problems With Horse Slaughter

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frontiers in Veterinary Science

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"Feelings and Fitness" Not "Feelings or Fitness"–The *Raison d'être* of Conservation Welfare, Which Aligns Conservation and Animal Welfare Objectives

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Increasingly, human activities, including those aimed at conserving species and ecosystems (conservation activities) influence not only the survival and fitness but also the welfare of wild animals. Animal welfare relates to how an animal is experiencing its life and encompasses both its physical and mental states. While conservation biology and animal welfare science are both multi-disciplinary fields that use scientific methods to address concerns about animals, their focus and objectives sometimes appear to conflict. However, activities impacting detrimentally on the welfare of individual animals also hamper achievement of some conservation goals, and societal acceptance is imperative to the continuation of conservation activities. Thus, the best outcomes for both disciplines will be achieved through collaboration and knowledge-sharing. Despite this recognition, cross-disciplinary information-sharing and collaborative research and practice in conservation are still rare, with the exception of the zoo context. This paper summarizes key points developed by a group of conservation and animal welfare scientists discussing scientific assessment of wild animal welfare and barriers to progress. The dominant theme emerging was the need for a common language to facilitate cross-disciplinary progress in understanding and safeguarding the welfare of animals of wild species. Current conceptions of welfare implicit in conservation science, based mainly on "fitness" (physical states), need to be aligned with contemporary animal welfare science concepts which emphasize the dynamic integration of "fitness" and "feelings" (mental experiences) to holistically understand animals' welfare states. The way in which animal welfare is characterized influences the way it is evaluated and the emphasis put on different features of welfare, as well as, the importance placed on the outcomes of such evaluations and how that information is used, for example in policy development and decision-making. Salient examples from the New Zealand and Australian context are presented to illustrate. To genuinely progress our understanding and evaluation of wild animal welfare and optimize the aims of both scientific disciplines, conservation and animal welfare scientists should work together to evolve and apply a common understanding of welfare. To facilitate this, we propose the formal development of a new discipline, Conservation Welfare, integrating the expertise of scientists from both fields.

Keywords: conservation welfare, animal welfare assessment, wildlife conservation, environmental ethics, wild animal welfare

INTRODUCTION

Conservation biology and animal welfare science are both multi-disciplinary fields that use scientific methods to address concerns about animals (1, 2). Both also require decision-making in complex ethical milieu and in the face of significant uncertainty (3, 4). While animal welfare science has traditionally focussed on the welfare of domestic animals living under human care, there is increasing recognition of the potential for human activities to also impact on the welfare of wild animals (5, 6). In particular, various human activities aimed at conserving populations, species, ecosystems and, ultimately, biodiversity can influence the welfare of individuals and groups of wild animals (4, 7, 8).

Briefly, there is growing evidence of animal welfare impacts associated with *in situ* conservation activities, such as habitat management, field research, and management of rare and overabundant native animals, as well as, of invasive species [e.g., (9–27)]. Likewise, *ex situ* conservation activities including captive breeding, holding animals indefinitely in zoos as "insurance populations," wildlife rescue and rehabilitation, reintroductions and research on captive animals can influence animal welfare [e.g., (2, 13, 28–37)].

Such conservation activities are strongly supported by many in society, reflecting the value placed on concepts, such as "naturalness" and "biodiversity," the continuing existence of current species and retention of "evolutionary potential" (38–41). However, activities impacting detrimentally on the welfare of individual animals may ultimately threaten their survival and fitness and thus the viability of valued populations and species [e.g., (2, 9, 12, 15, 21, 34, 42, 43)], thereby negating some of the intended conservation benefits (3). In addition, growing public awareness of, and concern about, the welfare of individual wild animals necessitates improved transparency and justification of conservation activities (1, 3, 41, 43–46). Thus, the growing urgency for conservation brings with it an equally urgent need for conservation and animal welfare scientists to engage in genuine discourse in support of collaborative research to underpin welfare-focused conservation practices.

Animal welfare is a difficult concept to define but the term is now widely used to reflect how an animal is experiencing its life (47, 48). Dominant theoretical models for understanding animal welfare have focussed on the animal's physical state or biological function (Biological function orientation), the mental experiences, both positive and negative, the animal may have as a result of its physical state/biological function (Affective state orientation) or the naturalness of its environment and/or its ability to express natural behaviors (Naturalness or Natural living orientation) (49, 50). It is now widely agreed within the field of animal welfare science that no single orientation on its own is sufficient and that components of all three theories must be integrated to holistically understand and scientifically assess animals' welfare states (48, 50, 51). Further discussion and illustrations of the limitations of focussing on only one aspect of animal welfare, in the context of conservation, are presented below in The Need for Common Language and Understanding Relating to Wild Animal Welfare.

Conflicts between those working to achieve the goals of conservation biology and those aiming to safeguard the welfare of individual wild animals are apparently on the rise (20, 45, 52). As noted, this may be because of the growing urgency and thus volume and range of conservation research and practices, as well as growing public awareness of conservation activities (3, 12, 43) and, more generally, of animal welfare [e.g., (53–56)]. This is exemplified by the moratorium imposed by the Tasmanian state government in 2000 on hot-branding as a method for identifying individual elephant seals (*Mirounga leonina*) for research purposes on Australia's Macquarie Island after media attention and public outcry about perceived animal welfare impacts (3, 57). In the scientific arena, growing concerns about the effects of conservation activities on wild animal welfare may also be attributed to our increasingly detailed, robust and evidenced-based understanding of what animal welfare is and how it can be evaluated (48, 58–62) (see below).

Such conflicts have often been attributed to incompatible ideologies [e.g., (1, 38, 52, 63–65)]. For example, McMahon et al. (20) suggested that prioritizing concerns for the welfare of individual animals, as "animal welfare advocates" seek to do, stymies the generation of scientific knowledge critical to stemming the extinction of species and the consequent loss of ecosystem services and evolutionary potential. However, the position often cited for "animal welfare advocates" is actually one of animal rights, an ethical stance that no amount of benefit from conservation activities can justify any level of individual animal suffering [e.g., (20, 39, 66, 67)].

In contrast, the role of animal welfare scientists in the conservation context is to use scientific principles and methods to evaluate impacts on the welfare of animals, both positive and negative and at individual and population levels, to inform ethical conservation decision-making and practice (42, 68–70). Accordingly, they advocate approaches to achieving conservation goals that minimize negative welfare impacts [e.g., (64, 70–72)] and, where appropriate and possible, realize or maximize any welfare benefits (44, 46, 61). In some cases, animal welfare scientists may use the outcomes of scientifically robust evaluations to recommend that an activity not proceed if the predicted or actual welfare costs are considered to outweigh the likely conservation benefits (42, 44, 70, 73, 74). For example, application of an identification marking method that would cause significant tissue injury, pain or behavioral alteration and that would not facilitate animal identification at a level (individual or group) or distance or for a duration required to achieve the objectives of the research programme would be discouraged (44).

This kind of informed decision-making is equally recommended by conservation scientists [e.g., (2, 20, 21, 43, 75, 76)]. Thus, the starting positions and goals of conservation biology and animal welfare science do not appear to be inherently incompatible. Given that activities impacting detrimentally on the welfare of individual animals often also hamper achievement of some conservation goals (2, 43, 77) and that societal acceptance is imperative to the continuation of conservation activities (3, 20, 44), it is clear that the best outcomes for both disciplines will be achieved through collaboration and knowledge-sharing. Despite this recognition, cross-disciplinary information-sharing and collaborative research and practice in conservation are still relatively rare [e.g., (2, 20, 34, 78)], with the exception of the zoo animal context (see below), so that substantial scope for synergy between the activities of conservation and animal welfare scientists remains.

The aim of this paper is to summarize key points developed by a group of conservation and animal welfare scientists discussing scientific assessment of wild animal welfare. On the basis of those discussions, we propose the formal development of a new discipline, integrating the expertise of scientists from both fields, to progress our understanding and evaluation of wild animal welfare and optimize the aims of both disciplines: this is "Conservation Welfare," an appellation coined in the World Association of Zoos and Aquariums' Animal Welfare Strategy document in 2015 (46).

PARTICIPANTS AND WORKSHOP

Workshop participants were invited from those attending the third International Compassionate Conservation Conference in Sydney, Australia in November, 2017. The over-arching purpose of the 1-day workshop was to explore the various roles of science in "Conservation Welfare." Fourteen participants from Australia, New Zealand and the United Kingdom attended, and the workshop was facilitated by the two lead authors. The participants were animal welfare scientists, conservation scientists, scientific representatives of non-governmental animal welfare organizations, wildlife veterinarians and wildlife rehabilitators.

The workshop comprised a series of group discussions exploring the meaning of animal welfare and how it might be assessed, as well as, the ways in which conservation activities might impact upon wild animal welfare. In addition, the challenges associated with understanding wild animal welfare and integrating that kind of understanding into conservation policy and practice were explored. Following the workshop, the lead authors distilled from those discussions key principles for optimizing the aims of both scientific disciplines. The dominant theme emerging from the workshop was the need for a common language to facilitate cross-disciplinary progress in understanding and safeguarding the welfare of animals of wild species.

THE NEED FOR COMMON LANGUAGE AND UNDERSTANDING RELATING TO WILD ANIMAL WELFARE

There are two key reasons why conservation and animal welfare scientists should work together to evolve and apply a common understanding of welfare as it pertains to animals of wild species. First, the way in which animal welfare is conceived influences the way it is evaluated and the emphasis put on different features of welfare. This is important because rigorous, defensible and transparent assessment of "animal suffering" is key to making informed and ethical decisions in conservation practice (2, 16, 20, 69, 70). Secondly, the conception of animal welfare influences the importance placed on the outcomes of such evaluations and how that information is used going forward, for example, in policy development and decision-making.

CONCEPTION OF ANIMAL WELFARE INFLUENCES ITS EVALUATION AND EMPHASIS–"FITNESS"

The theoretical characterization of animal welfare directly influences both the approach to its assessment and the dimensions or features emphasized in such evaluations. Specifically, what welfare is considered to be dictates the indicators measured, the level of measurement (e.g., individual vs. population level), the aspects of welfare prioritized and how the data are interpreted (61). This can be illustrated by examining the apparently different characterizations of welfare in conservation and animal welfare sciences and the practical implications of these differences.

Logically, current conceptions of welfare in conservation biology often appear to align to the immediate goals of the discipline, that is, to keep genetically valuable individuals alive and reproducing and to maintain genetic diversity within and between populations [e.g., (21, 26)]. In accordance with this, welfare is often evaluated at the population level and using variables chiefly related to the physical state or biological function of the animals. At the most general level, welfare may be extrapolated from measures of survival and reproductive success, i.e., "*fitness*" [e.g., (79–86)].

Other conservation evaluations focus on variables that reflect the animals' physiological or health status in finer detail, i.e., specific attributes of their "*fitness*," and may be undertaken at the population or individual level, depending on the purpose and on practical considerations (44). Examples include body condition, weight, coat, plumage or skin condition, injury or pathology, altered gait or the occurrence of abnormal behaviors [e.g., (26, 84, 87–90)]. Likewise, blood, saliva and fecal components indicating nutritional status or energy reserves, immune or reproductive function or "stress" may be evaluated [e.g., (13, 91–94)]. In rehabilitation, translocation and reintroduction contexts, when animals are under closer human control for longer periods, clinical examinations may be performed to evaluate the health status and potential survivability of rescued, captured or captive wild animals [e.g., (35, 95–100)]. Similarly, in the research context, the impacts of manipulations, such as identification marking or capture are commonly evaluated using measures of physical status, such as injury severity or healing, changes in body weight, condition or temperature, energy expenditure, behavior or survival estimated by likelihood of re-sighting/recapture [reviewed by (11, 22, 101–106)].

More detailed evaluations of wild animal behavior are generally undertaken to understand features of the social and ecological interactions of animals, as well as the impacts of human interventions or changes to the ecosystem on fitness and ecosystem function, rather than to understand their welfare state *per se* [e.g., (2, 9, 34, 107–112)]. Notable exceptions are the detailed studies of behavior often undertaken in the zoo context for the explicit purpose of assessing welfare state [e.g., (113–116)] and systematic evaluations of wild behavior to improve the efficacy of strategies to control invasive species [e.g., reviewed by (117–119)].

Conception of Animal Welfare Influences Its Evaluation and Emphasis–"Feelings"

In the field of animal welfare science, welfare is generally conceptualized as a property of an individual animal. More specifically, welfare is a property of individuals of species considered to have the capacity for both pleasant and unpleasant mental experiences, i.e., experiences that matter to the animal itself; this capacity is otherwise known as sentience (58, 120–122). Such experiences are generated by processing of information about the animal's internal physical state and/or its external circumstances and are variously called affects, affective states, emotions or "feelings" (48, 51). Thus, while welfare can be assessed at the population level (as routinely occurs in assessment of farm animal welfare), the underlying assumption is that population-level indicators reflect the mental experiences of the various individuals within the group (62, 123, 124), rather than a population collectively possessing welfare *per se*.

In accordance with this conceptualization, there is now wide acknowledgment in this scientific field of the importance of animals' mental experiences as the feature of ultimate relevance for understanding their welfare (48, 58, 121, 122, 125). Related to this is recognition of the importance of assessing the potential for both negative (unpleasant) and positive (pleasant) experiences to holistically understand welfare state at any point in time (58, 59, 61, 62, 126). Thus, animal welfare evaluations aim to interpret the indicators of physical/functional state, i.e., biological function or "fitness," in terms of the mental experiences that those indicators are likely to reflect, i.e., "feelings."

In support of this approach, there is growing understanding of the neurophysiological bases of mental experiences, such as thirst, hunger, pain, breathlessness, nausea, fear and others, as well as evidence of the links between measurable indicators of physical/functional states and the occurrence of such mental experiences in some non-human animals [e.g., (126–132)]. Importantly, affective states can also influence physical/functional states; thus the two are inextricably and dynamically inter-related and should be interpreted as such (48, 50, 133). For example, it is well-established that dairy cattle, pigs and poultry which are more fearful of their human handlers exhibit lower productivity and/or reproductive success than their less fearful cohorts (134). This advancing biological understanding and evidence facilitates cautious interpretation of the kinds of data already collected in some conservation research as reflecting the mental experiences of the animals and thus their welfare state, e.g., hydration status or changes in body condition (94) as indicators of thirst and hunger, respectively (132).

Conception of Animal Welfare Influences Its Evaluation and Emphasis–"Feelings" and "Fitness"

Framed in this way, the limitations of using survival and reproductive success as proxies for welfare are clear. Simply surviving until the point of evaluation does not guarantee acceptable or desirable welfare, as animals can survive despite experiencing chronic unpleasant states (13, 135–138). This recognition may influence decisions between lethal and non-lethal population control strategies or attempts at rehabilitation and release vs. euthanasia for rescued wildlife [e.g., (15, 17, 18, 23, 24, 28, 35, 139, 140)]. Likewise, measures of survival and reproductive fitness are not useful for evaluating welfare impacts when animals are intentionally killed for conservation purposes [lethal control of invasive species or culling overabundant or nuisance native animals: e.g., (18, 25, 32, 73, 141–143)], or when they die due to unintended effects of conservation activities [e.g., (9, 15, 72)].

Alternatively, although low reproductive success might reflect physiological states that align with poor welfare, such as malnutrition or severe stress [e.g., (13, 15, 17, 144)], failure to reproduce, *per se*, is not necessarily indicative of a specific negative experience that would compromise welfare (4) and vice versa (137). This point might be important when considering the welfare both of valued animals that are not reproducing [e.g., cheetahs in captivity; (145, 146)] and when reproductive control is used to manage wild populations [e.g., (23, 147)].

Likewise, a sole focus on biological function can lead to interpretation of "normal" health or function as sufficient evidence of good or acceptable welfare or lead to emphases on "inputs" (i.e., good husbandry or care) that may not translate into acceptable "outputs" (i.e., good welfare) (34, 46, 133, 148). To illustrate these risks, many domestic farmed animals have good biological function and are highly productive, in terms of survival, growth and reproduction, but have poor welfare due to limited opportunities for normal behavioral expression and the attendant unpleasant mental experiences (135, 136, 149). Healthy wild animals may have unpleasant experiences too. Examples include significant anxiety or fear during capture, captivity or after transfer to a new location or social group (2, 12, 34, 125), or less well-understood experiences, such as loneliness, boredom or frustration in captive environments (61, 150, 151). Focussing only on indicators of physical status or biological function can also result in failure to look for or recognize indicators of the wide variety of unpleasant experiences that can compromise welfare (48, 152). Related to this, there is also a danger that the theoretical underpinnings of welfare evaluations may be conceived, *post-hoc*, to fit the limited data that can currently be collected in practice, rather than the preferred strategy of the established conceptual framework of welfare guiding the approaches to data collection and the identification of gaps to advance knowledge for future assessments (153).

In the context of killing, a focus on biological function may lead to over-estimates of welfare impacts. One commonly held view amongst animal welfare scientists is that death *per se* does not equate to poor welfare [cf. (154)]; an animal's experiences of its welfare state exist only while it is alive and able to consciously perceive features of its internal state and/or the world around it (42, 48, 51). Thus, negative welfare impacts take the form of unpleasant experiences, such as pain, breathlessness, nausea or fear before the irreversible loss of consciousness [i.e., the point at which experiences are no longer possible (152)]. Measures of physical state (i.e., behavior or physiology) made after this point no longer reflect conscious mental experiences and, although they are often aesthetically unpleasant to observe, they do not reflect welfare state (25, 32).

Previously Proposed Concepts to Unite Conservation and Animal Welfare Sciences

Several authors have previously indicated the need for a common language to unite conservation and animal welfare sciences and have attempted to identify common metrics to do so and to more clearly delineate the point at which biological fitness and welfare converge. "Stress" was proposed as that unifying concept, and measurements of stress have been widely used to evaluate the fitness and welfare impacts of human-generated conditions and procedures on animals of wild species [e.g., (42, 75, 78, 91, 92, 94, 155, 156)]. Stress has usually been characterized according to physiological responses, primarily activation of the hypothalamic-pituitary-adrenal (HPA) axis elicited by external threats or disruptions to internal conditions, i.e., homeostasis (157, 158).

As such, measurements of stress are often used to infer how well the animal is "coping" with its environment (159). But the affective significance of such stress, coping or lack of coping and thus the relationship to welfare state, is not clear (160). For instance, "stress responses" can also occur in situations actively sought out by animals and which would intuitively appear to be related to positive experiences e.g., hunting, mating (91). In addition, the responses and responsiveness of the HPA axis can change depending on the pattern and duration of the stressors [e.g., (92, 113, 161)], stress may have negative (e.g., reduced reproduction) or positive (e.g., escaping a predator) consequences for fitness (75), and behavioral strategies may be used instead to "cope" with conditions that are nonetheless unpleasant to the animal, e.g., hiding, expressing abnormal repetitive behaviors (114, 162).

In response to these limitations of using "stress," several authors proposed "distress" as the point at which physiological stress becomes intense and/or prolonged enough to be detrimental to both welfare and fitness (75, 113). Distress is variously defined as "a chronic condition reflecting the biological cost of repeated or cumulative stressors" (157) or "when stress induces allostatic [homeostatic] overload or becomes pathogenic" (163). So defined, distress reflects some point toward the extreme end of the continuum of physiological stress; the point at which stress becomes distress is empirically identified as when diversion of resources away from core functions, such as reproduction, feeding or immune function can be quantified (13, 157, 164). The concurrent measurement of stress (i.e., HPA activation) and biological cost (e.g., suppressed reproductive function) makes this concept valuable for assessing the conservation implications of stressors that may also impact on welfare (13, 137).

In contrast, in the field of animal welfare science, distress is generally characterized as "one or more negative psychological states indicative of poor wellbeing or that decrease wellbeing" (165) or as a "wide range of unpleasant emotional experiences" (166). Thus, distress in this field unequivocally represents the extreme end of a continuum describing affective, mental or psychological states while stress (and distress in the conservation context) appears primarily to represent a physiological response, with ambiguous relationships to affective state. Accordingly, these concepts do not occupy the same continuum. An important corollary of this is that the absence of evidence of extreme stress responses and/or fitness costs is not evidence of the absence of unpleasant experiences and poor welfare state.

This affect-related concept of distress is more consistent with the current conception of welfare favored by the majority of animal welfare scientists (48). However, given that distress is characterized as a range of different unpleasant experiences and that different mental experiences reflect different problems for the animal to solve via their behavioral and physiological responses (61), there is unlikely to be one single empirical metric of both reduced fitness and poor welfare nor even a single set of measurable indicators that can be used to practically evaluate distress (167). It is more meaningful to evaluate welfare according to the evidence about the intensity and duration of specific unpleasant experiences, such as breathlessness, pain, thirst and hunger (62). Doing so also facilitates the development and implementation of strategic approaches to avoiding or mitigating those specific experiences (152).

Thus, the problem with concepts, such as stress, distress and others like "suffering" is the lack of clarity about their meaning and their relationships to the mental experiences of animals and the associated lack of a scientific framework for assessing these scientifically nebulous concepts (51, 168). Interestingly, although such pragmatic models have been advocated for more than 15 years, there has been limited uptake in practice, and collaborative research and activity between animal welfare and conservation scientists is still rare (2). Perhaps this is because, more fundamentally, a common understanding of what animal welfare is conceived to be must be achieved first.

CHARACTERIZATION OF WELFARE ALSO INFLUENCES THE SIGNIFICANCE ASSIGNED TO, AND THUS THE APPLICATION OF, OUTCOMES OF WELFARE ASSESSMENTS

As well as influencing the approach to, and emphasis within, scientific assessments, the conceptual foundations of welfare influence the ways the outcomes of those assessments are interpreted, prioritized and applied. Specifically, how welfare is understood may influence the following: decisions about whether welfare is assessed at all; how strongly minimization of negative welfare impacts is emphasized; how information from welfare assessments is integrated into conservation decision-making; and how that knowledge informs the development of policies, guidelines and legislation. Salient examples from the New Zealand (NZ) and Australian context are presented below.

Overall, it is argued that understanding welfare as what is experienced by, and thus what matters to, the animal itself increases our responsibility in three areas: to systematically evaluate welfare impacts; to genuinely include that knowledge in conservation decision-making practice; and to give it more appropriate prominence in those decisions than is currently apparent (52).

Whether or Not to Devote Resources to Welfare Assessment and How Strongly Minimization of Negative Welfare Impacts Is Emphasized

Many kinds of conservation activities proceed without explicit or formal scientific evaluation of potential welfare impacts. In NZ, these include routine management of threatened native animals (such as captive breeding and release, intensive monitoring, and regular movement between populations), control of invasive animal populations, wildlife rescue and rehabilitation, and permanently holding native and exotic wild animals in captivity. Decisions about whether to undertake formal welfare assessment may be made implicitly or explicitly by various stakeholders with various objectives; such decisions may sometimes involve conflicts of interest, i.e., not wanting to know about the welfare impacts of activities considered to be desirable for other reasons, including for the achievement of conservation objectives. While it may be argued that many such activities are "routine" or based on "best practice," the lack of ongoing welfare assessment limits opportunities to update practices as scientific understanding and technical capacity grow (169), thereby limiting opportunities to minimize negative welfare impacts.

Characterization of welfare may also influence the emphasis put on minimizing negative impacts in the context of conservation research. In NZ, research on wild animals must be approved by animal ethics committees (AECs) authorized under the Animal Welfare Act (1999) (170); approval depends on demonstrating an understanding of the potential negative impacts on the subject animals' welfare as well as the likely benefits of the research. However, there may be unrealized opportunities for minimizing welfare impacts associated with research procedures, and it behooves AECs and the applicants seeking approval to regularly challenge the status quo in terms of what might be considered to be "unavoidable" negative welfare impacts. As a parallel, while surgical procedures performed on laboratory animals almost inevitably cause some degree of pain, NZ AECs put the onus on applicants to demonstrate how such pain can be minimized and that pain relief strategies are the best currently available [e.g., (153, 171, 172)]. Likewise, academic journals in the field of animal welfare science are increasingly demanding evidence, above and beyond appropriate regulatory approval, of strategies to avoid, mitigate or minimize negative welfare impacts on research animals [e.g., Animal Welfare journal; (43)].

To better realize these sorts of opportunities, research directed at minimizing existing welfare impacts associated with conservation activities should be encouraged and specifically funded (153). As one example, systematic evaluations of the effects of identification marking techniques on the welfare of subject animals are still rare relative to the number of studies applying such techniques to wild populations [e.g., reviewed by (11, 22, 101, 103)], and more are needed (169). Whenever the type, severity, duration, distribution or variability of negative welfare impacts are not well-understood, preliminary studies that formally assess the impacts of the proposed procedures should be required by AECs before granting approval for major studies applying those procedures in wild populations (44, 153, 169).

WHETHER AND/OR HOW TO INTEGRATE INFORMATION FROM ASSESSMENTS INTO DECISION-MAKING

In line with the points made above, decisions about whether and how a wider range of conservation activities proceed should be informed by impacts on the animals involved (20). As noted, such decisions are complex and involve multiple stakeholders with differing priorities [e.g., (1, 45)]. However, such decisions cannot be taken knowledgeably and ethically if welfare impacts are not rigorously and transparently evaluated (72, 74). Assessments that emphasize the importance of mental experiences to an animal's welfare and that cautiously interpret measured physical/functional variables accordingly may result in greater weight being given to the welfare outcomes in conservation decision-making. Alternatively, there is a risk that evaluations focussing only on "objective" clinical indicators of biological function will inspire less concern for animal subjects of conservation activities, leading to prioritization of other objectives in conservation decision-making.

To illustrate, despite rigorous scientific research demonstrating the negative experiential impacts of poisons used to lethally control various invasive mammal species in NZ and Australia [e.g., (18, 25, 32, 173, 174)], both small-scale domestic applications (e.g., household rodent control) and mass poisoning programmes continue to use the least humane agents because they are effective and safe for humans (118, 175). In the last 30 years, relatively little progress has been made toward developing effective and safe alternatives that are demonstrably more humane for the millions of sentient animals so affected (175, 176). Perhaps explaining those welfare impacts in terms of the severely unpleasant and protracted experiences that the animals may have before loss of consciousness (25, 32, 174) would influence the weight assigned to welfare when deciding to continue to use those agents.

Framing welfare impacts in terms of the unpleasant experiences animals might have may therefore also be useful for informing public sentiments and political decisions regarding lethal vs. non-lethal control of both native and introduced species. With regard to non-lethal methods, wild animals clearly demonstrate species-specific indicators of experiences, such as extreme fear, anxiety, rage and/or frustration during the processes of capture and transport for purposes, such as relocation, re-homing or permanent penning [e.g., (2, 15, 34, 92)]. Other unpleasant experiences, such as pain or exhaustion may arise due to physical injury or capture myopathy [e.g., (177, 178)].

Importantly, scientific studies now provide evidence of ongoing negative welfare impacts in animals relocated rather than humanely killed [e.g., (15, 17, 24, 139, 140)]. Other studies compare potential impacts associated with all components of lethal vs. non-lethal methods to allow holistic decision-making (18, 24). Impacts occurring after the period of capture, temporary holding and release may take the form of extreme hunger due to unfamiliarity with foraging opportunities (34), or fear and pain due to the animal's reduced ability to escape predators in the new location or because of aggression from resident conspecifics (2, 15, 140). For animals captured from the wild and brought into captivity, for example, for permanent penning or taming, there is undoubtedly a period of severe fear and anxiety as they habituate to confinement and human proximity (78, 179); some individuals never successfully acclimate, meaning such experiences likely persist to some degree [e.g., (33, 180–183)]. Disruption of social groups and restricted movement may lead to other, less well-understood unpleasant experiences, such as loneliness, frustration, boredom, depression or grief [e.g., (62, 150, 151)].

Similarly, decisions about whether to rehabilitate or promptly euthanize "rescued" wildlife should not be evaluated only in terms of the conservation status of the species and the genetic merit of the individual, but also by considering the potential for significant and/or chronic unpleasant experiences, such as pain, sickness and fear, both during and after the rehabilitation process [e.g., (30, 95, 97, 184–186)]. In both cases, the potential for longer-term negative welfare impacts is often not formally evaluated in conservation decisions, and, in any case, the significance of such impacts for the animal itself may be overwhelmed by public sentiment about the value of sustaining life at any cost over a humane death [e.g., (96, 187)].

WHETHER AND/OR HOW TO CONSIDER INFORMATION IN DEVELOPMENT OF POLICY AND LEGISLATION

As well as influencing current conservation decision-making, research and practice, the conceptual basis of welfare may also influence development of policies, guidelines and laws that, in turn, guide future practice. In particular, emphasizing that some animals experience unpleasant (and pleasant) states which affect their welfare highlights the significance of legislative discrepancies and the limitations of using survival or biological function to infer welfare in conservation and other policies and guidelines.

In NZ's Animal Welfare Act 1999 (170) and Codes of Welfare enacted under that Act, persons in charge of wild animals held for the purposes of exhibition, containment or rehabilitation are obligated to meet the animals' physical, health and behavioral needs and to act to avoid or alleviate any unnecessary or unreasonable pain or distress [e.g., (188)]. Other wild animals are variously recognized and treated under the law (see below). Although there is general reference in the law to one specific unpleasant experience, i.e., pain, and an amalgamation of others under the appellation "distress" (54), the importance of unpleasant experiences for animal welfare is not explicitly articulated, which may encourage emphasis on physical state, the limits of which have been discussed above. The importance of interpreting observable or measurable indicators as reflective of animals' mental experiences in the legal context has recently been exemplified in a number of successful legal prosecutions for animal welfare offenses in Canada and the UK (168, 189, 190).

For free-living wild animals or animals living in a wild state (i.e., feral domestic animals), there exist incongruities among NZ laws or even among sections of the same Act that appear to facilitate de-prioritization of animals' mental experiences in certain contexts (41). These "exemptions" to general requirements to safeguard animal welfare become more difficult to defend for economic, conservation or practical reasons if the experiences of the animals themselves are central to our collective conception of welfare. To illustrate, under Section 30A of the NZ Animal Welfare Act, "a person commits an offense who wilfully ill-treats a wild animal." Ill-treatment is defined as "causing the animal to suffer pain or distress that, in its kind or degree, is unreasonable or unnecessary." However, it is legally acceptable to purposefully use control methods scientifically demonstrated to be relatively less humane than existing alternatives for some sentient wild animals, either because of their classification as "pests" or because it is "generally accepted" to treat them in that particular way (170). Some of these exemptions relate to fulfillment of the purposes of other acts, such as the Conservation Act 1987 (191) and the Biosecurity Act 1993 (192) (Animal Welfare Amendment Act (No.2) 2015 (193) subsection 30A4) or the Animal Welfare Act, Section 181, relating to the Agricultural Compounds and Veterinary Medicines Act 1997 (194), when the activity involves the use of any substance for direct management or eradication of vertebrate pests. Nonetheless, the question arises: "is the suffering caused to these wild animals 'necessary'?" (41, 52, 195).

There are also examples of animals of the same species being treated differently under the law when they are classified differently for human purposes. For example, feral cats (*Felis domesticus*) are designated as pests and are thus exempt from certain welfare protections under various NZ laws, as described above. In contrast, owned cats of the same species (*Felis domesticus*) and cats used for the purposes of research, which presumably have the same biological capacity for unpleasant experiences that compromise their welfare, are much more strongly protected under the NZ Animal Welfare Act. These categorizations and legal exemptions serve to reinforce existing species and contextual biases (41, 74) and are likely to stymie progressive development of more humane methods for managing wild populations, both of which are detrimental to wild animal welfare overall.

EXAMPLES OF "CONSERVATION WELFARE" IN THE ZOO COMMUNITY

As noted above, collaborative research and practice among conservation and animal welfare scientists occur only sporadically. Explicit and deliberate evaluations of welfare occur in some specific areas of biological conservation, particularly in context of research involving wild animals, when approval from a regulatory body is required, and for animals kept in zoos.

Zoos arguably play roles in *ex situ* conservation by providing genetic repositories for threatened and endemic species and by educating the public about animals and conservation [e.g., (196–198)] [but cf e.g., (199)]. In these roles, the zoo community is demonstrating a commitment to "Conservation Welfare" in various ways, most notably by adopting a contemporary characterization of animal welfare and scientific principles and methods of assessment to guide zoo design and practices [e.g., (115, 200–204)]. Two key examples are the World Association of Zoos and Aquariums Animal Welfare Strategy (46) and the Zoo and Aquarium Association (Australasia) members' accreditation programme [(205, 206); n.d.]. Both documents are based on a characterization of animal welfare and assessment framework reflecting the centrality of animals' mental experiences. To become accredited ZAA members, Australasian zoos and aquaria must demonstrate the ways in which they provide care and husbandry practices and habitats designed to minimize unpleasant experiences and maximize opportunities for animals to have positive experiences [(205, 206); n.d.].

For various reasons, this approach may be easier and also more pressing for the zoo community to action than for biologists working in other areas of conservation practice. Maintaining public support is of primary importance for the continued existence of zoos, and zoo practices, including those reflecting a commitment to animal welfare, are under increasing public scrutiny (204). Zoo scientists are able to evaluate welfare at the level of the individual animal over time and are able to collect much more detailed data than field biologists usually can (10, 75). Increasingly, this kind of information and a focus on animals' mental experiences is guiding habitat design [e.g., (133, 207)] and the evolution of zoo policies and guidelines (116), ZAA's Animal Welfare Position Statement (205) and is being given greater weight in conservation decision-making in the zoo community [e.g., Periera (208) "Tiger returned to SF zoo after transfer to Sacramento made her homesick"; Anon (209) "Zoo pays tribute to much-loved lions"; Johnston (210) "Auckland zoo puts down 'unhappy and agitated' gibbon"]. Individual zoo organizations, and increasingly the zoo community as a whole, are showing leadership in this regard, and there is great potential for zoo biologists and welfare scientists to collaborate more closely with their field research colleagues to optimize policies and practices to better achieve both welfare and conservation goals more broadly [e.g., (211, 212)].

CONCLUDING REMARKS: A FUTURE OF CONSERVATION WELFARE

To address some of the challenges identified above, the establishment of a new discipline of "Conservation Welfare" is recommended. Its major role would be to reveal key synergies between the sciences of conservation and animal welfare with the aim of providing an integrated foundation upon which the two could interact constructively to further the objectives of both. Finding common ground has apparently been hindered thus far by notions that these are competing disciplines or schools of thought, or even ideologies. In part, this has been due to different ways members of the two disciplines have understood animal welfare, with conservation scientists generally emphasizing "fitness" and welfare scientists "feelings," as illustrated here. This dichotomy has led to apparently incompatible views on the nature and significance of animal welfare impacts and the related implications for wildlife policy and management. Some of these difficulties have been considered here, and these observations raise the question of how this impasse can be resolved.

It is concluded that to make progress scientists in both disciplines will need to arrive at compatible understandings of animal welfare; in other words, it will behoove both groups to use a common language when considering welfare matters in the conservation context. Thus, instead of reinforcing the existing "fitness" *or* "feelings" dichotomy, cross-disciplinary progress may be achieved by recognizing the scientifically current and widely accepted animal welfare conceptual framework that integrates these two elements as dynamically interacting components within animals, i.e., that animals embody a "fitness" *and* "feelings" unity. Understanding this unity underpins the conceptual foundations of animal welfare and rigorous and robust science-based methods used to assess animal welfare impacts in circumstances that compromise and/or enhance welfare.

It is still necessary to consider various matters in more detail than was possible here. They include: what the precise implications will be for informed decision-making in the conservation arena; what will constitute humane conservation practices and/or management; how public perceptions and values will evolve to interact with welfare and conservation decision-making and practice; and how high standards of individual and/or group animal welfare can be monitored and achieved practically in conservation biology whilst most effectively meeting both conservation and animal welfare objectives.

AUTHOR CONTRIBUTIONS

NB and DM designed and facilitated the workshop during which the ideas expressed in this paper were collectively generated by all authors. NB wrote the first draft of the paper and all other authors provided critical review of the drafts.

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Appendix 6

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Maternal protectiveness in feral horses: responses to intraspecific and interspecific sources of risk

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In most mammalian species, mothers must protect offspring from multiple sources of risk. In Australia, feral horses, *Equus ferus caballus*, have naturalized in many ecosystems, and foals are at risk from both predation by dingoes and sexually selected infanticide by nonpaternal stallions. This study tested maternal responses to these two forms of risk: risk of predation through dingo call playbacks and infanticide risk through a comparison of maternal protectiveness of foals in single- and multistallion bands. Mares were more vigilant and spent more time close to foals in bands with multiple resident stallions, where there is a higher risk of infanticide, relative to bands with a single stallion. Dominant stallions spent more time close to foals following the dingo call playbacks, indicating that stallions may play an important role in detecting and protecting foals from interspecific sources of risk. There was no significant increase in maternal protectiveness in response to dingo call playbacks, indicating that mares did not perceive dingo calls to be an immediate threat to foals. While predators were present, infanticide risk appeared to be the most significant modifier of maternal behaviour in this study.

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Final Report of the Kosciuszko Wild Horse Scientific Advisory Panel

Advice to assist in preparation of the Kosciuszko National Park 2020 Wild Horse Management Plan

September 2020

This report was prepared by the Kosciuszko Wild Horse Scientific Advisory Panel (SAP)

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Executive Summary

The Kosciuszko National Park (KNP) Wild Horse Scientific Advisory Panel (SAP) reviewed previous management plans for wild horses in KNP and management strategies used for other populations of wild horses, as well as the most recent literature on horse ecological impacts and control

methodology, in addition to KNP site visits, with a view to providing scientific advice to the Minister and NPWS to assist in the preparation of a new Wild Horse Management Plan.

A reduction in wild horse distribution and density is required to minimise the considerable negative impact that wild horses are having on the natural values of KNP. However, as the precise quantitative relationships between horse density and negative impact across different areas of the park are not fully understood, a target population size is not advised. Rather, an adaptive management strategy is recommended with continual monitoring of performance against objectives and adjustment of actions depending on feedback from monitoring.

The SAP recommends that the new management plan should follow the International Consensus Guidelines for Ethical Wildlife Control, which incorporates modifying human practices, justification for control, clear and achievable outcome-based objectives, optimising animal welfare, social acceptability, systematic planning, and decision making based on specifics rather than labels.

The systematic planning recommended by the SAP comprises strategies for:

- identifying a horse population that will not diminish environmental values
- identifying management zones
- monitoring of the horse population
- monitoring impacts including environmental, Aboriginal heritage and horse welfare
- a decision-making process in choosing control methods for different zones and circumstances
- assessing the animal welfare impacts of control methods, with an adaptive management plan to prioritise the use of methods that have the least negative impact on animal welfare

Suggestions have been made where community involvement can be incorporated into each of these stages.

The advice aims to ultimately achieve environmentally sustainable populations of genetically viable heritage horse populations, managed with control methods that have the least negative impacts on animal welfare, with a management program that is socially acceptable and incorporates ongoing community involvement.

1. Introduction

The wild horse population in KNP has substantially expanded, both in distribution and density, during the last 30 years (Cairns 2019; Cairns and Robertson 2015; Walter 2003; Dawson 2009; Dyring 1990). This has been associated with increased concern for the negative impact on natural and cultural values (see Appendix 1 for further detail).

The SAP was formed to provide scientific advice to assist in the preparation of a new wild horse management plan. Members of the SAP are keen to ensure that the new plan will result in ethical and effective management of wild horses in KNP, ensuring the protection of natural values while also protecting the heritage value of the wild horses. The SAP has worked alongside the Community Advisory Panel (CAP) to listen to their views, address their questions and concerns, and incorporate their local knowledge and expertise. This report describes work conducted by the SAP to review available scientific and other information to provide advice that will contribute to the preparation of a successful KNP wild horse management plan.

SAP members reviewed the 2008 KNP Horse Management Plan and the 2016 KNP Draft Wild Horse Management Plan (Appendix 2) and the accompanying 2016 Independent Technical Reference Group Final Report and control method humaneness assessments (Appendix 3), as well as more recent scientific documents. Previous management plans for wild horses in Kosciuszko National Park (KNP) and management strategies used for other populations of wild horses were considered to identify reasons for failure or success (Appendix 2).

2. Recommendations by the SAP

Based on these reviews, the SAP has developed recommendations for wild horse management in KNP which follow the International Consensus Principles for Ethical Wildlife Control (Dubois et al. 2017). Specific recommendations are made with regards to estimating horse population densities, estimating environmental impacts of horse populations, horse control methods for reducing negative environmental impacts, animal welfare impacts and decision making within control methods, impacts on Aboriginal heritage, and community engagement.

2.1. Ethical Management Decision Making

To ensure that the new wild horse management plan is based on sound ethics, the SAP proposes that the new management plan follows the 'International Consensus Principles for Ethical Wildlife Control' (Dubois et al. 2017), and incorporates a 'One Welfare' framework (Pinillos 2016; Fawcett et al. 2018), in addition to aligning with the principles of 'Fraser's Practical Ethic' (Fraser 2012; Fawcett et al. 2018), and 'Conservation Welfare' (Beausoleil et al. 2018; Beausoleil 2020), which are summarised in Appendix 3.

The International Consensus Principles for Ethical Wildlife Control

1. Modifying human practices

The SAP has carefully considered each negative impact related to horses in KNP (danger to people at campgrounds, road traffic accident risks, and negative environmental/cultural heritage impacts; (summarised in Appendix 1) and evaluated first whether the impact can be mitigated by modifying human practices. The predominant negative impacts from wild horses that are not able to be further mitigated by modifying human practices are those of negative environmental and Aboriginal heritage impacts, and in restricted areas, road traffic accident risk and risk to people at campgrounds. Thus, further action for managing these negative impacts is required.

2. Justification for control

Justification for the need for increased management of wild horses in KNP is based on scientific evidence of the rapidly growing horse population in KNP, and the environmental degradation to which horses are contributing (see Appendix 1.2). There is a lack of robust evidence regarding the precise relationship between negative environmental impacts and horse densities, across different habitats. Consequently, the identification of what would be an environmentally 'sustainable' population of horses is imprecise. We define 'environmentally sustainable population' as a population size at which there are no or negligible, negative environmental impacts. The SAP, therefore, proposes rigorous monitoring during horse management to determine the relationship between horse density and negative impacts, and be able to identify such environmentally sustainable populations for different regions. Details of proposed monitoring are outlined below (as well as in greater detail in Appendix 5). Further, based on optimal welfare outcomes for wild horses, the SAP recommends against inaction on implementing horse population reduction as inadequately managed wild horse populations are likely to have a large proportion of horses with poor welfare as their numbers exceed available food, and horses are pushed out into woodland areas of the park where food availability is limited.

Horse populations in KNP also have impacts on other aspects of the park, including Aboriginal and European Australian heritage (see Appendix 1.3 for discussion of the impacts of horse populations on Aboriginal heritage). The heritage value of the horses and the importance of horses to community members are recognised. Nonetheless, of high importance are also other species, unique aspects of the environment, ecosystems functions, and Aboriginal heritage. The 2020 Wild Horse Management Plan should, therefore, seek to reach a balance in protecting all these assets and reach compromises between conflicting values.

3. Clear and achievable outcome-based objectives

The SAP advises that the wild horse management plan has clear and achievable outcome-based objectives, i.e. adaptive management (Allan & Stankey 2009), as outlined in the below sections of this report. As such, the SAP advises rigorous monitoring throughout management including of environmental impacts and horse population demographics and welfare. The SAP recommends that management is adaptive based on lessons learnt from ongoing monitoring, as is detailed below. The end goal is to reach an environmentally sustainable population of wild horses in KNP.

4. Animal welfare

The need for more systematic evaluations of the welfare outcomes in Australian horse management was recently identified by Scasta et al. (2020). Thus, the SAP recommends welfare outcomes of control methods be empirically evaluated. Management methods used should cause the least animal welfare harms to the least number of animals. To identify those methods, systematic scientific evaluation of the possible animal welfare harms is required (see sections 2.2.6, 2.2.7 and Appendix 3).

5. Social acceptability

No wild horse management program will be successful if it is not socially acceptable (Howard et al. 2019, see Scasta et al. 2020 for a recent review of the social issues faced by wild horse managers). Social acceptability is dependent on education, transparency, and trust (Scasta et al. 2020). Having a management plan that follows the described ethical frameworks will hopefully instil transparency and public trust in the plan. Incorporating a strong program of environmental impact and animal welfare impact monitoring, with adaptive management informed by empirically assessed results, should further increase trust and transparency. Ongoing community involvement in the management plan will further help to ensure wider social acceptability. Community involvement in monitoring may instil greater transparency and trust in the data being used to guide horse management. Areas where community engagement can be incorporated into management are identified in each respective section and community engagement recommendations further summarised in section 2.2.8. Previous lack of true community engagement was identified by the SAP as a reason for the failure of previous management plans.

It must be acknowledged, however, that different people have different values; successful coexistence in any society is dependent on mutual trust and respect among people with different views and values, as well as those people with similar views and values. Successful and ethical management is thus further dependent on understanding and appreciating alternative views and values to reach compromises that strike a balance between these alternate views. It is also important that representative views of the broader community are considered, and not just those of the most vocal minority groups. The SAP recommends that communications specialists with knowledge and experience of gaining social license to operate with animal management issues are engaged. It is vital that a strategy is developed for effective communication with, and education of, the broader public, and that misinformation disseminated on social media, and mainstream media is effectively mitigated. For example, misinformation/misinterpretation among the public regarding population estimates and reproductive control has been identified as a social issue hindering effective horse management (Scasta et al. 2020). Thus, providing easily understandable information regarding management actions and the scientific justification for those actions to the public may improve community support of management actions (see Supplementary Material for some examples of lay explanations of the science behind management decisions).

6. Systematic planning

The SAP recommends that the management plan is systematic and adaptive to most effectively minimise negative environmental impacts of horses while optimising welfare outcomes of horse management. The recommended systematic planning comprises strategies for identifying an environmentally sustainable horse population, identifying management zones and horse heritage areas, monitoring of the horse population, monitoring environmental impacts and Aboriginal heritage, a process for decision making in choosing control methods for different zones and circumstances, and assessing the animal welfare impacts of control methods, with an adaptive management plan to prioritise the use of methods that have the least negative impact on animal welfare. The SAP recommendations for this are detailed below (section 2.2).

7. Decision making by specifics rather than labels

The SAP recommends an evidence-based management program, as already highlighted in principles 1-6. The steps being followed in developing the management program would be the same for any wildlife species where management is required as based on scientific evidence. Management decisions are therefore not being suggested based on horses being an introduced species or based on any groups of people having a bias against horses. The heritage value of wild horses in KNP and the importance of the horses to elements of the local and wider community are recognised. The SAP seeks to introduce a management plan that will achieve a balance, to reach an environmentally sustainable population of wild horses, protecting the heritage value of the horses whilst also protecting and retaining other values of KNP.

2.2. Systematic Planning

2.2.1 Recommended strategy for identifying environmentally sustainable horse populations

As the precise quantitative relationships between horse density and negative impact across different areas of the park are not fully understood, a target population size is not advised. Rather, an adaptive management strategy is recommended with continual monitoring of performance against objectives and adjustment of actions depending on feedback from monitoring. As part of adaptive management, controlled experimental studies (e.g. Lenehan 2011) should be conducted during management activities to better assess the changes in environmental impacts as horse densities are reduced. This will inform the horse densities that can be retained whilst minimising negative impacts. Preliminary targets for specific zones could be determined based on historic data of horse densities at a time when there was little evidence of negative impacts.

The SAP recommended strategy for identifying environmentally sustainable horse populations includes the following steps:

1. Define environmental and Aboriginal heritage impact.

This should include measurements of water quality, vegetation and faunal biodiversity and abundance, stream morphology, and Aboriginal archaeological surveys. More detailed methodology will need to be developed and included in the final plan. See section 2.2.3 below and Appendix 5 for specific monitoring methodology recommendations.

2. Define horse management zones.

Starting with the already defined management zones developed in the 2016 Plan, incorporate new information regarding areas of high conservation concern and value, high Aboriginal and European Australian heritage value, and habitat suitability for horses, other herbivores, and key endangered species, to further refine management zones (section 2.2.1, Appendix 4).

3. Set preliminary horse density targets within each zone.

Preliminary horse density targets should be guided by current estimated densities within each zone, and past densities when there was little evidence of negative impact (these targets will be refined as the adaptive management process continues).

5. Establish monitoring sites.

Monitoring sites within each management zone (sampling all habitat types) should be selected. At each site record indices of abundance/activity of horse (section 2.2.2, Appendix 5.1) and other grazing species (e.g. deer, pig), and record the *a priori* selected environmental and Aboriginal impacts at each site (section 2.2.3 and 2.2.4, Appendix 5). This will provide data on horse densities and impacts, prior to commencing management; monitoring should continue as management proceeds.

6. Initiate horse population control

Starting with horse reduction zones (section 2.2.1, Appendix 4), begin horse control, initially targeting areas of highest environmental and Aboriginal heritage concern within the zone. Trial initially recommended control methods and monitor efficacy, practicality, and welfare outcomes (section 2.2.6, 2.2.7, Appendix 3) to inform how management can best proceed in other zones.

7. Assess the relationship between reduction in horse density and negative impacts to inform sustainable horse densities in each region within zones

Resurvey sites for horse densities and impacts regularly once or twice per year as management progresses. Conduct water, vegetation, and faunal surveys (e.g. spring, autumn or summer, depending on the species involved) per year to assess recovery (Appendix 5.2). More frequent surveying is likely unnecessary due to the time lapse required for recovery (i.e. vegetation and fauna will require a period of time to respond to the change in horse density and recover).

8. Adjust density as necessary to reach a sustainable target density

As management progresses, scientific monitoring of environmental impact, horse density, and horse welfare (all discussed above) will enable an environmentally ‘sustainable’ target density to be determined.

2.2.2 Management Zones Recommendations

The 2016 Plan identified five types of horse management zone types: prevention; elimination; containment and population reduction; key environmental asset protection; and public safety. See 2016 Plan for further detail regarding each of these management zone types.

Working with these zones, the SAP considered the subsequent changes to reported negative horse impact up to 2020 and the damage caused by the 2019-2020 fires (Adaminaby Complex, Dunns

Road, Marys Hill, and Rolling Ground Fires) to indicate three areas where immediate horse management should be prioritised. Boundaries were revised after discussions with NPWS and the CAP based on operational and heritage considerations.

Three management zones are proposed for the northeastern section of the park where fire damage has been severe and wild horse populations are at high densities (Figure 1). The rationale is that the post-fire recovery of the vegetation and its dependent native fauna may be hindered by the high horse densities that have become established in this area of the park, especially over the last decade (see Appendix 4). Additionally, there is a need to protect environmental assets that are currently being negatively impacted by horse activity (see Appendix 4). Horses also need to be managed in places where collisions between motor vehicles and horses pose a significant safety risk to humans and horses.

Zone 2 has additionally been identified to incorporate an area of potential horse heritage value in the Kiandra region. This population of horses is important to the local communities, and individual horses are well known through the work of local photographers. Anecdotal evidence from the community suggests that this population was reduced during the 2019-2020 fires. Therefore the SAP recommends that the Kiandra region be utilised as the key region for a pilot community engagement study (detailed in section 2.2.8) prior to further management planning for this region.

Beyond the three zones identified above as priorities, the SAP recommends the management zones and types specified by the 2016 Plan be incorporated in the 2020 Plan, while acknowledging that these may be refined through the adaptive management process. Additional horse subpopulations of particular community value should be identified and included in the consideration of management zones. Further detail should be provided for the justification for each zone to improve transparency (as per Appendix 4).

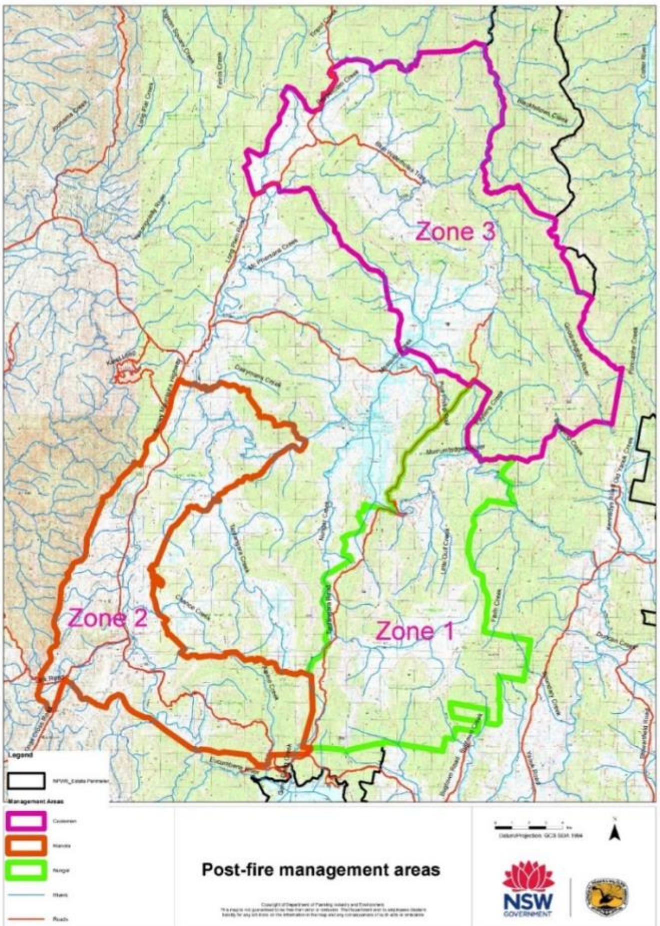

Figure 1. Priority post-fire management zones recommended by the SAP.

2.2.3 Horse Population Monitoring Recommendations

To monitor the effectiveness of horse management on a KNP wide scale, the SAP recommends that existing aerial line-transect surveys are maintained for a KNP wide population estimate (Cairns and Robertson 2015, Cairns 2019). The scientific methodology used in these surveys is scientifically robust and has provided accurate estimates of the horse population within the survey area at the time of the survey (see Supplementary Material 1.1 for background information). While the methodology of the aerial count should be maintained to allow for comparison across years of sampling, improvements that can be made include attaching video cameras and/or thermal cameras alongside human observers. Eventually, drones may replace helicopter surveys, but further development of this new technology is required.

A study of the movement behaviour of horses during an aerial survey should be conducted to measure the influence of flushing horses from one transect to the next. Satellite tracking of collared horses on the edges of the survey area would be valuable to help provide estimates of movements during aerial surveys and control operations.

The SAP recognises there are some parts of the community in disagreement with the KNP wide population estimates. Community engagement in population surveys, in addition to further education regarding the methodology (e.g. Supplementary material 1.1), may help address this issue. This is likely to remain a challenge given the complexity of performing large scale population estimates in a scientific manner. The SAP emphasises that management decisions should not be directly based on the KNP wide population estimates, but rather on environmental impact monitoring (see 2.2.4 below) and smaller scale population estimates within management zones. While aerial surveys provide an estimate of the entire horse population across KNP, there is a need for greater use of population estimates at smaller scales. The relationship between horse densities and environmental impact will likely differ for different ecosystems within the park (Appendix 1) and thus within the different management zones. Determining this precise relationship for each management zone is crucial. To obtain these smaller scale population estimates, we recommend the following methods:

- Line-transect dung count surveys
- Dung DNA sampling (genetic analysis)
- Aerial line-transect surveys using drones
- Ground surveys (line-transect and mark-recapture)
- Camera traps

Detailed methodology for each of these methods, and opportunities for community involvement, are provided in Appendix 5.1. Smaller scale population estimates within management zones will not only be more useful for directing immediate management, but estimates at this scale will be more likely to achieve community agreement. Therefore, the SAP recommends that the main focus for population estimates is shifted to smaller scale estimates within management zones.

For management zones where horse populations will be retained, horse welfare and genetic health will need to be monitored as part of the identification and maintenance of a genetically viable healthy horse population. However, as the population in KNP is not currently considered a small population and subpopulations are not geographically isolated, the SAP does not see genetic diversity within the population as an immediate concern. Genetic work as described in Appendix 5 should provide further information in this regard. Regular genetic monitoring would be required to monitor for evidence of declining genetic health over time. If this arises, management strategies would need to be altered accordingly. Additional genetic analyses may be used to identify horses outside of the park that would be suitable to introduce into these heritage herds to genetically reinvigorate the population (see National Research Council 2013 for detailed discussion of managing genetic viability in horse populations).

2.2.4 Environmental Impact Monitoring Recommendations

The SAP recommends that horse management goals be focused on reducing negative impact rather than a target population size, as was also advised by the ITRG in 2016. To achieve these goals, negative environmental impacts need to be identified and monitored to ensure that horse population reduction efforts are having the desired outcome.

Several methodologies have been developed to assess the negative impacts of wild horses on different ecosystems (e.g. Cherubin et al. 2019; Robertson et al. 2019; Thiele & Prober 1999; Tolsma & Shannon 2018; Worboys et al. 2018). The SAP review of horse impacts in KNP indicates that high densities of horses have the greatest negative impact on waterways and bogs (see Appendix 1.2). Growing horse populations can also have negative impacts on key endangered species, directly and indirectly through loss of habitat (Appendix 1.2). Thus, these impacts should also be a focus of monitoring effort. The SAP recommends monitoring include the following areas.

- Vegetation. Monitoring to include changes to vegetation structure and composition, weed abundance, endangered flora abundance, proportion of trampled or eaten vegetation.
- Stream morphology. Monitoring to include water quality, water turbidity, stream bank morphology, degree of pugging.
- Key endangered fauna. Monitoring to include presence and/or abundance of Corroboree Frogs (especially Northern species *Pseudophryne pengilleyi*), Broad-toothed rats (*Mastacomys fuscus*), and other key species, and impacts on the habitat of these species.

Further detailed recommendations regarding methodology for each of the above areas are provided in Appendix 5.2. Opportunities for community engagement should also be identified.

With reductions in horse densities, it is likely that not only will negative environmental impact be mitigated, but some positive impacts may be identified at moderate or low horse densities in some management zones (e.g. Fahnestock & Detling 1999, Austrheim & Eriksson 2001, Ostermann-Kelm et al. 2009, Stroh et al. 2012). Alternatively, even at very low horse densities, unacceptable negative environmental impacts may remain in some management zones. An adaptive management process as described here will inform either situation.

2.2.5 Aboriginal Heritage Monitoring Recommendations

The impact of horse populations on Aboriginal heritage has been woefully understudied in KNP and across Australia (see Appendix 5.3). The SAP recommends that NPWS take this opportunity to examine the impacts of horses on Aboriginal heritage and evaluate how best to preserve Aboriginal heritage into the future. The SAP understands that NPWS have also engaged an anthropologist to undertake indigenous community engagement, and that a separate advisory report will also arise from this.

The effect of wild horses on Aboriginal heritage objects and places is hypothesised to be detrimental over the long term, even if short-term observations do not see large or obvious changes in site integrity (see Appendix 5.3). There are no known studies that address the issue of the effect of horses on hunter-gatherer archaeology. Therefore, as well as contributing to the effective management of KNP, the initiation of such study would represent a potential contribution to knowledge that would be of international importance.

Monitoring the effect of horses on Aboriginal heritage will require approaches predicated on the range of Aboriginal site types likely to be encountered in KNP by wild horses (Appendix 5.3).

The broad approaches should be enacted in each of the designated management zones and should encompass:

- Archaeological survey of known/suspected areas of higher horse density to determine the presence of archaeological material and horse impact (direct or indirect) thereon.

- Investigation of known key locations within KNP of high Aboriginal archaeological/heritage significance to assess potential impacts of horses, or their exacerbation of disturbance partly or wholly attributable to other factors.

- Design of controlled experimental locations that would allow measurement of the impact of horses on prevalent site types (see Appendix 5 for more detailed methodology).

At monitoring locations, an inventory should be undertaken of potential Aboriginal food plant resources and note of any impact to these by horses.

A further area on which to focus will be the potential effect of managing the horses on Aboriginal objects. For example, the creation of even temporary yards has potential to create a congregation of horses that might not normally occur there - and a consequent degree of scuffage and topsoil disturbance that could break and displace artefacts. Similar effects could be hypothesised if horses were to be buried in pits or roads/tracks needed to be constructed or upgraded to allow for trucks to remove captured horses. Each proposed measure for horse control should be assessed for its potential to disturb archaeological material.

2.2.6 Control Methods Recommendations

Available control methods

Rehoming and reproductive control are the only ‘non-lethal’ options of wild horse management - noting that rehoming can terminate in lethal control in some circumstances as some horses removed for rehoming may subsequently end up at sale yards and/or knackeries. Reproductive control does not reduce the population substantially or in a short period of time (see Supplementary Material 2 for an explanation of how reproductive control influences population size versus growth rates). Therefore, of these two methods, only removal for rehoming has the potential to reduce the population in the short-medium term. Figure 1.2 illustrates that when locations are truck accessible for live transport of horses out of the park and there are rehoming opportunities, then passively trapping or mustering horses for removal and rehoming is the preferred method as this has the widest community acceptance. Community involvement in rehoming efforts has the potential to increase the success of rehoming captured horses. Many people have gained considerably from their partnership with rehomed wild horses and there are many examples of horses being successfully rehomed. However, it should be noted that there is little information available on the welfare outcomes of rehoming, and on the welfare impacts of the domestication, training and rehoming processes. There is the potential for significant adverse welfare outcomes during this process, in addition to the potential for a lethal end outcome that may also be associated with significant negative animal welfare impacts. Strict conditions for rehoming should therefore be in place to increase the likelihood of acceptable animal welfare outcomes. Furthermore, research is advised to obtain further information about the welfare impacts of the rehoming process and end outcomes of rehomed horses.

There are, however, often limited rehoming opportunities, necessitating additional methods for reducing the population, of which there are only lethal methods available (Table 1). These methods are also necessary for locations that are either not truck accessible, or do not have truck access suitable for live horse transport (e.g. very long, rough, bumpy, and/or hilly tracks). Some lethal control methods require capture of the horse first, whilst others do not (Table 1).

Table 1: Lethal methods available for population reduction

Lethal control methods requiring capture		Lethal control methods not requiring capture
Capture methods	**Control methods**	
Passive trapping Mustering Brumby running	Transport to abattoir or knackery Shooting in trap yard Tranquilising in trap yard followed by euthanasia with captive bolt or lethal injection	Ground shooting Aerial shooting

The SAP recognises that some sections of the community strongly oppose lethal control methods but at the current time it is unlikely for successful management to be achieved without them. The primary objective should be achieving an environmentally and genetically sustainable horse population that can be managed without the need for ongoing lethal control methods. By utilising lethal control at this stage, fewer horses will be impacted in the long term than if ineffective management continues and the population continues to rise. Therefore, at the current time, when rehoming possibilities are exhausted, lethal control methods are advised, focusing on those methods with the least detriment to animal welfare.

For lethal control, the worst welfare outcomes are likely to be those where there are multiple stages (e.g. trapping, mustering, loading and transport, holding periods) prior to death, and those where death is associated with more extreme or prolonged anxiety/fear and/or pain. **It should be noted that ALL methods have the potential for moderate to extreme adverse welfare outcomes,** and so reducing negative welfare outcomes relies on adhering to strict conditions and protocols upon which each method is used. Furthermore, assessment and auditing of actual animal welfare outcomes, and alteration of methods accordingly, is required to continually strive for the lowest negative welfare impacts.

Ethical decision-making on control methods

Welfare impacts of management are a key consideration in choosing management techniques. It should be noted that, in line with current recommendations, the SAP does not advise using the term 'humane' since this is a vague non-specific term that incorporates ethical values in addition to animal welfare impacts, and thus is open to variable interpretations (Hampton et al 2020 *in press*). Rather the SAP recommends using the term 'welfare impact', or more specifically 'negative welfare impact' as this is very specifically referring to the welfare impacts on the animal, which can be assessed scientifically and objectively (more detail in Appendix 3).

A range of management options will be needed to accommodate different variables, such as whether the location is a heritage area, whether it is truck accessible, truck accessible for live horses, whether carcasses need to be removed if horses are shot and what carcass disposal options are available, herd sizes, herd approachability, the terrain and habitat. Different methods will be better suited to different locations depending on these variables (see Figure 2). Additional variables such as efficacy, cost, practicality, operator safety, target specificity, environmental impact, and public acceptability also require consideration (Table 2).

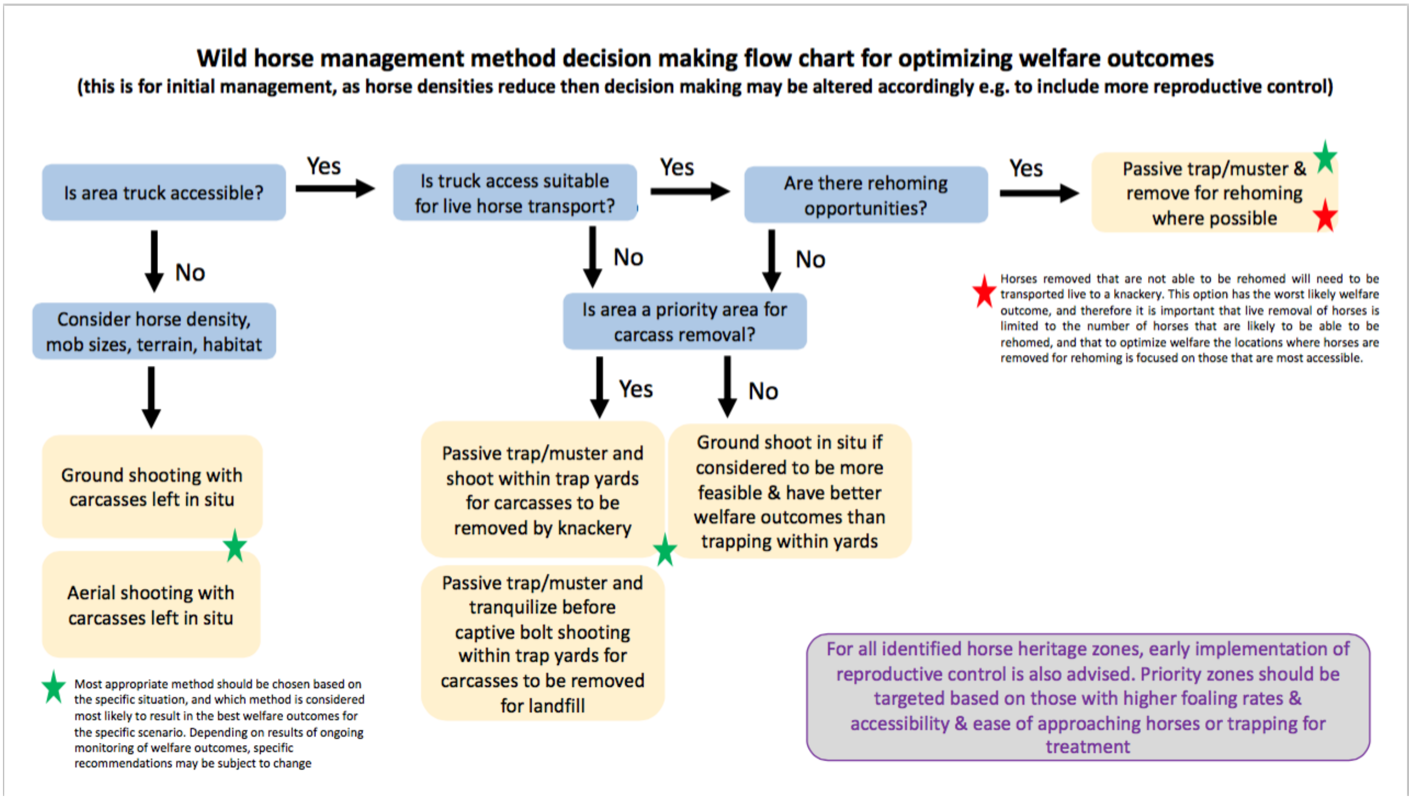

Figure 2. Wild horse management method decision making flow chart

Table 2. Variables other than horse welfare impacts that influence control method recommendations for specific areas

Rate of horse removal required for mitigating negative environmental impacts & preserving heritage populations
Truck accessibility (live horses vs carcasses)
Carcass disposal options
Herd characteristics – size, approachability
Environment characteristics – terrain, habitat type, weather conditions
Efficacy & cost
Practicality & safety issues
Potential welfare impacts on non-target species
Practicality

The actual animal welfare outcomes of the available control methods as applied in KNP has never been formally assessed. Formal studies of actual animal welfare outcomes for euthanasia of horses in trap yards and ground shooting have never been performed. Only one published study of animal welfare outcomes of aerial shooting in horses has to date been carried out (Hampton et al. 2017). Therefore, there is insufficient evidence upon which to base firm recommendations for one lethal control method above another. Consequently, rather than making firm recommendations regarding lethal control methods, the SAP advises choosing future control methods, based on preliminary pilot studies of animal welfare outcomes with a range of available options, as outlined below.

Development and refinement of Standard Operating Procedures (SOPs), and auditing of animal welfare outcomes, should be performed with ongoing involvement from the SAP/newly appointed steering committee and/or additional veterinarians as required, who have specific animal welfare expertise (e.g. MANZCVS in animal welfare and/or animal welfare PhD) in addition to experience in wild horse management.

Recommended selection of control methods based on welfare impacts

1. The ITRG assessments should be used as a starting point (see Appendix 3). From these assessments, management options could be identified where there is high confidence of severe/extreme welfare impacts, and these management options should not be considered or should be a last resort with additional investigation into how welfare impacts could be further mitigated. These assessments also enable identification of specific stages of a management option that may have severe welfare impacts and thus should be avoided (e.g. options that includes separation of and/or mixing of mobs, or involve withholding food and water for > 12 hours). This may not preclude the whole management technique but may mean aspects of the technique need to be improved for welfare impacts to be acceptably reduced

2. SOPs for current methods of capture and population reduction should be reviewed to identify areas where animal welfare outcomes may be able to be further improved. Some management options (e.g. shooting in yards, tranquilising in yards prior to captive bolt or pentobarbitone euthanasia) were not assessed by the ITRG as no SOPs had been developed at that time. SOPs should be developed for **all** available methods, utilising expert advice, and theoretical animal welfare assessments made.

3. Any methods deemed worthy of further consideration based on likely acceptable animal welfare outcomes from theoretical welfare assessments should undergo pilot studies to assess the actual animal-based measures of welfare impacts (as recommended by Hampton et al. 2016; further information in Appendix 3). Results can then inform best future management options and/or further refine management techniques to further reduce welfare impacts – a critical aspect of ensuring acceptable animal welfare outcomes.

4. Every stage of each control method needs to be considered. Many management techniques first require capture of horses and the welfare impacts of this process need to be considered in addition to the welfare impacts of post-capture control methods. Management options with fewer stages (e.g. that does not require capture of horses) are likely to have less negative welfare impacts (see ITRG humanness assessment report).

5. A welfare assessment framework should be devised specifically for each stage of each management option (see 2.2.7, Appendix 3).

6. Consideration also should be given to the welfare impacts on remaining horses, as well as impacts on other species (e.g. indirect effects of large-scale mustering on other species).

Recommendations for control methods that should undergo further evaluation

In line with the above, the following methods for capturing and lethal control should be further evaluated for implementation in appropriate circumstances (considering variables in Table 1.2) with potential alterations to any existing SOPs to further minimise negative welfare impacts (further info Appendix 3). There may be opportunity for community involvement with some capture methods.

- Capture
  - Passive trapping (suggested alterations to existing SOP in Appendix 3)
  - Mustering (either aerial or ground) of small groups of horses performed over hours, in small areas (maximum 2 km)
  - Brumby running
    - Note that this capture method had the highest negative welfare impacts in the ITRG assessment. However, this was based on a SOP from Victoria, and there is potential for many of the negative impacts associated with this method to be mitigated (see Appendix 3). Where substantial welfare impacts can be acceptably mitigated in the development of an alternative SOP, this method could be trialled under strict conditions with actual monitoring of animal welfare outcomes.
- Lethal control
  - Capture followed by transport (of maximum 6 hours) for slaughter at an animal welfare-audited knackery (See Appendix 3).
    - Note that this is only recommended where transport out of the park was performed with a view to rehoming, and was possible to transport the horses out of the park with minimal negative welfare impacts, but where rehoming did not eventuate.
  - Capture followed by euthanasia at the trap site
    - This should be undertaken when no rehoming options are available, or where it is not possible to transport the horses out of the park for rehoming without unacceptable negative welfare impacts (e.g. if journey out of the park is a long and turbulent journey).
    - Various options are available for euthanasia at trap site and the SAP advises that all these options are further evaluated to identify which has the least negative welfare impacts in specific circumstances.
      - Tranquilising (dart or hand injection) followed by captive bolt shooting
      - Tranquilising (dart or hand injection) followed by lethal injection
      - Tranquilising (dart or hand injection) followed by shooting with a firearm
      - Shooting with a firearm without prior tranquillisation
        - Recommendations for trials and further guidelines on minimising negative welfare impacts associated with these options are detailed in Appendix 3.
  - Shooting with a firearm without prior capture
    - Options include ground and aerial shooting
    - The SAP recognises that some sections of the community are strongly opposed to shooting, particularly without prior capture. Methods not requiring prior capture of horses (such as these methods) will likely have less welfare impacts, provided that the control method is carried out according to strict protocols with auditing of animal welfare outcomes (see Appendix 3).
    - The SAP would only recommend use of any of these methods in very specific circumstances, and only if preliminary trials demonstrated better animal welfare outcomes than achieved with other methods that require prior capture.
    - Shooting methods may be particularly preferable for locations where horse density, habitat, and terrain are not favourable for mustering or trapping of horses, and removing from the park.

- More detailed recommendations of important considerations in SOPs and animal welfare auditing recommendations for these control methods are included in Appendix 3. The SAP recommend trialling of both methods under strict conditions to determine animal welfare outcomes, with the ongoing method used to be that with the least negative impacts on animal welfare, alongside consideration of other variables as outlined above.

Recommendations of methods that should not be further considered

- Capture by mustering of large groups, over a period of days, over long distances outside of their home ranges
  - This is in line with ITRG assessments, based on negative impacts associated with mixing of herds, and time period without rest, food and water (see Appendix 3 for further detail).
- Slaughter at abattoirs (see Appendix 3 for discussion of the difference between abattoirs & knackeries)
  - Due to the limited number of abattoirs that process horses, this requires long-distance travel.
  - Due to the number of animals and different species being processed at abattoirs, and the method of killing (captive bolt in a conscious horse), extreme negative welfare outcomes are likely to occur, particularly as independent animal welfare auditing has not previously been permitted (see Appendix 3 for further detail).
- Slaughter at knackeries that requires > 6 hours transport and/or where independent animal welfare auditing is not permitted, or where audits reveal unacceptable animal welfare outcomes.
- Relocation requiring > 12 hours transport without rest, food and water
  - Risks of dehydration, gut stasis, muscle fatigue and delayed urination will be significantly increased for journeys beyond 6 hours without rest, food and water, so journeys of less than 6 hours (without rest/food/water) are advised, with the shorter journeys being preferable. No journey should exceed a maximum of 12 hours without rest, food and water (see Appendix 3 for further detail)
- Relocating wild horses from ‘more sensitive’ to ‘less sensitive’ areas of the park
  - Based on currently available evidence, this would be expected to have a low success rate with poor welfare outcomes, in addition to exacerbating negative environmental impacts in ‘less sensitive’ areas of the park (see Appendix 3 for further detail).
- Establishment of large off park holding facilities to allow more time for rehoming
  - Based on the experience of this practice in the USA, this is unlikely to be successful in increasing rehoming. Further, the SAP is highly concerned about the negative welfare impacts of longer term holding of horses, ability to provide appropriate care in this setting, and increased difficulty in decision making around these horses when no rehoming opportunities are identified.

Rate of population reduction

An issue in past horse management plans has been a failure to consistently remove horses at the rate required to reduce the population. To reduce the horse population size in specific areas, it will be necessary to remove a greater number of horses each year than are produced through reproduction and immigration; the number of horses that need to be removed will vary from year to year. When the population size is substantially reduced, horse breeding is likely to increase because under good conditions at low density, the age at first breeding in mares decreases and the frequency of foaling and foal survival increases (Grange et al. 2009), resulting in more rapid birth rates and higher juvenile recruitment rates (see Supplementary Material 2). This would result in a greater number of horses needing to be removed per year to continue reducing overall numbers (see Appendix 6 for further explanation). Monitoring at each site is required to determine whether the rate of removal is sufficient.

Reproductive control recommendations

Reproductive control alone will not reduce the population substantially in a short-medium timeframe (see Appendix 6 & Supplementary Material 2). However, once the population has been reduced to a lower level, reproductive control may then assist in maintaining this population level, therefore reducing or removing the ongoing requirements for lethal control methods. Earlier implementation of reproductive control trials may be desirable within heritage areas. In particular, the SAP recommends that the Kiandra population be further assessed as an initial early trial site for reproductive control. As the SAP has recommended that this population be used as a key area for a community engagement pilot study, the collection of horse population and demographic data required to instigate a reproductive control program would already be underway. If the population is found to have already been reduced from the 2019-2020 fires, and the horses in this population are already more habituated to human presence, then this may be the ideal location for an early reproductive control trial.

The SAP has discussed a range of reproductive control methods for potential trialling (see Appendix 6). It is important to note that no reproductive control method developed yet is highly effective, easily delivered, affordable, and does not alter the behaviour or physiology in some way (Kane 2018; see Appendix 6 for further detail). Criteria for selecting reproductive control methods are: delivery method, availability, efficacy, duration of effect, and potential physiological and behavioural side effects (see Appendix 6 for a review of each currently available method for mares). Currently, the most efficacious immunocontraceptive vaccines for horses are PZP and GonaCon (see Appendix 5). Neither are licensed for use, nor available, in Australia at the current time. SAP recommends that NPWS submit applications for APVMA approval for the importation and use of both of these immunocontraceptives.

Preliminary studies of approachability and flight distances of horses in various locations in KNP have suggested that only a minority of horses in KNP can be approached close enough for safe and effective dart administration of immunocontraceptives. The SAP recommends that data are collected on horses in management zones identified for horse retention (e.g. Kiandra horses in zone 2 – see section 2.2.1) in regard to approachability/flight distance, band sizes, sex ratios, reproductive rates, and identification of individuals. Once preliminary data have been obtained, further plans can then be made (see Appendix 6 for more detailed proposed steps of implementation). Efficacy and choice of possible immunocontraceptive agents are increased if hand injection is used. Therefore, at least in the initial stages, administration would require horses to be trapped first. The SAP also recommends consideration of trialling other reproductive control methods, such as Intrauterine Devices (Appendix 6). A new oocyte growth factor immunocontraceptive is also currently under investigation in the USA, and the SAP has read the researchers preliminary summary. It has yet to be concluded as to whether this will be an effective immunocontrapetive, but the SAP advise following this research and consideration be given to incorporating this into future reproductive control trials if the opportunity arises.

The SAP advises that any such reproductive control programs are developed by biologists and veterinarians in conjunction with NPWS with involvement of community members where appropriate. There is much expertise within Australia that can be drawn upon including within the SAP, and also outside of the SAP with a range of veterinary reproductive specialists with specific expertise in equine reproductive control, reproductive biologists, and wild horse researchers, in addition to veterinary darting experts. Some community groups have also acquired experience from overseas reproductive control programs. There are several opportunities for community involvement in obtaining essential demographic data for reproductive control, including recording the number of mares, stallions, sub-adults (1 to 2-year-old) and foals (< 1 year old) in subpopulations and identifying individual band and horse characteristics to assist in reproductive control planning.

Please see Appendix 6 for further background information and recommendations.

2.2.7 Animal Welfare Monitoring Recommendations

As outlined in section 2.2.6, theoretical animal welfare assessments should first be performed for each method, using the SOP for that method, and incorporating both best- and worst-case scenarios (as per ITRG assessments 2016). For those methods that are deemed to have likely acceptable animal welfare outcomes based on the theoretical assessment, then trials of the methods with assessment and monitoring of actual animal welfare outcomes should be performed. There are currently very limited data on actual animal welfare outcomes for all of the available methods.

For those management options that require multiple stages (e.g. capture, loading and transport, holding), welfare impacts need to be assessed for each stage of management. In assessing negative welfare impacts, both the **severity and duration** of the impact need to be assessed.

Use of The Five Domains Model for assessing welfare is recommended (Mellor & Reid 1994, Beausoleil & Mellor 2015, Mellor & Beausoleil 2015, Mellor 2017, Harvey et al 2020). The Five Domains Model comprises four interacting physical/functional domains of welfare; 'nutrition', 'environment', 'health' and 'behaviour', and 'mental state' (affective/mental experience). Following measurement of resource and animal-based indices within each physical domain, the anticipated negative or positive affective consequences are cautiously assigned to Domain 5. It is these experiences that contribute to descriptions of the animal's welfare state.

A welfare assessment framework should be specifically devised for each stage of each management option. This should include assessment of impacts within each physical domain, for example:

Domain 1: Restricted food/water

- Time without access to any food
- Time without access to foraging and grazing
- Time without access to water

Domain 2: Environmental challenge

- Degree & duration of physical exertion during high ambient temperatures
- Duration of holding in high ambient temperatures without shade
- Duration of holding in low ambient temperatures without shelter
- Presence and duration of shivering (mild/moderate/severe)
- Presence and duration of sweating (mild/moderate/severe)

Domain 3: Health challenges

- Presence of wounds
- Presence of injuries
- Presence of lameness
- Sickness behaviours: e.g. colic signs, non-ambulatory, inappetence, separation from other horses

Domain 4: Behaviour challenges/restrictions

- Time and severity of restricted normal behaviours
- Time and severity of restricted positive social interactions (e.g. separation from mob members), and increased negative social interactions (e.g. fighting)
- Time and severity of flight behaviour e.g. during musters or helicopter chases

Additionally, welfare impacts associated with methods of lethal control need to be considered separately, to objectively assess measures that indirectly relate to the degree and duration of anxiety and/or pain prior to death. It is a common misconception that non-lethal methods cause less severe animal welfare harms than lethal methods, but this is not always the case (Dubois et al. 2017, Beausoleil et al. 2018, Beausoleil 2020, Hampton et al. 2016). Lethal methods can have less animal welfare impacts if death is instantaneous, particularly if prior capture is not required. The term ‘euthanasia’ means a ‘good’ death, i.e. one associated with minimal anxiety and/or pain. This is clearly desirable in comparison to a death that may involve substantial anxiety and/or pain. The following are examples of key measures that can be assessed (Aebischer et al. 2014, Hampton et al. 2017, Stokke et al. 2018):

- Time to unconsciousness following gunshot
- Time to death following gunshot
- Distance moved between gunshot and loss of consciousness
- Presence of non-fatal wounding
- For aerial shooting, helicopter chase time

Finally, where environmentally sustainable populations of horses are being retained in the park, monitoring of the welfare of these horses is advised. Population densities, that are too high for the food available within a particular habitat, may have poor welfare associated with malnutrition. Methods for monitoring welfare in free-roaming wild horses have been previously described (Harvey et al. 2020). In some regions with very poor nutrition, sustainable populations may not be advisable based on welfare grounds.

Refer to Appendix 3 and Supplementary Material 4 for more information regarding ethical decision making and welfare in horse management.

2.2.8 Community Engagement Recommendations

Due to the adaptive nature of the recommendations being made by the SAP, with the need for substantial ongoing monitoring of horse populations, environmental and Aboriginal heritage impacts, and animal welfare impacts of control methods, engagement of a scientific and community steering committee is advised to assist in successfully implementing and executing the management plan. With a scientific and community committee working together on an ongoing basis, the management plan is much more likely to be successful.

The SAP considers that true community engagement is critical to the success of horse management in KNP, and that a lack of true community engagement is one key reason for failures of previous management plans. Recommendations have been made within sections above where there are opportunities for community involvement. This may occur across a range of management zones. In particular, the SAP recommends that in the early stages, the Kiandra region within Zone 2 is used as a key area for an initial pilot community engagement program. The reasons for choosing the Kiandra area for this are outlined in section 2.2.2. This community engagement program could involve detailed horse population monitoring including local-scale population estimates, collection of population demographic data, systematic evaluation of the heritage value of the horses in this region, individual identification of horses, assessment of horse approachability (to inform future management options), monitoring of horse welfare, and potential genetic monitoring. Involvement in environmental impact and aboriginal heritage monitoring would also be a key feature. Once initial data is collected, optimal management decisions can then be made for this region in collaboration with the community groups. The SAP also suggests that the Kiandra herd might also be an ideal population for instigating a reproductive control trial early on in the course of implementing the new management plan (see Reproductive Control Recommendations in Section 2.2.6). The results and success of this pilot community engagement program can be used to inform further community engagement programs within other management zones as management progresses.

Appendix 1. Review of Horse Population and Impacts

1.1 Horse Population and Demography

It is clear that horse numbers within KNP have increased over the last 40 years. In the late 1980s, there were several hundred wild horses in KNP (Dyring 1990). Based on an aerial survey in 2001, there were at least 5,200 wild horses in KNP (NPWS 2008). After the 2003 bushfires the wild horse population estimate dropped to 2,300 (NPWS 2008). By 2009, there were an estimated 7,700 wild horses in the same area surveyed by helicopter in 2001 and 2003 (Dawson 2009). In response to criticism of the aerial survey methods by some community sectors, as well as to improve the precision, accuracy, and Work Health and Safety of the survey methodology, a new survey design was developed (Cairns 2015). This survey in 2014 produced an estimate of 9,200 horses (Cairns & Robertson 2015) within the area surveyed. The 2014 survey methodology was repeated in 2019, providing an estimate of nearly 20,000 horses in the surveyed part of KNP (Cairns 2019). While the methods used in all aerial surveys since 2001 are scientifically robust (refer to Supplementary Material for further information), estimating the growth rate of the horse population is confounded by the changing methodology from 2009 to 2014 (see ITRG 2016 for further discussion). Also the number of horses within the survey area will change between surveys due to the combined influence of reproduction, immigration, deaths, capture and removal, and emigration. Nevertheless, the changes in population estimates derive from aerial surveys between 2001 and 2019 show clearly the numbers and density of wild horses have been growing substantially within the surveyed areas in KNP.

Counts from helicopter in the open grassland areas of north KNP between 1998 and 2013 by NPWS showed horses were increasing at 23% per year (75 to 1674 horses). The number of horses in north KNP increased from 3,255 in 2014 to 15,687 in 2019, a rate of increase of 37 % per annum (see below for explanation). The annual rate of increase in horse population size depends on how close the population is to the carrying capacity (see Supplementary Material for further explanation), ranging from about 20-23% per year when population size is low, down theoretically to 0% when the population is at carrying capacity (Garrott et al. 1991). The high rate of increase suggests the population is not yet near carrying capacity and may continue to increase at a rate of 20-23% until density-dependent factors (e.g. resource availability) reduce this rate of increase. The rate of increase in north KNP between 2014 and 2019 is above the biologically possible rate of reproduction (Garrott et al. 1991). The likely explanation for this is that there is considerable movement of horses between surveyed and unsurveyed areas that is not accounted for in the survey methodology (Cairns 2019). When horses move between survey areas, this may affect the apparent rates of population growth in a particular surveyed area. The helicopter surveys of north KNP and Big Boggy conducted by NPWS in 2015 provide useful information to this regard; it was observed that an increase in density occurred as conditions became drier, while reductions in density occurred when seasons were wetter (OEH 2016). Thus, horses may be moving into the survey area and onto the moister habitats during dry times and spreading out during wetter conditions. These conclusions are based on a cursory evaluation of the relationship between cumulative rainfall residual and horse density, and thus require further empirical investigation.

1.2 Environmental Impact

The environmental impacts of wild horses, both worldwide and in Australia, were comprehensively reviewed by the ITRG in 2016. Since then there has been a surge in research activity documenting various aspects of the impact of wild horses in the Australian Alps (Worboys et al., 2018). This recent work reinforces the ITRG conclusion that wild horses cause considerable negative impacts on the environmental values of KNP and therefore need to be managed to reduce those impacts. The SAP agrees with the ITRG that most research, including recent studies, are correlative and inferences can be improved by controlled, manipulative, experimental measurement of impacts (Hone 2007).

This can be achieved as part of the management programme to better measure the progress of control operations with respect to minimising negative impacts. The SAP concludes, as did the ITRG, that there is a need to emphasise the importance of measuring environmental impact (instead of just horse numbers) over time, given that reducing negative impact is the ultimate aim of the management plan.

The negative environmental impacts of wild horses have been of concern since the 1950s (Costin 1954) but their impacts were not examined scientifically until the late 1980s (Dyring 1990). Horses can have negative environmental impacts, especially along trails and at stream crossings, through soil compaction and erosion (Dyring 1990; Robertson et al. 2019; Kauffmann & Krueger 1984; Hope et al. 2012; Whinam & Hope 2005). 20–50 passes by unshod horses are sufficient for dry soil compaction to occur (Dyring 1990). This means that a group of four horses would only need to travel along the same route twice a day for five days to cause significant compaction. Dyring (1990) found that soil erosion is greater on compacted tracks than non-compacted soils. In wet, peaty areas, erosion occurs through runoff and soil displacement to the side of trails as hooves sink deeply into wet soil (Marshall & Holmes 1979, cited in Dyring 1991; Lance et al. 1989). The negative impacts along trails also include changes in plant species diversity and cover. While these impacts are of concern, trails often cover less than 1% of a specific region. More work is required to measure compaction and indirect impact on flora and fauna away from trails in Australia, as has been measured in North America (Beever et al. 2003).

KNP contains the most extensive peatlands in the Australian Alps. These ecosystems are unique in Australia and are more sensitive to the impacts of large hard hooved herbivores than other ecosystems (see Worboys et al. 2018). Studies specifically in the Kosciuszko ecosystem show a variety of negative impacts in wetlands. Among the chief threatening processes impacting peat communities in the Australian Alps is physical damage by trampling (e.g. from large hoofed animals), which leads to loss of vegetation cover and altered hydrology and channelling of water (McDougall & Walsh 2002; Hope et al. 2012). Near wetlands and bogs this is linked to declines in sphagnum and sedges, increased erosion, and increased probability of wetland draining, which in turn will negatively affect soft plants (e.g. sphagnum moss) and fauna with specific requirements for well-aerated soils (Dyring 1990; Hope et al. 2012; Rogers 1991).

Negative impacts of horses on bogs and waterways are probably the greatest concern in KNP, particularly because they are important habitats for a range of Commonwealth and State threatened species (summarised in Robertson et al. 2019). Broad-toothed rats (*Mastacomys fuscus*), for example, depend on these habitats and evidence suggests these habitats are being degraded due to the negative impacts associated with a high density of horses (Cherubin et al. 2019; Schulz et al. 2019). While these data are correlative and the inference is weak, differences in rat abundance were attributed to grazing of the tussocks and trampling of the inter-tussock spaces (Schulz 2019). Damage to Sphagnum moss bogs also threatens the Northern Corroboree Frog (*Pseudophryne pengilleyi*) as these habitats are critical to breeding for these frogs (Evans 2018; Scheele and Foster 2018). Habitat loss arising from horses could also threaten the Mountain Pygmy Possum (*Burramys parvus*) by exposing them to fox predation (OEH 2013); however further investigation is required to determine the nature of negative impacts on these species.

While it is clear that horses at a high density have a significant negative environmental impact, the precise relationship between horse density and negative impacts specific to different areas in KNP is not yet known. There may even be positive environmental impacts of horses, at least when their densities are low. Positive impacts observed under light grazing regimes in drier areas could include recycling of nutrients and maintenance of patchy habitat and improve floristic diversity (Menard et al. 2002). For example, higher plant species diversity was maintained by wild horse grazing in the Australian Alps (Wild and Poll 2012; Williams et al. 2014). Horse and cattle grazing is beneficial for an endangered daisy in Tasmania (Gilfedder and Kirkpatrick 1994). The potential benefits of horses, such as reducing fire severity, are not supported by studies of cattle grazing in the Alps (Williams et al. 2006; Williamson et al. 2014) but more thorough studies specifically assessing fuel reduction by horses in grassland are required. The 2016 ITRG acknowledged that there are potentially both positive and negative impacts from horses but concluded that positive impacts are not supported so far by science in the Australian Alps. Monitoring of wild horse control, if designed in a controlled experiment, will confirm or refute this.

1.3 Aboriginal Heritage

The Aboriginal heritage of KNP is referred to in both the 2006 and 2016 Management Plans and widely acknowledged to be diverse and abundant. Traditional connections to the area are strong among the Traditional Owner descendants, as attested in several consultation and planning documents (Sullivan and Lennon 2003, Melville et al. 2015, but also see below extracts of Local Aboriginal Land Council correspondence). Flood pioneered archaeological research in the high country (Flood 1973, 1980), suggesting seasonal occupation built around the abundance of Bogong Moths. Aboriginal occupation at Birrigai Rockshelter in the northern Namadgi foothills has been dated to ~25,000 years before present (Flood et al 1987) and site Y258 in the Yarrangobilly caves area has evidence of visitation between 9,700 and 9,120 years ago (Aplin et al. 2010). Further north on the margins of the high-country, occupation in the Wee Jasper area has been dated to ~14,000 years ago (Theden-Ringl 2016, 2018). Recent work in the Namadgi high country (e.g. Gudgenby, Bobeyan) demonstrates “people were actively utilising the Namadgi high country from almost 8,000 years ago” (Theden Ringl 2016). Since Flood’s initial work, further studies have contributed to a refinement of our knowledge of Aboriginal occupation in KNP, although very few have had fully alpine areas as a basis for study. Commercial heritage studies for environmental assessments have been undertaken in selected high-pressure areas, and these often find evidence of Aboriginal occupation. As a consequence, there are numerous sites recorded around recreational facilities and associated tracks and infrastructure. These are mostly scatters of stone artefacts of varying density (e.g. Geering 1982, Paton and Macfarlane 1988, Feary and Niemoller 2015). The most recent work occurring in KNP has been heritage assessment related to the Snowy-Hydro II project, where artefact concentrations and isolated artefacts were found at 44 locations, and test excavation yielded 2,306 stone artefacts from 180 50cm x 50cm widely spaced excavation squares (Dibden 2018).

Certain areas in KNP contain relatively dense archaeological material, indicating intensive occupation: c.f. Paton and Macfarlane (1988): 246 artefacts at 39 artefacts/m^3 and potential 2 million at the one site; Kamminga et al. (1989): 661 artefacts from test excavation at Lake Crackenback resort with deposit dated to ~4000 before present; Dibden (2018): 2306 artefacts at an average of 51 artefacts/m^2, up to 524 artefacts/m^2. Kamminga (1992) postulated that the Thredbo valley was a major thoroughfare for Aboriginal people moving into the higher mountain peaks from ceremonial grounds at Kalkite and the Wollondibby valley and the base of Mount Crackenback; those sites can be expected to occur throughout the valley (Kamminga 1992). He interpreted the archaeology of the Thredbo valley as a continuous archaeological site, comprising many activity areas and postulated that flaking of quartz pebbles at locations along the valley floor and lower slopes over millennia have produced a high background count of flaking debitage. Dibden concluded a similar pattern at Lobs Hole (Dibden 2018), noting the presence of numerous identifiable individual ‘flaking floors’[1]. The importance of the presence of these features for the purposes of this report is to demonstrate the potential for relatively intact archaeological signatures to be extant in KNP. These signatures can include quite dense clusters of archaeological material and a relatively small disturbance footprint (e.g. horses) has the potential to disturb a large number of Aboriginal objects (see also Plates 1-3 below).

At the time of preparing this report, there are a total of 1003 Aboriginal sites recorded in KNP (see Table 3)[2]. Some of the recorded site locations have more than one feature recorded - for example, to observe an artefact scatter at the same location as a scarred tree is relatively common. The vast majority (90.6%) of these registered places are related to the occurrence of stone artefacts, or their potential to occur. These sites occur throughout the park and their recorded locations will be in large measure due to cultural heritage assessments of proposed development projects or park

[1] A flaking floor is a cluster of stone artefacts produced in one session of flaking stone - it is usually dense and will cover anything up to ~3m^2, but can be larger (see plate 3, this appendix).

[2] ESRI .shp file provided by Mr D.Gordon to Douglas Williams 15/06/2020.

works. They are generally located close to watercourses/sources, but also on more level ground in more elevated locations (ridge crests and saddles, high plains). As might be expected, modified trees have a similar distribution to open artefact scatters, being remnants of everyday living activities.

'Ceremonial Ring' sites occur mainly in the northern end of the park, where higher horse populations occur. This type of site, if remaining extant, would be particularly vulnerable to trampling and scuffage. Stone arrangements are also vulnerable to disturbance through the movement of stones in the arrangement by trampling or kicking. While these sites are found at various locations in the park, they can also cluster at localised landscapes - for example, three stone arrangements recorded in 350m ~11km east of Lake Tantangara.

Table 3. Aboriginal Sites Registered on the AHIMS database in KNP at 15/06/2020.

Site Type	**Description**	**%. records**
Artefact Scatter or Isolated artefact	Surface stone artefacts in clusters or in isolation.	81.20
Potential Archaeological Deposit	An area where stone artefacts may occur below the ground surface, sometimes recorded in associating with a scatter, but not exclusively so.	9.40
Modified tree	Tree from which bark was removed for a utilitarian purpose (container, shield, shelter, canoe) - commonly called a scarred tree, or where designs were cut into the heartwood to designate an important place - commonly called a carved tree.	6.20
Burial	An interment of human remains.	0.60
Ceremonial Ring (stone or earth)	Sometimes called a 'Bora Ring', a raised earth or stone ring used in ceremonies.	0.50
Grinding Grooves	Ground patches or grooves, mainly where stone hatchets were sharpened, sometimes spears.	0.30
Habitation Structure	The remains of an aboriginal dwelling such as a gunyah, but could also be on the record as a historical structure (e.g. a shepherd's hut), or a rock shelter,	0.50
Shell	Culturally transported o accumulated shell	0.08
Stone Arrangement	Stones arranged into a pattern or design.	0.73
Stone Quarry	A source of stone exploited by Aboriginal people, normally for material to make stone artefacts.	0.33
Resource and Gathering	Location of a natural resource other than stone (e.g. location of a bush food or medicine plant	0.08
Art (Pigment or engraved)	As described.	0.08
TOTAL		**100.00**

Plate 1. Artefacts from one 50cm x 50cm test excavation square at Lobs Hole (Dibden 2018:147)

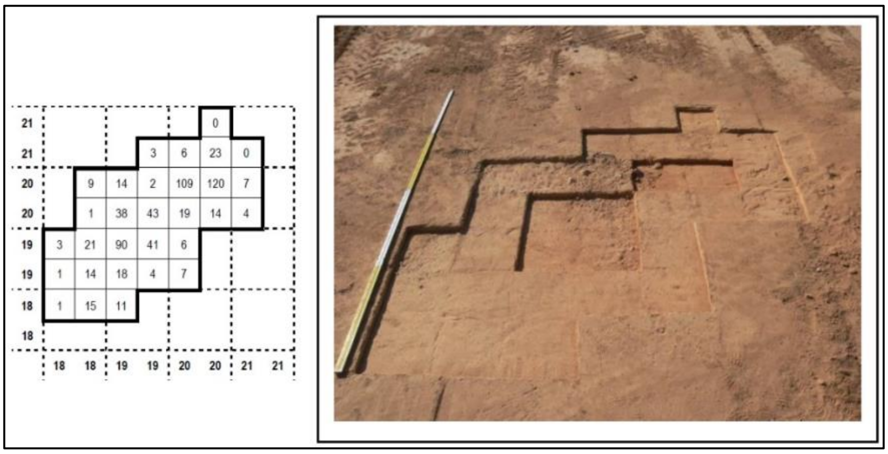

Plate 2. Example 1 of extent of stone artefact flaking floor (Williams 2008:102). Each small square is 50cm x 50cm. Numbers in squares are numbers of artefacts

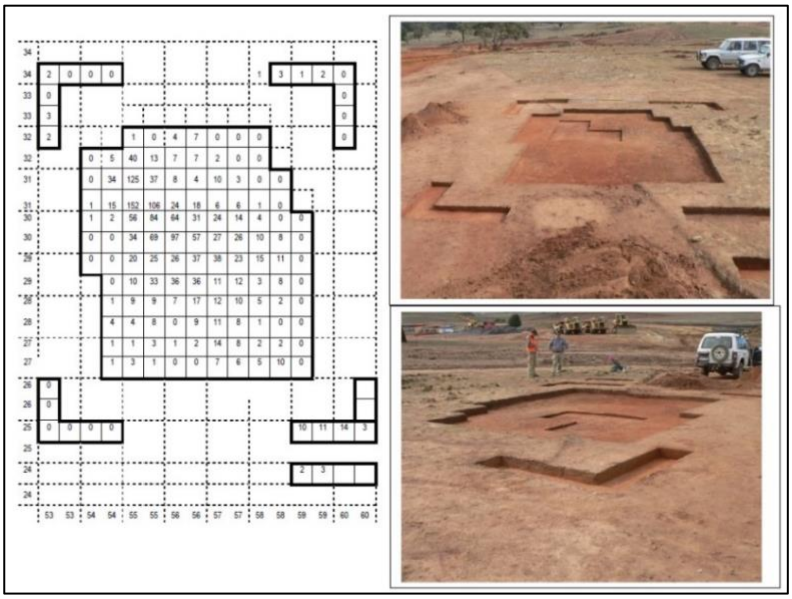

Plate 3. Example 2 of extensive stone artefact flaking floor (Williams 2008:105). Each small square is 50cm x 50cm. Numbers in squares are numbers of artefacts.

It should be noted that site recordings are denser where specific projects have required intensive survey and further investigation; this density of archaeological material might be found in many of the highland and alpine valleys if they were afforded investigation, which has not yet occurred. In dissected upland topography, a significant relationship has been demonstrated between the density of archaeological material and low slope and proximity to water (Cochrane et al. 2013, Dibden 2018).

Other archaeological site types found in the region are human burials. Archaeologically recorded (Feary 1996) and ethnographically noted (Helms 1895), quarries where stone materials were obtained, scarred trees, and occupation sites, such as rock shelters. Ceremonial sites, such as earth rings and stone arrangements, have also been noted. A more complete summary can be found on the Australian heritage Database (AHC 2008).

There has not been time to research the level of immersion of the local Aboriginal people in the pastoral industry of the high country, but references exist that would bear collation and examination (e.g. Wesson 1994, Young et al. 2000, Young 2005) it is certainly a subject worth study. Initial discussion with Ngarigo Traditional Owners highlights the complexity of the issue, for example, "the wild horse issue is … complex for us as our forefathers were renowned horseman on that Country yet still cared for and held and performed Cultural Obligation and Responsibilities for that Country" (White, pers. Comm. 2020).

In other areas of Australia, Aboriginal people found ready work in this industry as stockmen, drovers and shearers, and indeed excellent horsemen. Aboriginal women found employ as domestic staff. The CAP and SAP are referred to the 2015 'competing values study' (Melville et al. 2015), which considered the question of horses and Indigenous Tradition at the National Level. The assessment concluded that any relationship between Aboriginal people and horses in KNP did not meet the threshold of National Significance (Melville et al. 2015), although they also note that 'there are historical and contemporary heritage values held by members of Indigenous tribe and clan groups associated with the Alps and KNP that can be considered under other criteria, given the well-documented roles of Aboriginal people in contributing to the pastoral development of the region'. Along the same lines, it was also noted by Sullivan and Lennon (2003) that 'values associated with the contact period and post-contact Aboriginal life and history of the KNP landscape, including items from the pastoral era, are of potential significance, [could not] be identified because the required research [had] not taken place [at the time the study was completed]'.

Overall, concerns expressed in consultative studies suggest an overriding interest in the conservation of native species, native environments, and sites of significance above post-European Aboriginal heritage values. Recent (2020) consultation of Aboriginal communities with responsibility for land including KNP demonstrates a widely held view that protection of natural and cultural heritage should be prioritised over the retention of wild horses, as attested in the following extracts of letters to the NSW Minister for the Environment:

> The Bega LALC holds the view that the feral horses in the [KNP] negatively impact and threaten Indigenous heritage and cultural values...These values developed over millennia must not be lost through the trampling of feral horses and the destruction of precious life-giving waterways must stop…Indigenous heritage and culture are of greater significance than the plague of…feral horses that are decimating significant cultural landscapes in the [KNP]. Effective feral horse population controls should be implemented immediately to reduce the numbers and the ongoing future management of the mountains must protect Indigenous heritage and culture, so that it can be passed on to all Australians.
>
> Glenn Willcox, CEO, Bega LALC

> The KNP is the birthplace of streams and waterways that give life to the country and form part of the shared heritage for [all] Australians …The brumbies are relics of colonisation…The reality is that a romanticisation of these animals prevails over living

> Aboriginal culture and heritage and that parts of this landscape are so fragile and of such cultural significance to Indigenous people that they are in danger of being lost forever... We urge the authorities to hear our voice as we speak as one and undertake an urgent and humane culling program to preserve country and areas of cultural significance. Future management of these areas must protect Indigenous places so we are able to pass on our stories to all Australians.
>
> Craig Mills, A/CEO, Twofold Bay Aboriginal Corporation

> We are concerned that feral horses are impacting on the source of the Murrumbidgee River. This river is extremely significant to the Wiradjuri and Wolgalu people, both spiritually and culturally. I have seen the damage that horses have caused on the spring at the very beginning of this important river. We would like to see the horses removed from this fragile and important area.
>
> Sue Bolger, CEO, Brungle Tumut Local Aboriginal Land Council

> Indigenous people have lived in the Snowy Mountains for thousands of years. The mountains are unique and very important to Aboriginal Heritage and Culture. There is no other place like them on earth. The environment contains the only representation of many endangered species and it is being disregarded. Our culture is also unique because of the environment and the dreaming that has been given to us. Both are in danger of extinction and both must be protected. Feral horses are destroying our heritage and erasing our culture….The destruction of thousands and thousands of years of Indigenous heritage and culture, must not be lost through the trampling of feral horses. The destruction of our precious life-giving waterways must stop…Our heritage and culture are of greater significance than the plague of ever-increasing feral horses that are decimating our sacred mountains... We ask that we are respected and that the culling of feral horses begins immediately, and that culling is carried out to the degree that it is effective and results in the preservation Indigenous places. The ongoing future management of the mountains must protect our heritage and culture, so that we are able to pass on our stories to all Australians.
>
> B.J. Cruse, Board Chair, Eden Local Aboriginal Land Council

These extracts demonstrate several important points with regard to Aboriginal heritage considerations. Firstly, they eloquently express a connection to the land and environment beyond the somewhat narrow view of archaeological sites. The waterways (in particular), the landscape features, flora and fauna all interconnect in a holistic consideration of 'heritage'. Secondly, there is an exceedingly generous desire to share knowledge of that heritage with all Australians. Finally, and crucially, they express a fear that the ability to preserve and share aspects of their heritage is being lost through damage being done by feral horses.

Legislative Protection

In NSW Aboriginal Objects and Places are protected by the provisions of the *National Parks and Wildlife Act* (NSW) *1974* (as amended) (The Act). The following sections are particularly relevant:

- **Section 3**. The Act binds the Crown, with respect to NSW and "in all its other capacities"
- **Section 85 (2) (a).** The Chief Executive is responsible for the '*proper care, preservation and protection* of any Aboriginal Object or Aboriginal Place on any land reserved under [the Act].

- **Section 86 (1)-(8).** A person must not harm or desecrate Aboriginal objects or Aboriginal places - knowingly or unknowingly, though being guilty of knowing harm carries higher penalties.
    - Under the Act, harm is defined as any act or omission that:
        - (a) destroys, defaces or damages the object or place, or
        - (b) in relation to an object--moves the object from the land on which it had been situated, or
        - (c) is specified by the regulations, or
        - (d) ***causes or permits*** the object or place to be harmed in a manner referred to in paragraph (a), (b) or (c)
- **Section 87 and 87A**. There are exemptions from harm to Aboriginal Objects and Places under s.86, mainly:
    - If harm is permitted by an Aboriginal Heritage Impact Permit (AHIP)
    - If harm occurs during certain emergency activities
    - If harm is inadvertent and the person has exercised *due diligence* prior to undertaking the action.
- **Section 90**. Harm may be authorised by an Aboriginal Heritage Impact Permit.

In NSW there is currently no threshold of significance applied to the Aboriginal Objects or Places protected by the Act. - it is an offence to CAUSE or PERMIT destruction, defacement or damage to even one artefact however small or 'insignificant'. Permitting horses to proliferate unchecked or unmanaged in locations where Aboriginal Objects occur might be regarded as contra to these provisions and intent.

Impact of Wild Horses on Aboriginal Heritage

Unchecked numbers of horses in KNP have the potential to cause irreversible damage to Aboriginal heritage places in KNP. Aboriginal heritage places/values fall into a number of categories for the purposes of management in KNP. While Aboriginal people might rightly regard these as a continuum of values embedded in the overall landscape ('country'), these might be summarised as:

- Archaeological
- Natural
- Historical

Archaeological Resources

Aboriginal archaeological resources occur under, on, and above the ground. As indicated, the most common type of archaeological site is a scatter or cluster of stone artefacts. These sites are where Aboriginal people discarded stone artefacts in the course of either manufacture or general living activities. Table 4 (below) is a very preliminary summary of archaeological site types that might be expected in KNP and potential impacts from horses.

An important feature to note about archaeological resources, in comparison to many other ecological and environmental variables, is that once disturbed or destroyed, there is no way of it being undisturbed or undestroyed; there are no methods of regeneration. So, while environmentally degraded areas might regenerate following de-stocking or erosion control, the archaeology within it remains compromised. Archaeological research of open sites depends largely on the study of spatial relationships of elements within sites. When that spatial relationship is disturbed, it cannot be reestablished. This potential for damage is referred to in several planning documents, but there has not yet been a dedicated study on the impact of wild horses on Aboriginal heritage places. For example, the 'Feral Horse Impacts: The Kosciuszko Science Conference' (Worboys et al 2018) contains no assessment of the impact of horses on Aboriginal heritage. Given the demonstrated relationship between low slope, presence of water and high density of archaeological material (Cochrane et al. 2013, Dibden 2018), and given this mix of topography will also be highly attractive to horses, horse congregation likely has a negative impact on Aboriginal archaeological sites. However, to the best of our knowledge, there is no study of the effect of wild horses on Aboriginal archaeological sites. Such a study should commence as soon as possible.

Table 4. Archaeological Site Types Expected in KNP and Potential/Predicted damage from horses

Site Type	Frequency of occurrence	Predicted location	Vulnerable to	Predicted Likelihood of damage
Isolated stone artefacts (1 in isolation, say 100m from any other)	Very high	Flats, low to moderate slopes nearly anywhere	Breakage, moderate movement.	Low where horse groups are small. Moderate where groups are large and/or traffic is high or repeated
Sparse artefact scatters/simple sites (2-20)	Very high	Flats, low to moderate slopes nearly anywhere. Increasing likelihood closer to water	Breakage, moderate movement,	Low where horse groups are small. Moderate to high where groups are large and/or traffic is high or repeated, subsurface artefacts/site structure may be compromised where soil churning occurs. Consequent erosion/deflation is also a threat to site integrity
Dense artefact clusters/complex sites	Moderate	Flats and low slopes close to water, moderate to large plateaux or other wider level to gently sloping area in dissected topography,	Breakage, artefact movement, site deposit deflation or churning (loss of integrity)	Moderate to high, environmental factors leading to site location are also very attractive for horses. | Accumulation of horses leads to accumulation of impacts - churning of artefact bearing deposit, loss of site integrity, deflation/erosion.
Human Burials	Low	Creek flats	Erosion (destabilised banks), crushing	Overall low, as site frequency is low, but potentially high if encountered.
Scarred trees	Low	Almost anywhere, more frequent near water/environments that support larger trees	Rubbing, root destabilisation	Overall low, as genuine scarred trees are rare (or rarely recorded), but where they do occur, they will be old and potentially susceptible to external pressures. Often scarred trees are dead (standing or fallen), where they have not been destroyed by fires. Horses rubbing against a dead tree or destabilising around the roots of a dead standing tree would hasten deterioration

Rock shelters and caves	Low	Karst systems (caves) or large granite boulders forming shelters between	Erosion.	Overall low, no known karst formations where horses can shelter, and granite shelters are usually too low for horses to enter. However, numbers of horses sheltering around granite boulders could destabilise soil and cause external erosion, allowing for the erosion of internal deposit. Flat areas around granite boulders were also occupied, people using boulders as a windbreak. Horse treadage could potentially disturb significant archaeological deposit in some locations
Stone quarries	Low (but unknown and unresearched)	Anywhere useful stone sources occur	Breakage, artefact movement, erosion,	Overall low, quarries are rarely recorded so unlikely to be encountered. Their locations are also geologically specific rather than determined by day to day survival and comfort requirements. On balance, it might be predicted that quarries might occur in locations generally unattractive for horses (rocky outcrops), but not exclusively so.
Rock Art	Low	Caves, overhangs and granite boulders	Rubbing art off walls	Very low potential for impact due to very low potential for occurrence.
Stone Arrangements	Low	Level to gently sloping ground in secluded harder to reach locations	Displacement of stones, loss of patterning.	Very low, based on the rarity of these sites in the knowledge database, but also a low level of investigation. A high impact could be predicted if horses congregate at the location of a stone arrangement
Axe grinding grooves	Low	Where suitable stone occurs (sedimentary, mainly sandstone, but also granite in rare instances)	Wear, breakage	Overall low. Grinding grooves would not be expected to occur with frequency and would be resilient to an extent. However, any that did occur would be of high significance.

Natural Aboriginal Heritage Resources

This category of heritage might include the full gamut of environmental variables, including bush foods and medicines, water quality, and non-economic species with cultural significance (e.g. Corrobboree frog, Pygmy possum). While ecological and environmental conservation measures will conserve these values, the Aboriginal cultural element/connection to these resources should be more explicitly referenced as part of their value. Bushfood and bush medicines should be a particular focus of research, documentation, and monitoring for impacts (both negative and positive) by horses. The importance of this range of heritage values is demonstrated by ubiquitous opinion provided by four Local Aboriginal Land Councils surrounding and including KNP (see extracts above).

Historical Heritage

This phase of Aboriginal heritage is that of the European contact and post-contact period, and may indeed include a connection to horses and the pastoral industry. It may also include components of archaeological sites where there is material evidence of the adoption of European material culture into traditional Aboriginal life (e.g. glass flaked artefacts). Connections to this phase of Aboriginal history may be intangible and would bear research in terms of the impacts the removal of wild horses would have on the strength of community memory.

Appendix 2. Review of Past Plans

2.1 2003 Plan

Horse Management Plan: For the Alpine Area of Kosciuszko National Park January 2003 – January 2005 (20 pages)

Preparation of the 2003 Horse Management Plan commenced in the year 2000. The importance of involving the community was recognised. A Wild Horse Management Steering Committee was established, and the committee was "*instrumental in developing the draft plan*".

The primary objectives were:

- *"To conserve and protect the natural values of the Kosciuszko alpine area (above the treeline – approximately 1850 metres) by removing horses and to ensure the alpine area remains free from horse impacts; and*
- *To minimise the likelihood of horses causing a traffic hazard on the Alpine Way."*

The 2003 Plan examined the range of horse management methods available, including immobilisation using tranquillisers, fertility control, fencing, shooting and capture and removal methods. The plan proposed to trial trapping, roping and mustering (using horse riders) and these methods were to be evaluated, *"for their effectiveness in humanely removing horses from the alpine area and reducing environmental impacts."*

"To measure changes over time and responses to the implementation of management strategies, four sites that exhibit existing impacts from horses were chosen for monitoring." Monitoring sites included: Little Tin Mines Creek, Cascades Hut and the Big Boggy. At each site, there was to be an assessment of vegetation, water quality, horse population and the environmental impact of the removal process. Exclusion fenced areas (10 m x 10m) were to be built at each site.

A goal of the plan was to reduce horse numbers and collect information to allow NPWS to modify and improve management techniques as appropriate, i.e. adaptive management. The population was estimated to be 1400 in south Kosciuszko. They estimated that a removal rate of 100 horses per year would reduce the population to 800 horses in 30 years. A removal rate of 150 horses would reduce the southern population to 850 horses in eight years. There was no mention of north Kosciusko although since the total population for KNP was reported to be 2300, and 1400 were in south Kosciuszko, there must have been around 1000 in north Kosciusko at that time.

Community consultation at workshops and information sessions determined that "*management of feral horses is a very polarised issue"* with views ranging from total eradication to the view that "*horses have heritage value and should be retained in national parks*".

There was discussion about ground and aerial shooting. Some members of the Wild Horse Management Steering Committee were "*adamant that they would never endorse shooting as an acceptable option.*"

There was agreement that:

- The alpine area of Kosciuszko is a unique environment which must be protected from the impacts of horses.
- Control methods must be humane.
- Management of horses shouldn't be limited to the alpine area but should be extended across the entire park.

Achievements

- The Plan was implemented.
- Used estimation of total population size and recent ecological information (Michelle Dawson's PhD).
- Planned to monitor environmental impact and the benefits of control.
- Used community members and community consultation to prepare the Plan.
- Used a simple population model to predict the change in population size depending on the removal rate.
- Removed 206 horses from 2002 to 2007 with 64 horses being removed from November 2006 to February 2007 (NPWS 2008).

Performance with regard to objectives

- Horses continued to increase in the Alpine area (NPWS 2008; NPWS 2016).
- Horses are still a traffic hazard on the Alpine Way (NPWS 2016).

2.2 2008 Plan

Kosciuszko National Park Horse Management Plan December 2008 (36 Pages)

"In 2006 a Plan of Management for Kosciuszko National Park was formally adopted and one of its objectives is to reduce the distribution and abundance of introduced animal species found in the Park. The Plan of Management called for the exclusion of horses from key areas and for a Feral Horse Management Plan to be prepared for the whole of the Park." (NPWS 2008)

The 2008 Plan was part of the response to the commitment in the 2006 Plan of Management for Kosciuszko National Park. As with the 2003 Horse Management Plan, a Horse Management Community Steering Group assisted with the preparation of the 2008 Plan.

"*The Steering Group examined the range of horse management methods available, including fertility control, fencing, shooting and capture and removal methods and some of the issues associated with each of the methods in the document. After reviewing the different methods, the Steering Group recognised that different techniques are best suited to different situations depending on issues such as mob size, geography and season. The Group agreed that as with any vertebrate pest program, a combination of different techniques will give the most effective result." (NPWS 2008)*

The objective of the 2008 Horse Management Plan for Kosciuszko National Park is to exclude horses from:

- the Main Range Management Unit;
- the Yarrangobilly Management Unit;
- the Cooleman Plain Management Unit;
- Safety risk areas such as highways;
- Areas of the park where horses have not been or have only recently been recorded (e.g. Jagungal);
- Areas of the park adjoining other Australian Alps national parks and reserves; and
- Feeder areas for all management units.

"*To measure changes over time and how our management strategies are working, we will have an environmental monitoring program in areas across the Park. This program will involve assessing vegetation and water quality at 4 sites that show signs of existing impacts from horses within the proposed horse control areas.*" (NPWS 2008)

Achievements

- Plan was implemented.
- Used estimation of total population size and ecological information (Michelle Dawson's PhD).
- Identified where horses were to be removed completely.
- Planned to monitor environmental impact at four sites.
- Planned to established photo monitoring points.
- Planned to monitor impact on cultural heritage.
- Planned to train staff in the appropriate methods of trapping and removal.
- Planned to involve RSPCA.
- Used community members and community consultation to prepare the Plan.
- Removed 2977 horses from 2008 to 2016 (ITRG 2016; NPWS 2008; NPWS 2016).

Performance with regard to objectives

- Horses continued to expand their distribution and abundance (NPWS 2008; NPWS 2016).
- Horses have not been completely removed from any of the areas identified for elimination.
- Horses are still a traffic hazard on the Alpine Way and along the Snowy Mountains Highway (NPWS 2016).

2.3 2016 Plan

2016 Draft Wild Horse Management Plan (47 pages)

The SAP members conducted a detailed review of this draft plan and prepared advice on how to address issues.

The 2016 Draft Wild Horse Management Plan had three objectives:

1. *To reduce the impacts of wild horses on the natural and cultural heritage values of Kosciuszko National Park by reducing the overall population of wild horses using a range of cost-effective and humane control measures.*
2. *To reduce and mitigate the risk of adverse wild horse interactions or incidents with park visitors and the public more generally.*
3. *To involve the community in the ongoing management of wild horses in Kosciuszko National Park through active participation in research, monitoring, and control programs where possible.*

Key strategies to achieve these objectives was to reduce the wild horse population from 6000 to less than 3000 horses in five to 10 years; and to reduce the population to 600 (400–800) horses within 20 years.

Achievements

- Used estimation of total population size and available scientific information via the Independent Technical Reference Group (ITRG 2016).
- Reviewed 2008 Horse Management Plan.
- Assessed the national cultural heritage values associated with the Kosciuszko National Park wild horse population (Context 2015).
- The heritage value of horses was acknowledged.
- Accepted the ITRG advice that complete eradication is not achievable.
- Used community members and community consultation to prepare the Plan.
- Planned to involve community groups in ongoing management through active participation in research, monitoring and control programs.
- Identified zones where horses were to be removed completely.
- Defined Management Zones, Prevention, Elimination, Containment and Population Reduction, Key Environmental Asset Protection.
- Where horses were to remain, the plan was to adjust or determine densities of horses based on acceptable levels of impact.
- Planned to use Adaptive Management.
- Acknowledged there were significant knowledge gaps and with the help of the ITRG recommended research required.
- Planned to establish a scientific panel to design a wild horse survey methodology that quantifies the environmental damage caused by wild horses, in addition to estimating total wild horse numbers.

Performance with regard to objectives

- Was not implemented

Note on Community Engagement

The community must be truly included in the preparation and implementation of the Plan. Past management plans have all attempted some form of community involvement (Melville et al 2015), but true engagement appears to have not been achieved. Past efforts include consultation, education, workshops, information sessions, online surveys, and “consideration of community views”. A lack of involvement of community representatives in the preparation of the management plan is considered to have contributed in part to the failures of past Plans.

Detailed Review of 2016 Plan

The SAP reviewed the 2016 KNP Draft Wild Horse Management Plan and the accompanying Final report of the Independent Technical Reference Group in detail. There are many gaps in scientific knowledge identified by the ITRG and the SAP. SAP members considered the use of unpublished data and local expert knowledge to help fill some of these gaps. Still, the collection of the most useful new information will need to be by incorporating scientific experimentation into the management plan. Integrating science with management is termed ‘adaptive management’, which was the approach also recommended by the ITRG.

Table 5 (below) lists detailed issues, describes the problems identified by SAP members, and suggests possible solutions.

Table 5. SAP review of 2016 Plan

Issue	Problem	Solution
The Management Plan, the ITRG Report and SAP advice	The ITRG advice was not sufficiently incorporated into the 2016 Plan. The Plan should have been a ‘stand-alone’ document, with the ITRG 2016 review providing ‘supporting information’. It should not be necessary for someone to read the ITRG review, unless they desire to do so.	The SAP will ensure information from the ITRG 2016 Report along with more recent scientific and other evidence is incorporated in the 2020 Plan.
Basis for the plan	The conceptual basis for the 2016 Plan acceptable, but it is implicit rather than explicit.	The logical sequence should be explicitly stated as follows: • There are many wild horses in KNP and other nearby areas • These horses have been and are causing significant negative environmental impacts and risk • Therefore, numbers of horses should be reduced • There is spatial variation in horse density, impacts of various kinds, cultural value, and practicalities of reducing horse numbers (and hence density) • Therefore, KNP (and other nearby areas) should be divided into different areas that are subject to various horse management regimes (e.g. prevention, elimination, etc.)
Supporting evidence	Many statements lack appropriate substantiation, and the plan therefore is not scientifically adequate. No information or citation is provided to support many assertions.	Need to be transparent about where information is lacking. Information can be published or unpublished from personal observation, but the source of the information must be supplied. SAP to provide citations, identify sources of unpublished information, and make sure it is clearly stated where there is uncertainty, for 2020 Plan.
Strategy to reduce the population from 6000 to 600.	No strong justification or explanation for selection of the reduction from 6000 to 600. This antagonised horse protection groups and they were mobilised against the plan.	The ITRG 2016 report stated: *“The outcomes of management would ideally be monitored primarily through the effects on agreed impact measures or thresholds of concern, rather than just on horse numbers or densities”*. This statement from the ITRG report is consistent with recommendations from “Managing Vertebrate Pests: Feral

Issue	Problem	Solution
	Insufficient support presented that reducing the population size to 600 horses (the key tactic) will have the desired positive effects.	Horses" (Dobbie et al. 1993) and the SAP. Less focus given to a concrete ultimate goal number and more emphasis is given to reduction of negative impacts. The main goal should be to minimise the negative impact and maximise positive impact. The overriding goal needs to be one that all key stakeholder groups are happy with (a common goal).
Vagueness regarding control methods to be used	'The decision on when and where one or more of the seven methods … will be employed will be determined by NPWS based on a range of considerations, including [a list of conditions' (page 25 KNP 2016 Plan). Vagueness/lack of transparency here may be a point of concern for opponents.	The new plan needs to be much more explicit what methods will be used where and what initial targets in, say, the first 5 years would be. While it is not necessary to detail the exact operating procedures of each method, providing greater detail of how control methods will be selected may strengthen support of the plan. Incorporate welfare impact assessment into decision making. People may be less worried, having the knowledge that animal welfare outcomes are to be assessed and transparently reported before progression of the plan.
Monitoring horse welfare	Little mention of monitoring horse welfare	As density increases, and environmental impacts increase, horse welfare may reduce. Severe welfare impacts can occur in self-limiting/resource limiting populations, so there are good welfare arguments for keeping population densities lower. There are also specific habitats where there are more horse welfare issues due to lack of nutrition (e.g. the lower snowy region) (Harvey unpublished data). There will be positive welfare impacts for the remaining horses caused by reduction in horse density and negative welfare impacts if too many horses remain. Thus, monitoring of horse condition is required. Set 'welfare targets', such as what % of the local population should not be below a BCS of 3/9 for example, so if > 60% have a BCS > 3/9 it is an indicator that the population needs to be further reduced (Harvey et al. 2020).
Evidence for environmental impact	The plan makes statements such as 'the preference for grassland and heath habitat is likely to eventually alter the ecology (page 16)'. Everyone should be concerned that the removal of 1000's of horses is based on 'likely eventually alter'. 'Snow patch herb fields need protection from introduced mammals (Williams 2005)'. No data to support more specific role of horses. It is possible that adverse impacts of horses reflect their abundance, and this could guide management, but no relevant information is presented. Such information is therefore much needed. It is clear that wild horses adversely affect various natural values of KNP, but it is not clear how to integrate this information into park management. For example, the nature, extent, and spatial distribution of such	Update references with new/more relevant research which can then be used to strengthen language such as 'likely eventually alter'. For example, studies such as (Wild et al. 2012) that exclude horses but not macropods. Other recent and relevant literature to include (Cherubin et al. 2019; Robertson et al., 2019) and all studies presented in (Worboys et al. 2018) which are specific to wild horses in Australian Alps. The conclusions from the existing evidence of environmental impact will need to be presented. This evidence will need to be improved upon substantially to ensure the cost, effort, and horse welfare impact of horse removal activities will be certain to produce real benefits to the environment in KNP. While it is clear that horses have a negative impact, a criticism that is often raised is the lack of data demonstrating horse impacts independent of other species. The 2020 Plan should take the opportunity to fill this knowledge gap. The 2020 Plan should involve Adaptive Management where performance of work to reduce horse

Issue	Problem	Solution
	impacts are not described, making it impossible to know where to aim to reduce any impacts. ITRG 2016 Report: “controlled experimental studies are rare, and most rely on a correlational approach and are often complicated by the presence of other herbivores (Beever & Brussard 2000). This issue is not acknowledged in 2016 Plan.	density is measured by controlled experiments measuring changes in environmental impact. Adaptive Management “learning by doing” is required. That is, management monitored scientifically.
Impact terminology	There is inconsistent use of impact, adverse impact, and damage. Need to acknowledge that there are positive and negative environmental impacts and not assume all impact is negative. In some places, impact is written where negative (adverse) impact or damage should be written.	Refer to impact as positive or negative impact consistently throughout. Acknowledge the possibility that there could be positive impact.
Positive environmental impact	The ITRG and horse opponents believe there is no scientific support for the presence of positive environmental impacts caused by horses. There is, however, some support for the hypothesis that some native plants or animals may benefit from the presence of horses, particularly if horse density is moderate.	There are a reasonable number of studies that have found positive impacts of wild horses overseas (Soriano 1991; Zalba and Cozzani 2004; Loydi and Zalba 2008) and in Australia (Wild and Poll 2012, Williams et al. 2014) and there are theoretical reasons to expect positive impact at moderate grazing density (Connell 1978). Experimentation measuring impact at a variety of densities is required to prove or discount the presence of any positive impact.
Separating impact of horses from other animals and from natural factors	Little information provided regarding the impacts and control measures of other grazing species. While it is clear that horses can have negative impact, a criticism that is often raised by opponents of horse control is the lack of data demonstrating horse impacts independent of other species.	Acknowledge that other species have negative impacts and specify the control measures undertaken to mitigate their negative impacts. Monitoring must include measurement of other things that may cause environmental impact. Deer/pig dung or hoof print counts, rabbit/hare pellet counts, camera trap indices for all species. Control of these species needs to accompany the horse removal activities. If other animals such as deer, pigs, rabbits, foxes, cats or dogs are present, and not managed as well the removal of horses may have no benefits to native plants, animals, or soil.
Aboriginal cultural heritage	The 2016 Plan does not consider or address the issues of managing Aboriginal Cultural heritage to a sufficient extent. The description of the three management regions touches only once on cultural heritage (the central region), and the management strategies/priorities do not include any strategic assessment of the likelihood of horses causing damage to archaeological sites. It is an omission that none of the actions specified in the five proposed management zones include actions specific to understanding the effect of horses on archaeological sites. This might have been particularly important in management of the ‘containment zones’	A process for considering the effect of management actions on Aboriginal cultural heritage should be included in the revised plan. One particular aspect of managing horses that has not received consideration is the potential impact of management actions/techniques on Aboriginal cultural heritage places. Consider for example trapping or corralling. The installation of fences capable of holding horses requires posts inserted into the ground surface, this and related construction has potential to disturb Aboriginal objects. The accumulation of horses in a fenced area has potential to concentrate considerable numbers of horses in one location, and as a result, exacerbate trampling damage to Aboriginal Objects.

Issue	Problem	Solution
How much reduction in horse density is required	It is clear that where there are too many horses there is considerable negative impact. Most agree that a reduction in numbers is necessary, but the level of reduction required is unknown. No research conducted so far helps to determine this level.	The historical densities and impacts from photos or expert knowledge might, to some extent, help determine a starting goal for density reduction. This goal will be different for different management zones. Refining the appropriate density that is most suitable for the various management zones must be determined by monitoring horse impacts. Sites selected for monitoring must be sufficient in number to represent each management zone. Monitoring methods must be detailed enough to allow identification of acceptable target horse densities.
Movement between neighboring properties and between areas within the park	It is believed that the relatively high numbers and densities of horses in adjoining areas have been a factor in the movement of horses into the alpine area and expansion in some areas. Any management within the park might be made less effective by lack of management (or growth-positive management) in the adjoining properties. There is no clear plan as to how the park will deal with neighbouring landowners.	Assessment of neighbouring areas to determine population sizes and perhaps radiotelemetry is required to determine how much movement there is into and out of the park. At the very least home range sizes need to be determined by radio-tracking. Include some discussion regarding how much connectivity occurs between the four park populations as well as from outside the park (if known). Provide a plan for mitigating incursion to areas of where removals are being undertaken.
Horse Impact on Fire	Common claim from horse protectionists is that horses reduce fire intensity or spread by reducing fuel (ITRG Report). Horses are also accused of increasing the vulnerability of peat to fire by drying out the swampy areas. There is a lack of information about the impact of horses on bush fire fuel and the impact of bush fires on horses. There is also little information on the impact on horse welfare caused by bush fires. There is little consideration to the response after a bush fire, e.g. take advantage and further reduce density? Will there be greater impact of horses on native wildlife because of the fire and limited resources?	Identify where fire has damaged peat and show that the drying caused by horses was a contributing factor. Keep horses out of areas threatened by this process. It is assumed that horses have the same impact as cattle in that by eating predominantly grass they increase the number of flammable shrubs. Where has this happened and has it been documented? Presumably, there should be a correlation between horse density and increases in shrub density and then fire frequency or severity. Include other studies such as the report on fires in the Alps (Zylstra 2006) that suggests that the increased burning by European settlers changed the vegetation to make it more fire-prone not the influence of introduced ungulates. Furthermore, Walter (2003) found that horses were not congregating in unburnt areas after the 2003 fires. A better understanding of the relationship between bush fires, horses, and horse impacts will help inform the proper response to bush fires.

Issue	Problem	Solution
Community Engagement	While the 2016 Plan made an effort to engage community groups, many still feel that their views have been ignored and this likely contributed to the upheaval of the Plan. In the ITRG 2016 Report, Appendix B: Stakeholder submissions to the ITRG on horses in KNP, there were four stakeholder types identified (Groups A to D). Groups A and D are the extremes. Group A thinks there is no management required, and Group D believe eradication is required. The conflict between these groups polarises the issue, and the moderate stakeholders move one way or the other.	The SAP considers the conflict between these groups prevents sensible management. Group D activities provoke Group A and vice versa. The only way to achieve sensible management (reduction in horse numbers and reduction in negative impact whilst also protecting the horse heritage values) is for these groups to have a common goal and work together. Group A needs to be convinced that management is required. Group D needs to be satisfied with less than eradication. The whole plan needs to be owned and accepted by the major interest groups. These groups need to be involved in the preparation of the plan from the start. The objective proposed in 2016 to involve the community in ongoing management should be expanded to include involvement in the preparation of the plan. This is happening using the CAP working with the SAP. The CAP appears to represent key community interest groups.
Involving the community in ongoing management	There has been a lack of acceptance by the community of horse population estimates. This is in part due to their opinion that there is a lack of transparency in how these estimates are obtained.	Mobilising groups of volunteers to assist in quantifying important Kosciuszko-specific demographic parameters (i.e. death rates, lifespans, foal rates, foal mortality, sex ratios) as the population is reduced may be a good area for community engagement. Using volunteers to identify animals and monitor foaling rates has been successfully used for some populations of wild horses in the USA (e.g. Baker et al., 2018; BLM staff, pers. comm). However, this may not always be feasible due to practical constraints relating to park conditions. Community involvement in heritage herd identification. There are herds for which the public know individual horses and are very attached to them. This would include some areas of Long Plain, Tantangara, Kiandra, some areas down Barry Way such as around the Pinch etc. These may be herds where reproductive control is considered earlier on, as these horses are also more likely to be more habituated to people and thus easier to identify and capture or dart. It is these populations that are most known to the public and horse lobby groups. How these populations are managed will impact the most on how these groups/public feel about the plan.
Legislation	Legislation is interpreted differently by different people. Many believe that horses should not be in national parks. They appear unaware that this is not written into legislation. This needs to be made clear. Many believe the Wild Horse Heritage Act prioritises horses over natural values and that lethal control has been banned. This is also not the case and needs to be made clear.	The National Parks and Wildlife Act 1974 No 80 requires conservation of habitat, ecosystems and ecosystem processes and biological diversity at the community, species and genetic levels and identification and mitigation of threatening processes. The new Wild Horse Heritage Act wording should also be included so that it clarifies that it does not prioritize horses over natural values and that lethal control is **not** legislated against. Where there is a conflict between the new Wild Horse Heritage Act and the National Parks and Wildlife Act 1974 the Wild Horse Heritage Act will prevail but there is unlikely to be significant conflict particularly regarding prioritizing horses over natural values. What happens in areas where horses have been retained and managed for heritage reasons and there is still significant negative impact on natural values?

Issue	Problem	Solution
Horse biology	Information regarding horse biology should be relevant, but such relevance is unclear in the 2016 Plan. Highly relevant would be the rate of population increase and how this varies with circumstances, but no such information is provided. Ecological information is essential for determining how many horses need to be removed to reduce or maintain populations at desirable densities. There are better references available than those used and better information. There is considerable published and unpublished information directly relevant to the Australian Alps. This needs to be incorporated in the plan.	Greater detail is required of how horse biology will be considered in the plan. For example: which horses will be targeted for removals? Mares rather than stallion should be targeted to reduce population growth rates. Will the entire band be removed together? Will mares with foals be released? Horses are trapped and travel much more calmly when with their social unit. Removal of the whole band versus select horses in a band will have both implications for the effectiveness of the removal and the welfare impacts of removal. Removal of bachelor groups may reduce adverse interactions with park visitors but will have little impact on reducing population growth rates. An understanding of reproductive rates, survival rates, social behaviour, and movement patterns is necessary for complete removal of horses from some areas and for maintaining populations at certain densities. Demographic data such as survival rates (age-specific if possible), reproductive rates, age structure, and sex ratios should be obtained where possible for each management zone within KNP so that possible rates of increase at different densities can be predicted and how they might change as horses are removed. Useful information can be found in Australian published studies (Dawson 2005, Dawson and Hone 2012, Zabek 2015, Zabek, Berman et al. 2016). Some statements to be revised, properly cited, or specified for KNP. For example, page 11 states 'Mares are able to foal at one to two years of age and usually raise one foal every two years (Dobbie & Berman 1992; Wagoner 1977)' and page 11 states "it is likely that horses in Kosciuszko National Park would have longer lifespans than wild horses in other parts of Australia due to the more favourable conditions." These statements may not be true for the KNP population and should be assessed. For example, while mares can foal at 1 to 2, it is uncommon for mares under 3 years of age to foal, and furthermore, mares do not reach full reproductive potential until 5 years of age (Berger, 1986; Feist & McCullough, 1975; Goodloe et al., 2000; Linklater et al., 2004; Scorolli & Cazorla, 2010). This has in fact been quantified for the Alps: 'No 2-year-old females were observed with young (Dawson & Hone, 2012)'. Furthermore, Dawson & Hone's (2012) findings demonstrate annual adult survival that is lower than many other populations (however this is compared globally; not specified in comparison to the rest of Australia). Thus, the assertion that lifespans are longer should be supported or rephrased to be less assertive.
Horse distribution maps	Various figures in the Draft WHMP indicate where horses occur, but the basis for such illustrations is unclear. The figures presumably indicate horse presence/ absence rather than horse abundance, but should be specified. More informative if variation in horse abundance indicated.	If the raw data from the broad-scale aerial surveys includes coordinates for each group of horses counted, then kernel analysis could produce "hot spot" relative density maps (see Zabek 2015).

Issue	Problem	Solution
Management zones	It is not sufficiently clear how and why the different management areas were chosen. It is reasonably obvious to select areas that apparently lack horses at present as 'prevention' areas, but otherwise designation of management areas is unclear. Identifying areas of horse retention, prevention, and elimination is a strength of the 2016 plan, but the plan does not provide transparency as to how management zones were selected. What are the specific control methods to be used for each management region?	Provide more detail of how management zones were selected to improve transparency. This is clearly a point of concern among community members. Include the current population estimates after the new survey in each of these regions to have a clearer picture of how many horses in each region need to be removed. Specify which control methods will be used in each management region or outline how methods will be chosen. This will reassure opponents that lethal control will not be the only method used in elimination zones
Reduction in horse numbers in management zones	Numbers of horses present in each management zone and the numbers that need to be removed from each zone are not known.	Analysis of the aerial survey data or more detailed existing surveys (Camera Traps, horse counts, dung counts) may provide the size of populations within management areas. These initial estimates should be improved by detailed surveys (Camera traps, DNA from dung, dung density). These should be done before and after removal operations to provide measurement to ensure that it is known whether a sufficient number is being removed or not.
Target densities	Proposed target wild horse population densities have no justification. It is not clear, in particular, why horse density should be lower than 0.4 per sq. km in one area and lower than 0.2 per sq. km in another. It is also unclear whether such target horse densities might be achievable.	If these target densities are to remain in the report, then the basis for their selection needs to be included.
Sustainable horse population	The number of horses retained for heritage values must be large enough so that there are no inbreeding problems or risk of extinction due to bush fire or other catastrophes.	Since the park's aim includes maintaining a 'sustainable' population of horses in the park, quantification of the genetic diversity may be required at some point to ensure genetic diversity of residual horse populations is maintained.
Trapping	Can this method remove enough horses? How much can trapping be increased from previous plans?	The highest number per year was around 600 horses. Is it possible to increase the density of trap sites? Passive trapping using electric fencing has proven successful where lure trapping is not an option. This method may be useful for removal of the last relatively untrappable horses in areas where exclusion is required.
Ground and Aerial Mustering	Is this a real option? If large numbers of horses are captured how will they be processed? What are the risks for horse welfare and people's safety?	Do a trial. Horses can be transported out for rehoming where opportunities exist. Where lethal control is required explore the possibility of field processing for meat so carcasses are removed and utilised.
Ground shooting	Further information on how ground shooting will be carried out required.	If trapping/mustering and rehoming fails to remove a sufficient number of horses, ground shooting may be required in some areas. Where this is used it is important to shoot the entire social group in a quick time period. Critical that horses are not non-fatally wounded. Ensure well-trained and experienced shooters are employed. They must know how to determine which horse in a group is shot first so that the others stay around. It is important not to take a shot if there is any uncertainty that the shot will be accurate. A drone may be useful to help the shooter approach the group during daylight, but thermal imaging can be very effective at enabling close approaches at night-time without horses being spooked.

Issue	Problem	Solution
Aerial shooting	Aerial shooting not included as social licence is not available in NSW and the use of this method may jeopardise its use in other parts of Australia. Horse protection groups are unlikely to support the use of aerial shooting. What should be done with carcasses?	In some areas with limited accessibility, then as a last resort aerial shooting may be required in some circumstances. Horse protection groups should be involved in designing a trial. Animal welfare outcomes would need strict monitoring.

Review of ITRG Humaneness Assessment

SAP members reviewed the humaneness assessments from the ITRG 2016 report. The ITRG developed a separate impressive panel of scientists with animal welfare expertise to develop a humaneness assessment of a range of horse management methods. The report used a well-established published model previously developed for comparing the relative humanness of pest control methods. Assessments were based on the Five Domains Model for assessing welfare (nutrition, environment, health/injury, behaviour, mental status; see Appendix 3 for more detail). The assessments were made based on available evidence in the literature, and where this was not available, expert opinion/experience (panel of 9 experts specifically created for the humaneness assessments) was used (acknowledging that there was some subjectivity). Assessments were not based on actual measures of welfare impacts during any management activities. There were two parts of the assessment:

- Part A assessed the impact of all methods and duration of impact;
    - The welfare impact in each domain was assigned a grade on a scale of 'no impact' to 'extreme impact', and then an overall welfare impact grade was derived from these 5 grades.
- Part B assessed only lethal methods assessing the impact of the actual killing method (Part A assesses impacts up until the point of killing, e.g. trapping, mustering)
    - The level and duration of suffering during the actual killing process were assigned a grade.

A scoring matrix (based on previously published humanness assessment model) was then used to assign an overall welfare impact grade to each method specified within the 2008 management plan. Most methods have multiple stages; for example, passive trapping, followed by loading and transport, followed by a holding period, followed by loading and transport to rehoming facility or knackery. The welfare impact of each stage in multi-stage control methods was assessed separately. All options with multiple stages are more likely to overall have more significant negative welfare impacts. For example, rehoming may end in positive welfare impacts, but can also result in negative welfare impacts with more stages, and still end in a knackery death. Assessments were based on Standard Operating Procedures and Codes of Practice, so an assumption was made in the assessments that each method would be carried out according to 'best practice'. It was acknowledged that auditing of processes would need to be performed to ensure best practice was adhered to. Final humaneness scores incorporated the severity and duration of welfare impacts, so a severe impact of short duration could end up with the same score as a mild impact of long duration.

Management methods assessed:

- Passive trapping
- Helicopter mustering within a 2km area (not pushed out of home range)

- Roping/brumby running
- On-site euthanasia in trap yards
    - Only Part B was assessed since there was no SOP for euthanasia within trap yards. Considerations need to include separation of individuals, partitioning, visual barriers, sound suppressors, handling, chemical restraint, firearm vs captive bolt euthanasia
    - Detailed SOP incorporating these needed before welfare impact assessment could be performed
- Removal for domestication and rehoming - assessed loading and transport for short (< 4 hrs) and long (> 15hrs) journeys.
    - Domestication process itself couldn't be assessed as details of practices and outcomes would be needed from rehoming groups
- Removal for slaughter – assessed loading and transport for short (< 4 hrs) and long (> 15 hrs) journeys
- Lairage and slaughter – assessment performed for both slaughter at abattoir and slaughter at knackery
- Ground shooting – 2 assessments performed based on head and chest shots, and also assessment of impacts on surviving herd members.
    - No published studies to base assessments on so this was based on expert opinion, assumed higher wounding rates than aerial shooting because assumed harder to approach horses from the ground
- Aerial shooting – 2 assessments based on best case scenario (< 1 min chase and rendered unconscious after the first shot) and scenario 2 (> 5 min chase and 2 shots needed to render unconscious)
- Immunocontraception - only effects of actual treatment assessed
    - Welfare impacts of administration (e.g. trapping or darting) not included
- Fencing

The ITRG also highlighted that their assessments could be used to inform recommendations for how improvements may be able to be made within the various methods in order to reduce welfare impacts. For example, if water and shade/shelter were able to be provided in trap yards then this could reduce the welfare impacts of passive trapping. Details of all processes in the humaneness assessment and overall scores can be found in the ITRG report on assessing the humaneness of horse management methods.

In summary: Methods with the lowest welfare impacts were passive trapping, mustering of small groups (large groups were different and associated with more severe welfare impacts), short journeys, aerial shooting scenario 1, followed by ground shooting with a headshot of small groups or individuals

Advantages and limitations of ITRG humanness assessments

Advantages:

- Assessments were based on available evidence in the literature at the time and expert opinion/experience within the panel

- The assessments enable transparent assessment of the welfare impacts associated with a range of management methods enabling a comparison of the various methods

Limitations:

- Assessments are theoretical
- Assessments depend on having a precise SOP for the proposed method
- Assumption that best practice/SOP is adhered to
- Assessments do not utilise actual animal-based measures of welfare
- The model has the limitation in producing overall scores, but some aspects are not directly comparable, e.g. a severe welfare impact of short duration is not the same as a low welfare impact of long duration.
- Assessments are overly simplified, since, in reality, the welfare impacts associated with each method will vary depending on many variables such as terrain, habitat type, horse density, mob sizes, weather, time of year, personnel expertise etc. (See supplementary info on humane management).
- Not all possible management techniques were assessed. For example, the welfare impacts of euthanasia in trap yards, and impacts of no management were not assessed.

Appendix 3. Ethical Wild Horse Management & Improving Welfare Outcomes

Ethical decision making

As outlined in the main body of the report as well as Supplementary Material 4, the SAP advise following the 7 Principles of Ethical Wildlife Control, from the International Consensus Guidelines (Dubois et al 2017). Furthermore, following **'Fraser's practical ethic'** (Fraser 2012; Fawcett et al. 2018) can further help to guide ethical decision making in wild horse management. This also incorporates a '**One Welfare'** framework (Pinillos 2016; Fawcett et al. 2018), to seek to maximise the wellbeing of animals, the environment, and people.

'Fraser's practical ethic' comprises the following 4 principles:

1. **To provide good lives for the animals in our care**

   KNP is home to many species, and we should be mindful of the welfare of all species, and how some species may impact on others. The SAP advise a management plan that optimises the welfare of horses in KNP, and also the survival and welfare of other species, particularly those that are currently threatened.

2. **To treat suffering with compassion**

   Compassion is required for all species and appropriate management of the impacts of wild horses on other species is required. In doing so, unnecessary suffering should not be caused during wild horse management, and the SAP advise management methods are adopted that have the least impacts on animal welfare. There are also times that wild horses may need to be euthanised to terminate their own suffering whether that be through injury, disease or malnutrition.

3. **To be mindful of unseen harm**

   Unseen harms may include harms that horses are causing to other species in the park or may also be harms to horses themselves (for example malnutrition, illness & injuries) which may be greater if they are left unmanaged. The biggest welfare risk to horses, with no natural predator other than humans, is if their population continues to rise, it only becomes limited by food availability, and so malnutrition and starvation become a significant welfare issue. This can often be an unseen harm since those horses that are most vulnerable tend be living in more densely bushed habitats where they are consequently less likely to be seen by humans. Unseen harms can also result from management interventions. It is critical that unnecessary harm is not caused during management. Therefore, management methods with the least negative impacts on animal welfare should be employed, with ongoing welfare assessments and auditing, and alternation of management practices as necessary to ensure that unnecessary harms are minimised.

4. **To protect the life-sustaining processes and balances of nature**
   The key end aim of the new wild horse management plan is to reach a sustainable population of horses whilst protecting the other values of the park, life-sustaining processes and balances of nature.

Finally, **Conservation welfare** is a new discipline applying animal welfare ethic to conservation activities. This is a consequentialist ethic similar to utilitarianism, according to which the right action is the one that results in the greatest good for the greatest number. In practice, this equates to minimising harms to animals and maximining benefits of any harms caused. Conservation welfare applies scientific methods to inform an understanding of what harms and benefits animals might experience with different conservation activities, how they may be mitigated, and how this information can be used to inform decision making about what management actions are morally and ethically permissible. The SAP advise that the new management plan follows the principles of Conservation Welfare (Beausoleil et al. 2018; Beausoleil 2020).

Reducing welfare impacts associated with different management methods

The main body of the report addresses how welfare impacts can be assessed. The following summarises what welfare impacts occur with different stages of management options, and further considerations for how some of these impacts may be mitigated. For any method where welfare impacts are severe, and cannot be substantially mitigated, then this method should not be used. **This section is intended to be read alongside the IRTG humaneness assessment report.**

Methods of capturing horses

Passive trapping

The ITRG assessment scored passive trapping as the capture method with the least negative welfare impacts, although it still scored a moderate impact for a duration of hours, with an overall score of 5 (See IRTG humaneness assessment report). Considering that some of these impacts may also be further mitigated, passive trapping is the method of capturing horses that is most likely to have the least negative welfare impacts, and this is the preferred method for capture.

Impacts in Domain 1 (Food or water restriction) can be reduced if water is safely provided within trap yards. Water should not be restricted for more than 12 hours (particularly if mares are lactating). Consideration should be given to the feasibility of also providing hay within trap yards at the time the trap is set to reduce the duration of food restriction. Impacts in Domain 2 (environmental challenge) may be reduced by having trap yards in sheltered areas where possible. Provision of hay would further reduce the impacts of cold conditions. Thermal stress in hot conditions will be reduced if shade is available, and further reduced if water is available. Assessment of horses for presence and severity of shivering or excessive sweating will provide more information regarding thermal challenges during trapping, to highlight whether further mitigation of these challenges is required.

Impacts in domain 3 (disease, injuries) can occur; injuries are rare, and risks can be reduced by trap design and maintaining family groups within trap yards. It should be noted that horses may commonly have pre-existing illness (e.g. related to malnutrition, gastrointestinal parasitism, mineral deficiencies) that may become apparent following trapping (but is not caused by trapping). Impacts in domain 4 (behaviour) can be reduced if bonded groups do not become separated, and if personnel are careful to cause minimal disruption upon arrival for processing the horses, spending a little time to habituate the horses to human presence. Impacts in Domain 5 (mental status: anxiety, fear, thirst, hunger, hypo/hyperthermia) are reduced by addressing impacts in domains 1-4.

Mustering

The ITRG assessment scored mustering as another capture method with the likely least negative welfare impacts, although it still scored a moderate impact for a duration of hours, with an overall score of 5. Further, this was only when mustering was of small groups of horses performed over hours. If larger groups of horses are mustered over days, then welfare impacts will be greater (score 6). This also assumed mustering within a small area (i.e. maximum of 2km) so that horses are not pushed outside of their home range, and assuming up to 4 bands are mustered. If more horses were mustered, over a longer period, and/or over longer distances then welfare impacts would be expected to be greater. Therefore, mustering of smaller numbers of horses (1-2 bands) over hours, and not outside of their home range is advised. Mustering larger groups of horses (especially > 4 bands) over days, and/or moving them outside of their home range is not advised. If more than one band is being mustered, yards need to be able to appropriately separate bands maintaining family groups together. Food and water need to be provided in yards. Horses should be mustered slowly to reduce the negative impacts of exertion and risks of injury. Impacts once yarded can be similarly reduced as for passive trapping. Horses should not be without water and food for > 12 hours.

Brumby running

The ITRG assessment scored brumby running as the capture method with the highest negative welfare impacts, with a severe impact for a duration of hours, with an overall score of 7. Scoring was based on Parks Victoria SOP. Welfare impacts were classed as severe because horses may be without food and water for > 24 hours, they may experience heat stress during exertion, and potentially hypothermia if tied up for a long period during cold weather. Prolonged or excessive exertion can cause myopathy, neck ropes can cause injuries and interference with breathing, pursuit can cause injuries, the bands are disrupted, and horses separated from their family groups, captured horses may be tied up for 24 hours and be apart from other horses. Foals can also become separated from bands during the chase with subsequent extreme welfare impacts.

There is the potential for many of these impacts to be mitigated if strict protocols are followed, which would include a limitation on the pursuit time and tie-up time, that individual horses should not be left alone without another horse, horses should not be more than 12 hours without water (or not more than 6 hours without water if horses are sweating excessively), or food, neck ropes should not cause injury or interference with breathing, and bands containing young foals should not be targeted. Measurement/auditing of actual measures of animal welfare impacts has not been previously performed and would be advised for any pilot program before deciding if continuation of brumby running was acceptable on welfare grounds.

All these are simply methods of capturing horses, and need to be followed by further stages which may include the following:

Transport

There are significant potential welfare risks associated with transporting horses, even those that are acclimatised to the transportation process. These risks are heightened with wild horses that are not acclimatised to the transportation process. The most common and important complications are those related to dehydration and muscle fatigue (Doherty 1997, Friend 2000, 2001, Weeks et al. 2012, Padalino 2015).

Dehydration occurs through water deprivation during (and sometimes proceeding) transportation but is further exacerbated by higher ambient temperatures, the occurrence of diarrhoea which may be associated with transport stress, and increased sweating which can occur with anxiety and fear. Lactating mares, mares in the 3rd trimester of pregnancy, and foals < 6 months of age have higher water requirements and are therefore at an increased risk of dehydration during transportation. Dehydration can also lead to serious metabolic complications, reduced renal function, large colon impaction and laminitis (Padalino 2015). Severity of dehydration relates predominantly to journey duration, fear and environmental challenge with thermal stress described at ambient temperatures > 30 degrees C (Weeks et al 2012). Horses can tolerate water deprivation for longer periods in cool weather, but in hot weather, severe dehydration may occur with water deprivation of > 8 hours (Friend 2001).

Due to change in routine and confinement, wild horses are also more likely to have already had reduced food and water intake prior to transport, and are likely to take longer to eat and drink upon arriving at their destination. Therefore, it can be anticipated that these complications would occur in journeys of shorter duration when compared to domestic horses.

The muscular effort and physical exertion required for a horse to balance in a moving vehicle is similar to that of moderate exercise. Many physiological changes may occur during transportation including tachycardia, diarrhoea, increased cortisol, increased muscle enzymes and lactate, fluid and electrolyte imbalances, shipping fever, azoturia, reduced immune function, and physical effects such as weight loss and behavioural changes (Doherty et al. 1997). Horses can develop life-threatening levels of fatigue after 24 hours of exertion, but even after 8 hours horses have been reported to fatigue to the point of lying down in the truck, risking also getting trampled and injured (Friend 2001).

Additional transport stressors include separation from herd members, forced proximity to unfamiliar horses, exposure to pathogens, restraint of normal activities, forced adoption of an abnormal posture, extremes in temperature, reduced ventilation (Friend 2001), and lack of REM sleep (Weeks et al. 2012). Oxidative stress has also been shown to occur with 8-hour road journeys (Padalino 2015). Respiratory disease and weight loss are also common during long journeys. Significant weight loss is reported to occur even just after 6 hours of transport, mainly due to dehydration.

The IRTG scored welfare impacts of short journeys (< 4 hours) as overall moderate impact with a score of 5, and long journeys (> 15 hours) as an overall severe welfare impact, with a score of 7.

Every effort should be made to reduce the duration of journeys during which horses are not receiving rest, food and water. It is recommended that rest periods of 30 minutes are required every 4-6 hours to enable horses to urinate and drink (Weeks et al. 2012, Padalino et al. 2015). In many Codes of Transport, it is deemed acceptable to remove access to food and water for up to 8 hours (Padalino 2015).

The Australian Animal Welfare Standards and Guidelines - Land Transport of Livestock (http://www.animalwelfarestandards.net.au/land-transport/) stipulate maximum journey durations of 24 hours for horses > 6 months of age, and 12 hours for foals and lactating mares. However, additional considerations are needed after 12 hours off water for horses > 6 months of age, and after 8 hours off water for foals and lactating mares, and 5 hours in pregnant mares in their 3rd trimester.

Additional considerations include body condition, assessing the horses for temperature abnormalities, diarrhoea, lethargy and injuries.

Most horse transport guidelines are written with domestic, handled horses in mind, and wild unhandled horses pose additional difficulties in assessing horses while on the truck, provide food and water, or unload for rest. The SAP recommends reducing the maximum duration of journeys accordingly.

Risks of dehydration, gut stasis, muscle fatigue and delayed urination will be significantly increased for journeys beyond 6 hours without rest, food and water, so journeys of less than 6 hours (without rest/food/water) are advised, with the shorter journeys being preferable. Transport of foals, lactating mares and mares in their 3rd trimester of pregnancy should not travel for more than 6 hours without rest, food and water. If a journey is expected to exceed 6-8 hours, consideration should be given to body condition, period prior to travel without food and water, ambient temperature, presence of sweating and/or diarrhoea No journey should exceed a maximum of 12 hours without rest, food and water.

Slaughter

Slaughter requires further transportation and lairage. Overall due to combined welfare impacts of different stages, transport and slaughter has the worst potential welfare outcomes out of the lethal control methods. There are also important differences between slaughter in abattoirs vs knackeries and these are also regulated differently, and so will be addressed separately. The processes in abattoirs and welfare problems associated with horse slaughter have been described in detail (Harvey 2020). Abattoirs are licensed to slaughter animals for human consumption, whilst knackeries slaughter animals for pet food and other by-products, such as hides and bone meal. Since the market for horsemeat for human consumption is small and based overseas, all horses slaughtered for human consumption are done so at abattoirs licensed for exporting meat, of which there are 2 in Australia (Queensland and South Australia). Abattoirs and knackeries are regulated differently. Export abattoirs operate under both federal legislation, and requirements of the importing country, which includes EU legislation that incorporates some animal welfare regulations. There are specific requirements for export abattoirs under EU legislation such as requiring a veterinarian to be on-site, and a designated animal welfare officer to be present when animals are unloaded on arrival. Conversely, knackeries are state-regulated and have no requirements for a veterinarian or animal welfare officer, are audited much less frequently, if at all, and have no formal requirements for specific animal welfare audits.

Lairage and slaughter in an abattoir

The ITRG assessed lairage as mild welfare impacts, and the slaughter process up until the point of inducing insensibility being moderate impacts, with overall scores of 5 and 4 respectively. The method of rendering horses insensible in an abattoir is stunning with a captive bolt. When placed correctly insensibility occurs very rapidly. In this case, if they do not regain consciousness prior to death then there should be no suffering. However, problematically, the stunning success rate is unknown. A captive bolt only causes localised brain trauma and concussion, and to achieve insensibility, very accurate placement and positioning of the captive bolt gun is absolutely critical, and the gun needs to be held firmly over the intended site. If there is any error in placement when the gun is fired, it will cause extreme pain without inducing unconsciousness, and a further shot is immediately required. The brain of the horse is surprisingly small (about the size of a grapefruit), within a large skull, and so it can be challenging to achieve accurate placement, especially if there is any movement of the head. When fully conscious, most horses will tend to raise their head if they are anxious or an unfamiliar object is advanced towards their head, and this makes correct height and angling of the captive bolt even more challenging. Thus, in wild unhandled horses, accurate placement of the captive bolt gun is particularly challenging, and a higher than normal rate of ineffective first shots may be anticipated. Further, due to the number of animals being processed at an abattoir, and the size of the facilities, it is an inherently stressful environment for a wild horse.

Lairage and slaughter in a knackery

There are no animal welfare regulations for knackeries. However, that is not to say that animal welfare standards aren't high in some knackeries. Knackeries and much smaller and process much fewer animals than abattoirs, and therefore it is more likely that staff will be familiar with managing horses, and the environment is likely to be inherently less stressful. There are many more knackeries throughout Australia and therefore travel duration will also be much shorter. Knackeries may use a captive bolt or firearms. The advantage of a firearm is that it doesn't need to be held firmly against the horse's head, and instant death is more likely to be achieved. Some knackeries may allow independent animal welfare auditing.

Given the inherent potential welfare problems associated with slaughtering horses in abattoirs, combined with the long transport duration, the SAP does not advise slaughter of horses in abattoirs. Given that not all horses removed from KNP will be able to be rehomed, the slaughter of a small number of horses is unavoidable. The SAP advise the minimum number of horses possible should be slaughtered. When this occurs, the duration of travel without rest, food and water should not exceed 6 hours. Only knackeries that allow independent auditing of animal welfare outcomes should be used.

Euthanasia in trap yards

Euthanasia in trap yards has not been previously performed in NSW and so there is no existing SOP for these methods. Euthanasia in trap yards has the potential to have much less negative welfare impacts given that it avoids transport and horses will be habituated to the environment. It can be performed in the following ways:

- Tranquilising (dart or hand injection) followed by captive bolt shooting
- Tranquilising (dart or hand injection) followed by lethal injection
- Tranquilising (dart or hand injection) followed by shooting with a firearm
- Shooting with a firearm without prior tranquillisation

All of these methods have the potential to cause very rapid insensibility and death with no suffering. Tranquilising prior to applying lethal control has the advantages of keeping the horse still and reducing anxiety when the lethal method is applied, thereby minimising suffering. However, the disadvantage is that the process of tranquilising adds another stage where welfare impacts can occur, such as anxiety/fear and injuries. A veterinarian is also required to prescribe and oversea use of tranquillisers. The SAP advises trials of all these methods to identify the ones with the least negative welfare impacts.

Factors that require consideration in how to minimise anxiety and fear, and maximise the chance of instantaneous death, include separation of individuals, partitioning, visual barriers and sound suppressors for firearms.

Shooting with a firearm without prior capture

Due to the welfare impacts associated capturing horses, lethal methods that don't require prior capture are most likely to have the best welfare outcomes. However, they also have the potential for extreme negative welfare impacts. Aerial shooting, but not ground shooting, has been previously assessed in horses. These methods are only be recommended in the following circumstances:

- o Where there is a very low likelihood of significant animal welfare impacts, based on careful assessments of the points outlined above by the ITRG. An updated SOP would be advised, to incorporate these recommendations within the SOP to be used

- Use of extremely experienced shooters
- Where other methods are either not feasible, or more likely to be associated with higher animal welfare impacts (e.g. inaccessible locations or large numbers of horses in one area)
- Where a defined proportion/number of horses in the particular population are to be shot (i.e. indiscriminate use would not be recommended)
- Where animal welfare outcomes are monitored and results used for recommendations to further improve animal welfare outcomes, i.e. the initial recommendation would only be for a trial and targets for key welfare parameters such as chase time, instantaneous death rate, rate of non-fatal wounding etc, would be set. If targets considered to represent acceptable animal welfare outcomes could not be achieved, then continued use of the method would not be recommended.

More details about integrating ethics and animal welfare, and commonly asked Q&As about lethal control are detailed in Supplementary Material 4.

Appendix 4. Management Zones

Justification for horse management zones – starting in post-fire zones

Following the very large fires of December 2019 - February 2020, three priority locations in northern KNP have been identified by OEH with advice from the SAP for immediate horse management. The rationale is that the post-fire recovery of the vegetation and its dependent native fauna may be hindered by the high horse densities that have become established in the three identified areas of the park, especially over the last decade. Additionally, there is a need to protect significant scientific assets that are currently being damaged by horse activity, independent to the impact of the recent fires. Horses also need to be managed in places where collisions between motor vehicles and horses pose a significant safety risk to humans. Three Management Zones are proposed for the north-eastern section of the park where fire damage has been severe and wild horse populations are already at high densities. The Zones also include areas where horses pose a greater risk to motorists. They total 58,200 Ha or nearly 12% of the area of the park.

Similar zones were already identified in the 2016 Plan based on observed significant changes to the geomorphology (streams, soil cover, wetlands, and limestone karst) and ecology (floristics of grasslands, native rodents and invertebrates, bog and fen communities) and the desire to limit the spread of horses into new areas of the park. The SAP considered the subsequent changes to the reported negative impact of horses up to 2020 and the damage caused by the 2019-2020 fires (Adaminaby Complex, Dunns Road, Marys Hill, and Rolling Ground Fires) to indicate three zones where horse management should be focused to assist in the post-fire recovery and serve as a starting point for horse control efforts. After a review of the zones proposed by the SAP, NPWS recommended minor changes to the boundaries based on operational considerations. Boundaries were moved to align with a nearby track/trail or waterway (where it exists) so that boundaries are easier to identify on the ground.

After the 2019 bushfires, members of the SAP have only inspected the area near the Snowy Mts Highway and the link road to Cabramurra, but adequate information has been provided by NPWS and other sources. Our information is that horse mortality associated with the fires has been relatively minor and that horses remain active across the area, with little effect on the total population. However, it is acknowledged that community concerns and collection of horse data suggest that there could be significant horse loses in particular in the Kiandra area of Zone 2 and thus this should be further evaluated prior to management in this specific region.

Identification of Management Zones

Zone 1 Nungar Plain and south to the Snowy Mts Hwy. This zone corresponds to an area that was designated for horse elimination in the 2016 Draft Management Plan. This area extends south along Pockets Saddle Road from 5 km north of the Port Phillip Trail intersection, crossing the Murrumbidgee River downstream of Tantangara Reservoir south along Kellys and Nungar Creeks to the Snowy Mountains Highway at Gang Creek. Then east and north along the eastern park boundary to the north of the Murrumbidgee River the northwest on the spur west of Paytens Creek to the Pocket Saddle Road.

The southern part of the zone is forested and has a low horse population, thus making horse eradication feasible for this zone. It includes Nungar Plain, an upland open plain at 1340 m altitude which has extensive specialized grasslands (McDougall and Walsh 2002). The grasslands are of two broad types: 1) herb-rich and dominated by *Poa petrophila*, *Poa hookeri* or *Poa phillipsiana*, occurring on dry slopes and 2) species-poor and dominated by *Poa labillardierei* and/or *Austrofestuca hookeriana*, occurring on damp flats. The grasslands have high floristic richness and preserve species such as the rare daisies *Calotis pubescens* and *Taraxicum aristum* that have not been located at Kiandra or Long Plain. The plain had long-term cattle grazing until 1975 but has been recovering after the cessation of cattle grazing. If the horse population is allowed to increase, the recovery may be reversed. The growth of sedges and aquatics such as *Potamogeton cheesmanii* have occupied stream channels and the banks are well vegetated and stable, supporting riparian sedge fens. McDougall and Walsh (2002) noted significant pig damage in the grasslands, which NPWS has subsequently effectively controlled. The Nungar Plain would be regarded as having high archaeological potential. Several modified trees have been identified in this zone that should be revisited and assessed.

This zone is identified for complete removal of horses because historically, horses have been absent, or incursions have been recent. Complete removal from this area will provide buffer areas that will prevent the expansion of horse populations into areas that are currently horse free. These include land bordering the park and the area of Zone 3 lying northeast towards the ACT border.

Zone 2- Three Mile Dam. This includes an area that was originally identified as high risk for public safety but is expanded from that described in the 2016 Plan to include an area inhabited by the endangered broad-toothed rat. This area, consisting of a zone following Gang Gang Creek south across the Snowy Mountains Highway then west across the Eucumbene River to Kings Cross Road. South for 2km and west and then north along the divide between Eucumbene and the Tumut River to meet the western side of the Snowy Mountains Highway north to 1 km south of Rules Point. Then west across the Murrumbidgee River and up Dairymans Creek and down Hell Hole Creek then upstream to Hains Hut and southwest to Goandra Creek. Then southeast to the head of Wallers Creek and east over Tantangara Mt to Gang Gang Creek.

The zone is identified for an overall reduction in horse abundance to a level where traffic safety risk is minimised. Vehicle-horse collision is a significant risk. All horse groups that cross the highway should be removed to establish an initial horse-free buffer, approximately 4 km wide from the highway, be established. The reduction in horse numbers at the southern end also has the aim of preventing the spread of horses southwards into areas of the park that are currently horse free.

Reducing wild horse populations near popular campgrounds, such as at Three Mile Dam, is desirable as unsafe conditions have been noted with horses damaging tents, searching for food, and leaving piles of manure. NPWS advises that a small northward extension of the zone near Three Mile Dam would incorporate an existing trapping site.

A total of 86% (139 km^2) of this Zone was burnt by a very hot fire that removed the ground layer of plants and burnt into the humus layers of soils. The bare surfaces are prone to erosion and recovery to a dense and stable grassland will be a lengthy process as the seed store will have been destroyed.

Mastacomys fuscus, the Broad-toothed rat (Green et al. 2014) occurs in wet tussock grasslands and shrub bogs at Delaneys and Rocky Plain, despite a long pastoral history in the area. They are absent in areas of high horse density where grazing has removed the tall grass cover that they need. Only small patches of unburnt grass have survived in areas of former high broad-toothed rat density populations. Hence their post-fire survival and eventual recovery will require extremely low horse densities.

In this zone, horses should be excluded or removed from the most severely burnt areas and densities managed to reduce traffic risk. Fencing has been suggested as a solution, however, this would be expensive and require constant maintenance, but nevertheless should be further considered. The major riparian corridors running through the zone will have high archaeological sensitivity, including the locations of existing campgrounds (which have topography conducive to the existence of Aboriginal sites). Other locations of low slope in otherwise hilly country would be expected to exhibit a higher density of archaeological material.

In regard to Kiandra, horse removal should initially occur only at the very northern tip to provide a buffer zone to prevent horses in the Kiandra region from dispersing further north. In line with the proposal for Kiandra to form the key initial pilot study area for community engagement and reproductive control, horse removals would not occur until local population estimates and horse identifications have been performed with community involvement. The main population in the Kiandra zone is anecdotally less than 300 horses. A rigorous population and environmental impact assessment need to be carried out to empirically inform what an acceptable horse density is for this area. If substantial numbers of horses were lost in the fires, the current population may be able to be retained. This site may then be used as an initial key site for reproductive control methods (see Appendix 6) to prevent further population growth.

Zone 3 Upper Currango, Coolemon, Peppercorn and upper Long Plain. This zone lies to the north of Zone 1 and is considered an area warranting protection of karst and other key environmental assets in the 2016 Plan, as well as an area that could provide a buffer to prevent horses from moving into the ACT. This area, extending from Pocket Saddle Road south of Gurrangorambla Creek northwest across upper Currango Plain to Old Currango then to the Gurrangorambla Range and northwest to Cooinbil Hut then following the Cooinbil and Long Plain Roads to the Murrumbidgee Crossing then north along the western side of Long Plain and returning to the Long Plain Road and following it northwards to the Little Peppercorn Creek then eastwards down the creek to cross McLeod's Ridge north of Basin Creek to the Goodradigbee River. The boundary follows the river southwards to Rolling Grounds Creek where it turns southeast up the ridge to the ACT border. It proceeds southeast from Bimberi Gap to Murrays Gap and then follows KNP/ACT border to the west of Yaouk Gap then the Park boundary south and west to the Murrumbidgee where it turns north and west on the Murrumbidgee Fire Trail to join Pockets Saddle Road.

This complex zone is identified for a reduction in horse abundance to a level that protects key environmental assets. One of these is the karst (limestone) landforms and drainage integrity of Coolemon limestone, identified as vulnerable to horse impact (Spate and Baker 2018). Horse traffic has damaged an unusual karst weathering feature at Blue Waterholes, where slabs of limestone have naturally lifted into unusual tent-like structures. Because karst processes are affected by water quality, the entire catchment leading to a karst area needs to be as undisturbed as possible and this requires very low horse densities given their tendency to break down streamlines and increase turbidity. Spate and Baker (2018) note that the Blue Waterholes area supports some rare crustaceans adapted to the calcium-rich water. The removal of around 100 horses from the Blue Waterhole area at the end of 2019 did not solve the problem of karst catchment protection as horse densities are high at the southern end of Currango Plain.

The large montane sedge fens and drainage lines of the Mosquito Creek complex in northern Currango Plain are exhibiting increasing rapid erosion over the past eight years due to high horse densities (Driscoll et al. 2019, Hope et al. 2012). These peatlands contain 3-4 m of sedge peat which has acted as a sponge and infilled streamlines so that, after heavy rain, water flowed in a sheet through the sedges and not along channels. Sediments and nutrients were trapped by the peat and high-water quality resulted. The fens are of particular importance to wildlife during drought periods and after fires, as they remain green and the sedge resprouts quickly if burnt.

The entire fen complex has been taken over with the dominant sedge (*Carex gaudichaudiana*) replacing a 40-50 cm dense sedge land. The horse traffic has cut lines across the swamps and channelization has become marked, with some channels reaching down to the erodible silty clays below the peat. Consequently, the main streams are turbid, and the fen has drained and collapsed along streamlines. Substantially reducing horse densities is required to prevent a major collapse of the peatlands with large sediment losses to the Tantangara Reservoir.

The Peppercorn Hill area, including the source area of the Murrumbidgee and Peppercorn Plain, are similarly vulnerable to wetland loss and stream bank collapse with increasing horse activity. Small streamlines are progressively eroding up slopes, and shrub bogs are showing increasing horse trackways. These areas are under pressure from high horse populations migrating from the more resilient grasslands on Long Plain to the south. An unusual flark drainage complex of small linear ponds set across the slope occurs near the Murrumbidgee source and could be at risk from trampling.

In addition to widespread open artefact scatters and concentrations, significant archaeological sites occur at several locations in his zone. A human burial has been recorded at Blue Waterholes, and a cluster of stone arrangements occurs at Mt Morgan, ~11km west of Tantangara Reservoir. Seven kilometres NNW of these stone arrangements is a ceremonial ring recording (Oldfields Ring Site). These sites should be revisited and their condition assessed.

More than 22 km^2 (9%) of this Zone was initially burnt, although another fire from the east has more recently increased the burnt area, including burning over bogs along Dunns Flat Creek (east of the Goodradigbee). The peatland has been severely altered over the past 20 years from a *Sphagnum* shrub bog to grassland by horse trampling as evidenced by remnant peats under the grass. Horses in this area can cross into the ACT over Murrays Gap, itself an extensive wetland.

Zone three will have a reasonably high archaeological sensitivity, particularly areas like Currango Plains, Long Plain and the margins of wetlands (e.g. Murrays Gap).

Because there are no natural barriers across the northern parts of Currango and Long Plains the proposed boundaries are to provide a horse-free buffer zone of approximately 2 - 4 km that would be only slowly recolonised between the key assets and groups of horses. This buffer is also the aim in the Peppercorn Creek area to prevent further spread of horses northward into an area with the last remaining populations of northern corroboree frogs. NPWS suggest that extending the boundary westwards out to Peter's Hut on Long Plain (Murrumbidgee River) will allow the inclusion of 4 x existing trap sites, as these trap sites are the only ones with access over winter.

Within Zone 3 the question is what horse densities are environmentally sustainable such that the environmental damage can be reversed, and particular regions and herds within zone 3 that may have horse heritage value. Monitoring of the karst and fen water quality should provide a sensitive measure of stream erosion that will indicate if damage is continuing at various levels of horse densities. In addition to horse management, the zone will require rigorous control of deer and pig activity.

Appendix 5. Monitoring Methodology

5. 1 Monitoring Horse Populations

Below are more detailed recommendations for estimating horse numbers and their impacts.

Dung counts

- 1. *Distance*: Run a 200 m string line out and peg it tight and straight. Have an observer walk along the string line and call out when dung is seen from the string line. Another person with a tape measure measures the perpendicular distance to the centre of each dung deposit and records it. Only dung seen by the observer on the string line should be recorded. To calculate horse density using distance with an acceptable Coefficient of Variation, at least 80 dung points need to be recorded. The length of transect or number of transects needs to be increased if not enough dung is present. A simple strip transect may be sufficient under time constraints, at least for areas with low dung density.
- 2. *Strip*: Run two more string lines parallel to the first 200 m string line, 5 m either side making a 10 m wide strip 200 m long (Butler et al. UTAS *in preparation*). Search this strip and count every location with horse dung. Repeat the dung counts in each habitat.
- 3. Drive along tracks, recording the location of horse dung deposits (if time permits, record age: fresh (green or black) or old (brown or grey), stallion pile or single defecation). That will provide a broad-scale view of horse activity along vehicle tracks and the extent of the horse distribution. Select sections of vehicle tracks for more detailed sampling. Clear dung off these sections of road/track, record dung deposited in a set timeframe after clearing and before the control operation commences and then repeat clearing and recording after the horse control operation. Sections of the road should be chosen both close to where horse removals are being conducted and also far away from the control operations in a similar country where there are similar dung densities prior to control activities.
- Quadcopter drones can be trialled for use in dung counts. This could be more efficient than walking transects so a greater number of sites could be surveyed in more remote areas. Dung can be seen on drone video and can potentially be recognised by computer, which would require trials.

DNA samples from dung

- Collect samples of fresh (green or black) dung with a view to testing for horse DNA. This can provide an accurate estimate using genetic tags for individuals and mark-recapture estimation of actual numbers of horses in the area where dung is collected (see NHS 2014).

Ground Counts

- Twice a year (beginning and end of breeding season), count horses from vehicle or horseback (same tracks each time), recording at the very least, the number of foals and the total number of horses in each social group. Record the number of mares, stallions, sub-adults (1 to 2-year-old) and foals (< 1 year old) if time permits. This level of detail takes longer and may not be possible for NPWS staff, but community members may be able to do this. Also record identifying characteristics if time permits (community groups may have time and interest) to allow mark-recapture calculations. If possible, all areas in KNP with access should be covered. Staff and community members should be involved, and the observer noted. Apps are available on smartphones to do this quickly. Community counts on horseback would increase the area surveyed off vehicle roads and tracks. Data needs to be separated into community or NPWS staff records to control any biases.

Drone surveys

The SAP recommends that drone technology be trailed to replace the groups of people and transport devices and to record visual information that can be used to assess horse populations.

Specifically, we imagine surveys of horses (and other species such as pigs & deer) using drones and associated computer software configured as follows:

- Drone flies along pre-determined transect lines at a constant and relatively low height above the ground
- Drone has downward-facing cameras that record in normal light and infrared
- Camera settings (e.g. aperture, focal length, filter) will need to be adjusted to provide best-possible information, and then used consistently to enable appropriate comparisons
- All recorded information can be transmitted to a base in real-time and distributed from there
- Computer software can determine when and where a camera records an animal, estimates the distance of the animal from the transect line that is directly below the drone, and saves a sequence of photos for each such animal
- Computer software can be tested to determine if additional information about each recorded animal, such as species (e.g. horse, pig, deer, kangaroo) can be recorded
- Otherwise, each sequence of photos can be examined by a human for appropriate interpretation (e.g. species, sex-age class, behaviour)

Such information could then be analysed to obtain estimates of horse density using standard methods for 'variable distance transect counts'. However, it should be noted that it is necessary to assume that any animal that is directly below the drone is certain to be detected and that the likelihood of detection falls off with increasing distance from the transect line in accordance with a particular mathematical function (e.g. Gaussian or Normal).

Such estimates of horse density may then be compared with other measures of horse abundance (e.g. dung) and with various environmental attributes. This should lead, amongst other things, to evaluations of relationships between horse numbers and environmental impacts.

Implementing what we here propose will require funding to support (at least) the following:

- Purchase & maintenance of drones and associated equipment
- Computer & software
- Personnel (operate drones; operate computer software, including statistical analysis; interpret results; document results and conclusions; prepare scientific manuscripts)
- Scientific publications: this project would be novel and exciting research

Community involvement in horse monitoring

- Community counts on horseback or on foot could increase the area surveyed off vehicle roads and tracks.
    - Consideration should be made to potential biases introduced by surveying on horseback versus on foot.
- Community involvement in dung count estimates.
- The SAP advises that community monitoring occurs alongside park staff monitoring to control for potential biases in count data obtained by community members versus park staff.
- Community identification of individual band and horse characteristics to allow mark-recapture calculations and assist in future management (e.g. reproductive control). Apps are available on smartphones to do this quickly.

Monitoring genetic diversity

At the population level, genetic diversity can be measured as the mean number of variants of a gene (alleles) or as the proportion of individuals that have different variants of a gene (heterozygosity) (see National Research Council 2013). Inbreeding coefficients (the probability that genes at a randomly chosen location are identical by descent) is also a useful measure. There are no published genetic studies on the wild horses in KNP. Thus, baseline genetic diversity is unknown and thus, the effect of reducing the population on genetic diversity and health is not known. To indicate the genetic health of the current population, analysis for allelic diversity, heterozygosity, and inbreeding coefficients should be performed from genetic testing of samples collected from gathered horses. Recording the occurrence of genetic diseases and conditions could further provide an indicator of the genetic health of a population. The National Research Council (2013) discussion of maintaining genetic diversity in horse populations in their report to the Bureau of Land Management (BLM) provides further information on this topic.

5.2 Monitoring Environmental Impact

Methodology can be modelled from recent studies, such as those of Wild et al. (2012), Cherubin et al. (2019), and Robertson et al. (2019). For example:

- Selection of sites that are similar in landscape, soil type, habitat, and other factors that can potentially influence environmental impact measures. Select sites in each treatment (horses absent, horses removed, horses reduced, horses unmanaged).
- At each site, walk 200 m to the east, 50 m to the north, 200 m to the west then 50 m to the south and end up at starting point. Record the number of metres of impact (grazing, pugging, trampling) along the total 500 m.
    - Faunal survey can include recording the number of skinks observed, the length of each broad-toothed rat runway and the number of broad-toothed rat scats in each runway, wombat burrows, wombat scats, pig diggings, horse dung within 5 m either side, rabbit pills, deer pellets and cattle dung.
- At waterways, run a 50 m tape out along one bank. At 0, 10, 20, 30 and 40 m, record water depth, flow rate, siltation (percent cover and depth), turbidity, bank slope and height, and % veg cover. Each 10 m section of the creek, record the metres of bank disturbed and the likely animal causing it based on the most recent hoof prints, dung etc. This makes 5 x 10 m sections of the bank on either side.
    - It is also important to walk a longer section of the creek (200 m to 1 km) recording the length of bank disturbed to determine how representative the 50 m section is.

5.3 Monitoring Aboriginal Heritage Impact

Open Artefact Scatters/PADs

Controlled experiments at locations that would allow measurement of the impact of horses on prevalent site types, predominantly stone artefact clusters should be undertaken. Where possible these locations should be in complimentary locations to stations established for monitoring other variables. Foreseeable horse impacts might be breakage and displacement (either directly or through erosion/deflation). These potential effects could be measured through the creation of 'model' sites where change is measured over time. To replicate artefact clusters an initial method could be the use of metal tags (or something as simple as metal washers of different sizes), painted high vis, numbered and placed in pre-recorded locations. Where specimens were not observable, subsequent inspections to employ a metal detector to attempt to find them, and their movement thus able to be measured in relation to a pre-recorded datum (and/or differential GPS to sub centimetre accuracy).

Potential breakage could be measured through the creation of replica stone artefacts by a modern stone worker and inclusion in replica-controlled clusters.

A complementary approach would be to select several places in KNP where horses are known to frequent known Aboriginal open sites, and implement a regime of monitoring site/artefact condition, both through recording artefact condition and distribution and also measuring proxies of archaeological site condition (ground exposure, deflation/erosion).

Other Aboriginal Heritage places

As indicated in Appendix 1.3., open artefact scatters and PADs comprise the vast majority of sites in KNP, and can occur at nearly any location of low slope. The less common/more significant known places in KNP should be subject to a program of revisitation and re-recording to determine current condition and what (if any) factors are causing their deterioration. Determinations should be made at each of these sites regarding the necessity/feasibility of installing mechanisms to exclude horses. Assessment of the effectiveness of control measures on intangible cultural heritage is a less straightforward process but may be achievable through repeated surveys of Traditional Owner participants taken to particular monitoring locations and/or provided data on environmental changes. Such a process of recording and analysing changes in individual opinion would be best undertaken by a qualified anthropologist.

Appendix 6. Reproductive Control

Reproductive control alone will not reduce the population substantially in a short-medium time period. However, for populations already at a low density or once the population has been reduced to a lower level, then reproductive control may assist in maintaining this population level, therefore reducing or removing the ongoing requirements for lethal control methods.

Reproductive control is most effective in maintaining medium-sized populations (100-300 horses). For horse populations to be retained, such as the Kiandra horses, reproductive control could be used early on to maintain a population growth rate to be as close to zero as possible. It is important to note that no reproductive control method yet developed is highly effective, easily delivered, affordable, and does not alter the behaviour or physiology in some way (Kane 2018). Criteria for selecting reproductive control methods are delivery method, availability, efficacy, duration of effect, and potential physiological and behavioural side effects (see below for a review of each currently available method for mares). Currently, the most efficacious immunocontraceptive vaccines for horses are PZP and GonaCon (see below).

The size of the population would need to be determined by the density at which negative impacts are mitigated or considered acceptable. The applicably of reproductive controls would be determined by the ability to apply the control at the level necessary to achieve the goal (e.g. maintain the size or significantly reduce the number of removals required) and the cost of achieving that.

Steps to implement reproductive control program (adapted from Kirkpatrick & Frank 2005):

1) Identify goal
    a. Target density and locations, e.g. acceptable density regarding negative impacts
2) Gather information on the target population
    a. More accurate population counts
    b. Identification of bands and individuals
    c. Identify the rates of immigration and emigration

3) Assess feasibility of treatment(s)
    a. Selection of reproductive control method(s)
    b. Develop methodology for administration
        i. Vaccine by hand or other procedure (IUD or surgical) will require:
            1. Site for gathering
            2. Methodology for gathering
            3. Methodology for administration
            4. Infrastructure for administration
        ii. Vaccine by darting will require:
            1. Testing approachability of horses
            2. Estimate the daily number that can be darted
            3. Methodology for keeping track of darted mares
    c. Develop methodology for monitoring
        i. Monitoring treated mares' fertility and for any adverse side effects
4) Statistical modelling to inform the feasibility of using reproductive control to maintain this density, using the specific demographic data collected if possible
    a. Estimate number of mares to be treated
    b. Estimate the frequency of treatments
    c. Estimated effort: time and financial cost

If modelling shows reproductive control would be effective at reducing the population's growth rate to near zero (or enough to significantly reduce the frequency of removals required), and trials show the effort required is possible, then can go ahead with a plan to implement in that sub-population.

Some of the reasons why reproductive control could be less effective than planned

1. Reproductive control is not as effective in populations open to immigration (Ransom et al. 2014b). In an open population, new animals can come in from other populations and contribute to population growth. If no new animals are entering the population, more than half of the females must be treated to achieve a moderate reduction in growth rate (Ransom et al. 2014b). However, in open populations, where immigration can compensate for lower birth rate, more than 80% of females need to be treated (Hobbs & Hinds 2018; Ransom et al. 2014b). The sub-populations in KNP are open to immigration from other zones and treating more than 80% of mares would be a challenge.
2. Non-treated mares may compensate for the reduced breeding of their peers by producing more foals, meaning treatment may need to be more aggressively applied to achieve the same goal as time progresses. When fewer mares are foaling overall, there would be less competition for resources and mares will be in better condition. Non-treated mares may then start producing foals more frequently (e.g. each year) and those foals might be more likely to survive, thus increasing fecundity and juvenile survival (Kirkpatrick & Turner 2008). The rates used in the initial modelling to determine how many mares to treat would then be too low and more mares would need to be treated to achieve the same outcome.
3. Regarding immunocontraceptives, vaccines are generally less effective in the wild than in captivity (Miller et al. 2013; Powers et al. 2014). This is likely due to wild animals generally having lower immune response than captive animals, due to poor nutrition and more parasites and disease (Miller et al. 2013). If the body condition of the animals being vaccinated is low, there may be lower efficacy of the vaccine (Powers et al. 2014). Furthermore, immunocontraceptive vaccines, like all vaccines, have variation in how effective they are in each individual since immune function varies among individuals (Miller et al. 2013). There would be a portion of mares that will not respond to the vaccine, and depending on if this is a trait that is inherited by the daughters, the number of mares in a population who

won't respond to the vaccine could increase since these are the mares producing the next generation of females (Asa & Porton 2005; Massei et al. 2014).

Table 4 provides information on each of the reproductive control methods currently available globally for use in horses. Only methods for controlling reproduction in mares were considered, as male reproductive control in horses is far less effective as it would require nearly 100% of stallions to be made infertile (Bomford 1990; Eagle et al. 1993; Garrott & Siniff 1992; see Supplementary Material 3 for further discussion).

Table 4. Review of current reproductive control methods.

	PZP vaccines			**GnRH vaccines**			**Surgical sterilization**	**IUD**
	PZP-22	Spay-Vac	ZonaStat-H	GonCon	Improvac	Equity		
How effective treatment is at preventing pregnancy	>85% for 2 years; 50% in 3rd year. 2 to 4-year booster= efficacy for 3 years.	50-100% for 3 years (1st year 83-100%)	55-70% for 1 year; Up to 90% if booster at 2-4 weeks	40-94% for 4 years. Booster in 4th year = 91% for 5 years (still evaluating for 6 years +)	< 50%; 1 year or less; requires 2 doses, 4 weeks apart	<50%; 1 year; requires 3 doses, 4 weeks apart	100%; treating only 30% of mares can reduce foaling	Potentially 80-100% but can be expelled: More recent formulation, no expulsion in domestic trials
Delivery	By hand or dart, Hand more effective, darting less effective	By hand	By hand or dart	By hand or dart (Hand more effective) Current trialling of a remote delivery	By hand (dart probably possible)	By hand	Capture and restrain	Capture and restrain
Delivery time	Minimum of 1-3 months prior to breeding activity			Applied near the end of the breeding season for effects to occur after subsequent season			When not pregnant	When not pregnant
Ongoing management requirements	Every 1-2 years	Every 1-3 years	Every year	Every 4-6 years potentially; optimal schedule still being tested	Every 6-12 month	Every 6-12 years	None	Reapplication of expelled IUDs
Issues with delivery	Requires gathers and recognition of individuals High individual variability			Requires gathers and recognition individuals High individual variability			Requires gathers – need to be held for a week to	Requires gathers in a tight time window when non-pregnant

	Darting requires ability to approach within 40 meters			Darting requires ability to approach within 40 meters			ensure no medical issues	
Side effects – immediate physical	Uterine edemia; mild injection site reactions Little adverse effects if accidentally dosed more often Mild darting site reactions – more grievous injuries if darting inaccurate			Mild injection site reaction Little adverse effects if accidentally dosed more often Mild darting site reactions – more grievous injuries if darting inaccurate			Potential for infection – 2% mortality	Mild to moderate endometris; no long-term effects
Side effects- behaviour	Oestrous behaviour continues Increased agonistic and reproductive behaviour; Extended breeding season Decreased band stability			Suppresses oestrous behaviour Effects on band stability			Not demonstrated but limited tests	Maintain normal breeding behaviours
Safe during pregnancy	Yes			Yes, but may induce abortion in early pregnancy			No	No
Reversible	Yes, but long term (5 years) may induce permanent infertility			Yes, but long term (4 years) may induce permanent infertility (may also induce infertility in first dose for some individuals)			No	Yes
Cost	25 $USD per dose; additional costs with administration	24-35 $USD per dose; additional costs with administration	21-56 $USD per dose – need 2 for full efficacy; additional costs with administration	2-10 $USD; additional costs involved with administration	Approx. 4 $USD per dose; additional costs involved with administration	Approx. 200 $USD per dose; additional costs involved with administration	150 $USD includes vet costs	100-200 $USD for device plus vet costs
Availability	Must be made in AUS – is not currently. Would require formulation optimization and trials before ready for wild-scale administration			In process of being registered	Yes	Yes	Yes	Yes
Ecological feedback	Selection for non-responders Improved body condition and lifespan may affect fecundity Effects on band dynamics			Selection for non-responders Improved body condition and lifespan may affect fecundity Effects on band dynamics			Reduces genetic diversity as there is no reversibility	Improved body condition and lifespan may affect fecundity

Relevant references	(Bechert et al., 2013; Jones & Nuñez, 2019; Killian et al., 2006; Killian et al., 2008; Kirkpatrick et al., 2012; Kirkpatrick & Turner, 2003, 2007, 2008; Nuñez et al., 2010; Roelle et al., 2017; Roelle & Ransom, 2009; Rutberg et al., 2017; Turner Jr et al., 2007; Turner et al., 2008)	(Baker et al., 2018; Gray et al., 2010; Killian et al., 2004; Killian et al., 2008; Ransom et al., 2010; Ransom et al., 2014a)	(Botha et al., 2008; Donovan et al., 2013; Imboden et al., 2006; Schulman et al., 2013)	(Ealy et al., 2010)	(Collins & Kasbohm, 2017)	(Killian et al., 2006; Killian et al., 2004; Killian et al., 2008)

In addition to the above reproductive control methods, the BLM is currently developing a new immunocontraceptive, Oocyte Growth Factor (OGF) vaccine, which may safely prevent pregnancy for up to three years or longer from a single dose. Such vaccines prevent the growth of the oocyte (see Mauldin et al. 2007). This would have an advantage over current immunocontraceptives as it may only require a single dose versus two. However, the effectiveness is not yet known, and vaccination trials are currently underway in the USA (BLM).

Supplementary Material

1. SAP responses to CAP questions

Below are the written responses the SAP have provided to questions that have been posed by the CAP throughout the process of compiling this report. They have been included in this report as they are questions other members of the public may also have. These responses had been prepared for viewing by the CAP independent to this report and therefore, there is some repetition with the above report.

1) Quantitative methodology for measuring environmental impact – how, what and where? Include way to correlate numbers with impact

Environmental impact can be defined as any change to the environment, whether adverse (negative) or beneficial (positive), wholly or partially resulting from the activities of wild horses.

Why measure environmental impact of wild horses?

The goal of any horse management plan for KNP should be to mitigate identified negative impacts. While there is ample evidence to indicate that a large horse population in KNP has negative environmental impacts, the relationship between horse densities and these impacts are currently unknown. Since the goal is to mitigate these negative impacts, there is a need to measure the environmental impact of wild horses and the relationship between impact and horse density in KNP. In doing so, management will be able to a) assess whether reducing horse densities is having the desired outcome of reducing negative impacts and b) determine at which horse density the negative environmental impacts are at an acceptable level for the different areas of the park.

While there is valuable information contained in existing studies regarding how horses negatively impact the environment, improvements are required in experimental design to determine the relationship between horse density and impact; much of the previous research is correlative. Furthermore, exclusion studies (where large herbivores are fenced out of an area) may exaggerate the impact of horses due to confounding effects of other herbivores. Additionally, there may be some positive impacts, such as greater vegetation diversity. Impact, therefore, needs to be measured using controlled, manipulative experimental design.

What and how should be measured?

Horse density: horses densities need to be measured at a smaller scale in the different areas of the park to allow for the current horse density and any subsequent changes in horse densities to be linked to the current state and any subsequent changes to focal environmental impacts. Horse densities can be estimated with drone surveys, ground counts, dung counts, camera traps

Negative environmental impacts: Some of the environmental impacts that can be measured include trampled vegetation, eaten vegetation, change to vegetation structure and species composition/diversity, widened streams, broken stream bank, water quality, erosion along horse paths, change in native species abundance and distribution, change in native animal habitat quality (e.g. Dyring 1990, Thiele and Prober 1999, Beever and Brussard 2000, Prober and Thiele 2007, Beever, Tausch et al. 2008, Tolsma 2009, Wild and Poll 2012, Tolsma and Shannon 2018, Worboys, Driscoll et al. 2018, Robertson, Wright et al. 2019).

Where should it be measured?

In all management zones and different habitat types. The relationship between horse densities and negative environmental impact will likely differ for different habitat types. For example, alpine and subalpine habitats are likely more sensitive to even small numbers of horses than lower grassland habitats.

Correlate numbers with environmental impact: Suggested strategy

Using the experimental design described below, i.e. an adaptive management process, the impact of horses can be measured at different horse densities in different areas of the park and measured through time as density varies. If the negative environmental impacts measured (above) are reduced as horse densities are lowered (by control activates and verified by smaller-scale horse population estimates), that is good evidence that those negative impacts were caused, at least in part, by horses and management actions that lower densities are having the desired outcomes of mitigating negative impacts.

To identify environmentally acceptable horse populations will include the following steps:

1. Define environmental impact, as described above. Also define impact on Aboriginal heritage.
2. Define horse management zones. Starting with the already defined management zones developed in the 2016 Plan, incorporate new information regarding areas of high conservation concern and value, high Aboriginal and European Australian heritage, and habitat suitability for key endangered species to further refine management zones.
3. Set preliminary horse density targets within each zone. Preliminary horse density targets to be guided by the current estimated densities within each zone, and past densities when there was little evidence of negative impact (which can be informed by historic records). These targets will be refined as the adaptive management process continues.
4. Establish monitoring sites. Monitoring sites within each management zone (sampling all habitat types) to be selected. At each site record indices of abundance/activity of horse (dung counts, horse counts, etc.) and other grazing species (e.g. deer, pig), and record the selected impact variables at each site. This will provide data on horse densities and impacts, prior to commencing management, and monitoring should continue as management proceeds.
5. Initiate horse population management. Starting with horse reduction zones, begin horse control, initially targeting areas of highest environmental and Aboriginal heritage concern within the zone.
6. Assess the effect of the reduction in horse density on negative impacts. Resurvey sites for horse densities and impacts regularly once or twice per year as management progresses.
7. Adjust density as necessary to reach an acceptable target density. As management progresses, monitoring of environmental impact and horse density will enable an environmentally acceptable density to be determined.

2) *Using drones for population monitoring and mustering: Using drones to conduct an annual population survey over the entire park. Same methodology as the Alps survey. Drone replaces human observers. Data available in real-time to the community.*

Drones can sometimes be more accurate and precise than human observers for some situations but not for others. For example, drones have been shown to be more accurate and precise than ground observations by humans in estimating bird and large mammal populations, but less so than human observers in helicopters in estimating macropod densities (Guo et al. 2018, Hodgson et al. 2018, Gentle et al. 2019). But drones cannot always distinguish between species (Guo et al. 2018, Gentle et al. 2019). Moreover, the costs of labour for drone piloting and data processing can be more expensive than aerial helicopter surveys (Gentle et al. 2019).

At this stage it is unlikely that drones will be able to completely replace helicopters for surveys of the whole Alps, however, they may be useful for smaller-scale surveys of management zones.

Trials will need to be run to test drone capability for distinguishing between pigs, deer and horses and compare their accuracy and cost with other methods of population monitoring.

It should also be noted that the drone obtained data will still need to be statistically assessed. This means that horses counted in live-streamed footage do not equal the population estimate.

3) Types of effective and practical exclusion fencing

Exclusion fencing can be an effective tool to protect areas of high conservation value from threats posed by pest species and has been commonly used in Australia; for example, fencing can exclude herbivores to control erosion of streams and water supplies by preventing trampling, and regenerate vegetation by preventing grazing (Hayward et al. 2009; Kota et al. 2010). However, fence construction is laborious, expensive, and can present some unintended adverse effects: for example, blocking water sources limits water access for wildlife, non-native and native (Boone and Coughenour 2001), and can result in high mortality since animals can become dehydrated or entangled in fencing (e.g. Caughley et al. 1987; Hoare 1992; Mbaiwa et al. 2006). If some streamlines and water holes are to be fenced off to prevent horse access, there must be other water access options for horses and other animals in the area.

Some questions should be addressed prior to construction of fencing (adapted Hayward et al. 2009).

A. Will a fence provide benefits that outweigh both financial and ecological cost, including wildlife access? Exclusion fencing for introduced species in Australia costs between US$6700 and $9500 per km (Moseby et al. 2006).

B. Is on-going maintenance of fencing possible and practical? Once constructed, fences require annual monitoring and maintenance, thereby increasing financial costs.

C. Any failure in maintenance of fencing could have serious consequences such as entrapment in damaged fencing, and entrapment within the exclusion area (e.g. if this is a road, consequences would be very serious)

D. Will the fence solve the identified problem and will the problem be resolved? In the context of KNP, will the horses (and presumably other large, non-native mammals) causing the unwanted impacts to be eliminated or reduced to a density where impacts are acceptable so that fences do not have to be permanently maintained?

E. Will exclusion fencing from one area, increase negative impacts on an adjacent area?

F. Fencing introduces the opportunity to exclude other damaging animals such as pigs and goats, though it may be difficult to do so without excluding desirable animals such as kangaroos and wallabies. Fence design varies according to what animals are to be confined. Horses may be relatively easy to keep out with fencing while including native mammals, but may not effectively exclude pigs (Choquenot et al. 1996), goats (Parkes et al. 1996), or deer (VerCauteren et al. 2006).

G. Animal welfare impacts (some mentioned above) – blocking access to a range of resources for both the intended and unintended species, which may include water, food, con-specifics and potential mates, shade/shelter, escape from predators, escape from adverse environmental conditions (e.g. snow, fire), other potential negative impacts by altering home range, injuries and mortalities by entanglement in fencing, trapping within exclusion area if fencing fails

H. How will tampering of fencing (e.g. by illegal hunters) be prevented?

I. Will the establishment and maintenance of fencing have a detrimental effect on Aboriginal sites?

In conclusion, fencing may be an effective method to protect sensitive areas on a small-scale from horses. However, because we do not know the financial costs and practical constraints associated with fencing in certain areas of KNP, above considerations will need to be assessed before determining whether fencing is a feasible option in a specific area.

4) Significance of wild horses to Aboriginal cultural heritage.

Horses can have an impact on Aboriginal heritage. However, what these impacts have not been well-studied. For example, there is at present no known study of the effect of horses on Aboriginal archaeological sites in Australia. It is important therefore to establish baseline information on the effect of large, hard hoofed animals on Aboriginal sites in the region. Such information could be gathered as part of a suite of data built into holistic monitoring programs but also might require consideration of existing models of artefact accumulation for the high country and have certain dedicated monitoring locations

The impact of horses on Aboriginal heritage should be incorporated into the above strategy and may include the following:

- Archaeological survey of known/suspected areas of higher horse density with a view to the initial determination of the presence of archaeological material and horse impact (direct or indirect) thereon.

- Investigation of known key locations within KNP of high Aboriginal archaeological/heritage significance to assess potential impacts of horses, or their exacerbation of disturbance partly or wholly attributable to other factors.

- Design of controlled experimental locations that would allow measurement of the impact of horses on prevalent site types (see Appendix 5 for more detailed methodology).

- At monitoring locations, an inventory should be undertaken of potential Aboriginal food plant resources and any impact by horses.

5) Significant conservation areas where horses should be eliminated or reduced? Inc. threatened species

As identified by the ITRG and the Kosciuszko Science Conference, horses have the greatest impact in alpine ecosystems, in particular to the Commonwealth-listed Alpine Sphagnum Bogs and Associated Fens Endangered Ecological Community (Robertson et al. 2019) and unique karst systems of geological significance (Spate et al. 2018). Alpine and sub-alpine peatlands provide important habitat for a range of Commonwealth and/or State-listed threatened species including the Alpine Water Skink, Northern Corroboree Frog, and Broad-toothed rat among a variety of others (Cherubin et al. 2019; Worboys et al. 2018). These are areas in which even very low densities of horses are believed to cause considerable damage (Worboys et al. 2018). Sphagnum bogs, karst areas, and preferred habitats of sensitive endangered species would likely be the best areas for complete removal and exclusion of horses to provide the greatest positive ecological outcomes. The alpine and subalpine wetlands are listed as “Sphagnum bogs and associated fens” under the Commonwealth EPBC Act and the larger subalpine fens under NSW listed “Montane Peatlands and Swamps”. The karst areas are registered with NSW PWS for protection. The ITRG found that all groups interviewed agreed to exclusion of horses from above the tree line and in the Main Range in general. This is reflected in zones proposed in the 2016 Plan.

There are also areas in the park of high Aboriginal heritage, although further work is required to identify which areas of greatest concern. A high correlation is predicted between places where wild horses congregate, particularly in wet weather, and the location of larger more dense clusters of surface and subsurface archaeological material (more significant sites). These areas may occur within or peripheral to the areas of ecological sensitivity identified above but will also occur with frequency throughout the park.

6) Where are the heritage areas where horses should remain?

The SAP does not have particular expertise in this field, but valuable information is available in the National cultural heritage values assessment & conflicting values discussion report: 'The Wild Horse Population of Kosciuszko National Park' undertaken by Context Pty Ltd in 2015 and Jenny Dyring's Master's thesis.

Heritage areas are likely to be Kiandra, Byadbo, Long Plain-Coolemon and possibly the lower Indeegoodbee and other lower altitude drier grasslands which were formerly cattle grazing leases. Management of these populations will need to estimate the current population density and the associated impacts. Once an acceptable population size has been estimated with regard to negative impacts, the retained horse population can be managed with a mixture of removals and reproductive control.

Below are quotes from the National cultural heritage values assessment & conflicting values discussion report: 'The Wild Horse Population Kosciuszko National Park' by Context Pty Ltd in 2015.

"From the 1830s and 1840 when the Monaro and Snowy Mountains district was first settled, the occasional Thoroughbred and other breeds escaped from pastoralists, overlanders, or stockmen, or became lost, and inter-mixed with a growing wild horse population. A wild horse population was established in the Mt Kosciuszko area by the 1850s and probably by the 1840s. In 1861, during the ascent from Kiandra of Mount Inchcliffe, near Thredbo, the members of the climbing expedition sighted 'immense herds of wild horses, which would be impossible to break in' (Age 1861). The presence of 'immense herds' would suggest that the horses were well established in the mountain environment at that time." (Context 2015).

"There are various accounts of horses accidentally escaping at Mt Kosciuszko, including the horse/s belonging to Georg von Neumayer's scientific expedition of 1869. Domesticated horses were also intentionally released into the wild at Mt Kosciuszko from at least c.1900 by graziers and stockmen in order to 'improve' the wild horse population with fresh stock. Whereas in other pastoral districts of NSW the wild horses were culled on a large scale, the difficult terrain of the mountainous area of Kosciuszko provided a place of refuge for escaped and wild horses. By the 1890s, the wild horse population was probably greater in the Alps than in the Riverina and other districts. In 1890, Richard Helms noted 'A great number of unowned horses are found all over the ranges' (cited in Slattery 1998)."

7) Feasibility of mustering or brumby running with regard to animal welfare

The SAP has prepared some advice for reducing welfare impacts associated with different management methods, and so in addition to giving specific advice on mustering, have opted to also share advice being provided on passive trapping and brumby running, from the perspective of optimising welfare outcomes, and some general information on assessing welfare during methods of capturing horses. The preliminary evaluations of welfare impacts were based on the ITRG humaneness assessment report, and so it is advised that this is also referred to. The following summarizes what welfare impacts occur with different methods of capturing horses, and further considerations for how some of these impacts may be mitigated. For any method where welfare impacts are considered to be severe, and unable to be substantially mitigated, then this method is not advised.

Mustering

The ITRG assessment scored mustering as another capture method with the likely least negative welfare impacts, although it still scored a moderate impact for a duration of hours, with an overall score of 5. Further, this was only when mustering was of small groups of horses performed over hours. If larger groups of horses are mustered over days, then welfare impacts will be greater (score 6). This also assumed mustering within a small area (i.e. maximum of 2 km) so that horses are not pushed outside of their home range, and assuming up to 4 mobs are mustered. If more horses were mustered, over a longer period, and/or over longer distances then welfare impacts would be expected to be greater. Therefore, mustering of smaller numbers of horses (1-2 mobs) over hours, and not outside of their home range is advised. Mustering larger groups of horses (especially > 4 mobs) over days, and/or moving them outside of their home range is not advised. If more than one mob is being mustered, yards need to be able to appropriately separate mobs maintaining family groups together. Food and water must be provided in yards. Horses should be mustered slowly to reduce the negative impacts of exertion and risks of injury. Impacts once yarded can be similarly reduced as for passive trapping. Horses should not be without water and food for > 12 hours.

Brumby running

The ITRG assessment scored brumby running as the capture method with the highest negative welfare impacts, with a severe impact for a duration of hours, with an overall score of 7. Scoring was based on Parks Victoria Standard Operating Protocol (SOP). Welfare impacts were classed as severe because horses may be without food and water for > 24 hours, they may experience heat stress during exertion, and potentially hypothermia if tied up for a long period during cold weather. Prolonged or excessive exertion can cause myopathy, neck ropes can cause injuries and interference with breathing, pursuit can cause injuries, the bands are disrupted, and horses separated from their family groups, captured horses may be tied up for 24 hours or more and be apart from other horses. Foals can also become separated from bands during the chase with subsequent extreme welfare impacts.

There is the potential for many of these impacts to be mitigated if strict protocols are followed, which would include a limitation on the pursuit time and tie-up time, that individual horses should not be left alone without another horse, horses should not be more than 12 hours without water (or not more than 6 hours without water if horses are sweating excessively), or food, neck ropes should not cause injury or interference with breathing, and bands containing young foals should not be targeted. Measurement/auditing of actual measures of animal welfare impacts has not been previously performed and would be advised for any pilot program before deciding if continuation of brumby running was acceptable on welfare grounds. The SAP recommends that where substantial welfare impacts can be acceptably mitigated in the development of an alternative SOP, that this method could be trialled under strict conditions with actual monitoring of animal welfare outcomes.

8) Brumby Mare rates of reproduction per annum (i.e. how many foals is a mare likely to have in any 5-year window) i.e. it must be a less than 1 for every year to allow for infertility etc

In population studies of wild horses, this is usually quoted as ‘fecundity’ which is the number of females born per adult female per year, across the population. If food isn’t limited, fecundity is usually relatively stable across most wild horse populations, at around 0.4 in most published studies. This means on average, each adult mare has one foal every 2 years (or more precisely 4 foals over 10 years). Fecundity will vary however between individual mares with age, fecundity increases up to 5-6 years of age and foaling rates remain on average a foal every 2 years until the age of 15-18 years. Mares also produce fewer foals when they are in poorer body condition.

Since the number of foals that a mare produced over time is dependent on her body condition, which depends on how much food is available, this means, as the population grows larger, there is less food available for each mare, each mare is likely to be in poorer body condition and will likely have more years between each foal. This also means that when the population grows smaller (e.g. if horses are being removed from the population by management), each mare will be in better body condition and will be more likely to have a foal every other year, or even every year.

In the Australian Alps, population demographics were studied in detail by Dawson & Hone and over 3 sites (Big Boggy, Cowombat, Currango) fecundity varied from 0.24 and 0.21 at Big Boggy and Cowombat flat, to 0.31 at Currango plain. This is an average of one foal approx. every 3 years; or 3 foals every 10 years. There have been other more recent observations at Cooleman Plain suggesting that mares were on average having one foal every two years (Andrea Harvey, pers. obs).

In summary, depending on how good the season/habitat is in terms of food availability and how high the population density already is in an area, over a 5-year period, each adult mare will likely to have on average 2 foals. In a good season in good habitat (such as Northern KNP) they certainly can have a foal every year. In very bad seasons, poor habitats (such as some parts of Southern KNP), or at very high densities, they may only have a foal every 3 or 4 years. Because foaling rates can vary with population density, the rate of foaling will likely change as horse control methods are undertaken.

9) What is the survival rate for Brumby Foals, e.g. must be some disease, colic, health issues

In most wild horse populations without predators, foal survival is quite high. Survival for 0-2 year-olds is reported at being about 90% across most studies. In KNP, there are no predators of foals (dingoes or wild dogs may occasionally predate foals but evidence suggests this does not occur frequently), so that the most important factor influencing foal mortality is the amount of food available (which influences both the food available for the juveniles and the condition of mothers and their ability to produce milk).

In Dawson & Hone's study in the Australian Alps, average survival of 0-2 year-olds was 83 – 90% across the 3 study sites. In a more recent study at Cooleman Plain and Cowombat flat, foal survival appeared to be > 90% with monitoring over a 2-year period (Andrea Harvey, pers. obs). Moreover, since the proportion of foals and yearling was the same, foal survival was also likely high before these 2 years of observation.

10) What is the average life expectancy of a wild horse in this climate?

Life expectancy of a wild horse depends largely on the habitat, climate, and food availability. This means the life expectancy of horses in KNP is likely to differ within the different habitat types in the park and over time between good and bad seasons. Studies of most wild horse populations, including Dawson & Hone's study in the Australian Alps, have shown high adult survival (> 90%), but haven't specifically looked at life expectancy as this would require very long-term studies. This is a bit of a knowledge gap here. Some data from Northern KNP has been collected from ageing dentition of skulls found in the park, and ageing horses that have been removed from the park by dentition. Although not a precise measure of life expectancy, this offers some information on the ages of horses in KNP. Very few horses have been estimated at > 10 years of age, which may suggest that life expectancy is relatively short compared to domestic horses. We did find one skull of a horse well over 20 years old. However, this does need to be interpreted with caution; only a very small number of skulls were found and the cause of death was not known. The age of horses removed from the park is likely to be biased towards younger horses as older horses tend to be more 'trap shy'. We would expect life expectancy to be longer in northern KNP where food availability is higher, but this has not been tested. Life expectancy may also be shortened due to intestinal disorders associated with chronic gastrointestinal parasitism but there is no definitive information about this.

11) Explanation on the survey model analysis (how do they get 15,000 horses out of 1200 actual counted?)

Estimates of wild animal numbers are just that … estimates… because we can rarely record and count every animal. The KNP horse population estimates are obtained using a technique called distance sampling. This technique first estimates animal density and then converts to animal number by simply multiplying the estimated density (e.g. animals per ha) by the size of the area involved (e.g. in ha).

Suppose a friend (or fiend!) evenly scatters matchsticks across an area of your backyard lawn, telling you the boundaries of the area, but without telling you how many matches were scattered. Suppose also that these matches could sense your presence, if nearby, and were mobile, by virtue of little legs. Then imagine that your task is to estimate the density of matches and hence the total number of them within the demarcated area of lawn. This would be conceptually analogous to first estimating horse density within a certain area, and then extrapolating to estimate total horse abundance in this area. In this situation, estimating match density is plagued by the following two fundamental problems: it is increasingly difficult for an observer to detect a match with increasing distance between match and observer; matches may respond to observer presence by moving towards (i.e. attraction) or away (i.e. repulsion, which would be the case for horses).

The standard method for estimating match-stick density would be the transect-distance approach. In this case, one moves along a line (called the 'transect line'), possibly while walking but probably better on one's hands and knees, noting every match that is observed and estimating the distance between match and transect line. Statisticians have devised ways to convert such data into density estimates. Important assumptions here are that close to the transect line (e.g. perhaps about 20 cm either side, if the observer is crawling along) any match is certain to be detected, and that the likelihood of detection declines with increasing distance between match and transect line. This latter relationship can be estimated statistically: transect-distance data are converted into estimates of match density using computer software such as the program '*Distance*'.

This approach can be applied to estimating horse density through observations made either by people in helicopters or through recorded images from a drone. In either case, observations are made along transect lines with estimates of the distance between observed horse and the transect line. In either case, it must be assumed that horses relatively close to the transect line are certain to be detected and recorded. Estimates of horse density can then be converted into estimates of total horse population size for the relevant area, just by multiplying estimated density by the size of the area involved.

12) Feasibility of using translocation as a control method

Relocating animals with home range fidelity is generally a low success strategy because a) animals often return to where they were removed from and b) relocated animals suffer high mortality and thus a poor welfare outcome.

Relocated individuals often return to their home ranges, even when 100's km away (Fischer & Lindenmayer 2000; Linnell et al. 1997). Although horses don't defend territories, they show relatively high fidelity for home ranges (Cameron et al., 2001; Linklater et al., 2000). After large scale gathers, released horses will quickly return to their home range when possible (Berman pers. obs., Pirtle pers. obs. and pers. comm. with NPS and BLM staff). Depending on where bands are relocated to within KNP, it could be quite easy for them to return, potentially crossing roads to do so and presenting potential hazards and negative welfare outcomes for the horses.

Relocated animals tend to face high rates of mortality, in part, from harassment from residents or naivety of the new environments (Craven et al. 1998; Fischer & Lindenmayer 2000). Relocated animals tend to be harassed, excluded, and even killed by resident individuals. Horse bands often have overlapping home ranges and are familiar with neighbouring bands (Cameron et al. 2001; Linklater et al. 2000). Relocated bands into areas with established bands might face high levels of harassment and exclusion from prime resources by the other bands. Furthermore, relocated animals have no knowledge of reliable food and water sources throughout the season. In an extreme example, wild mares in Queensland that were moved from prime to less prime habitats, all either died or were in very poor body condition within 2 months (Hampson et al. 2011). The difference between areas in the park would not be as extreme but in drought conditions, naïve bands could face more difficulties in finding sufficient resources.

Relocating horses to 'less sensitive' areas on the park, even if successful, would only exacerbate the negative impacts on those 'less sensitive' areas. 'Less sensitive' areas would need to be identified and it would need to be determined that the environment could cope with additional, potentially hundreds, of horses. In conclusion, relocating horses within the park is likely to be an inefficient strategy with potentially poor welfare outcomes.

13) Numbers required for a genetically viable population

There is no magic minimum number at which a population can be considered infinitely viable (i.e. will maintain genetic viability indefinitely). How 'genetically viable' the population is will depend not only on the size of the population but also on how genetically diverse the population is already and how often it is infused with new genetic material (i.e. new animals immigrating into the population).

There are no current published genetic studies on wild horses in KNP, although we have been in touch with a geneticist recently with a view to such work commencing. This means baseline genetic diversity is currently unknown, so the effect of reducing the population on genetic diversity is not known. However, the population in KNP currently is not a small nor isolated population and thus there is little imminent risk of inbreeding and consequences to genetic health. This means that protecting genetic diversity of horses in KNP is not an immediate concern. However, genetic diversity can be routinely monitored as management progresses to ensure genetic health is maintained.

At the population level, genetic diversity can be measured as the mean number of variants of a gene (alleles) or as the proportion of individuals that have different variants of a gene (heterozygosity) (see National Research Council 2013). Inbreeding coefficients (the probability that genes at a randomly chosen location are identical by descent) is also a useful measure. To provide a measure of the genetic health of the current population, analysis for allelic diversity, heterozygosity, and inbreeding coefficients could be performed from genetic testing of samples collected from gathered horses. Recording the occurrence of genetic diseases and conditions could provide an indicator of the genetic health of a population as control measures are implemented and the population density reduced. If there is an indication of compromised genetic health, additional genetic analyses can identify horses managed outside of the park (e.g. in neighbouring parks/reserves) that could be suitable to introduce into these heritage herds to genetically reinvigorate the population (see National Research Council 2013 for a more detailed discussion of managing genetic viability in horse populations).

2. Estimating Population Size

Zen and the art of estimating wild animal numbers

Introduction to estimating wild animal numbers

Estimates of wild animal numbers are just that … estimates… because we can rarely record and count every animal.

Estimates of wild animal numbers will have inherent associated error, because many factors vary outside of our knowledge or control. However, it is often possible to also estimate the magnitude of such error and to be able to state, for example, that it is 95% likely that the number of animals within a certain area lies between one number and another (e.g., between 1,000 and 1,200). Such a range would be described as our 95% confidence interval.

We can decrease the size of the confidence interval, and hence be more confident in our estimates of animal numbers, by repeating procedures, and combining more and more data. If, for example, we were trying to estimate the number of horses within a particular area, then counting horses along 100 different transects or paths would be better than having just 50. In other words, the larger the sample size the better.

In estimating the number of animals in a certain area, we might aim to estimate this directly, or first estimate animal density and then convert to animal number by simply multiplying the estimated density (e.g., animals per ha) by the size of the area involved (e.g., in ha). We shall explain these two approaches below.

Direct estimate of the number of animals in a certain area

This task is conceptually similar to trying to estimate the number of marbles in a large jar, which is opaque except for a few clear windows on the side. These windows are like vantage spots, from each of which can be seen a few marbles. So, we know that there are marbles in the jar, but we have little or no idea as to how many marbles there are.

The first thing we would do is give the jar a shake or two, so that the marbles are well mixed with no biases in distribution (e.g., slightly larger ones nearer to the top and smaller ones toward the bottom, or perhaps vice versa).

The second thing to do is to remove a random sample of marbles from the jar. If the marbles are well mixed, we could just tip some out from the top. Otherwise, we would need to take marbles from throughout the jar, taking care not to create biases by inadvertently tending to pick one kind of marble over another. Here the aim is to get a sample of marbles that is 'random' with respect to any inherent differences amongst marbles.

Thirdly, we would mark each marble in the sample, perhaps with a dot of paint, count them (call the number 'n'), return them to the jar, and give the jar a few more shakes, to completely mix them up again. Marbles that were included in the sample could then be distinguished in the hand from other marbles in the jar.

Fourthly, we take a second random sample of marbles from the jar; it doesn't have to be the same number of marbles. We count the number in this sample, differentiating between specially marked and unmarked marbles. This gives up the proportion (call it 'p') of marked marbles in this second sample. Consequently, this approach is commonly referred to as 'capture-recapture'.

Finally, we estimate the number of marbles in the jar (call this N) by the following bit of simple mathematics:

$N=n/p$

In other words, the estimated number of marbles in the jar is the number in the first random sample divided by the proportion of marbles in the second random sample that were 'marked' as being included in the first sample.

For example, if the first sample consisted of 200 marbles (it is a big jar!) and 20% or one-fifth of the second sample were marked, and so included in the first sample, our estimate of the total number of marbles in the jar would be 200 divided by 1/5, which is the same as multiplying 200 by 5, which would equal 1,000 (yes, quite a large jar!). To check this result, you can confirm that the proportion of specially marked marbles in the jar should be 200 out of 1,000, or one fifth, which should and does correspond to the proportion of marked marbles in our second random sample.

Now, imagine that we are similarly trying to estimate the number of wild horses within an area.

In this case, the marble jar is assumed equivalent to an area within which horses mingle and move about and can be considered reasonably well mixed. It would require a somewhat more sophisticated approach if groups of stallions were in some parts of the area and mares in different parts. We would need help from the statisticians to cope with this. For now, let's assume it's not an issue.

There are a couple of ways in which we could take our first, hopefully random, sample of horses and identify animals in this sample. We could, for example, attempt to identify observed animals through patterns of colour. Or we could use DNA analysis to identify animals responsible for accumulated dung. We could assume, in either case, that the identified individuals constitute a random sample of the population. Otherwise, we would once again have to seek assistance from the statisticians.

We would similarly take a second random sample, allowing time (equivalent to shaking the marble jar) for horses to become well mixed.

We would then proceed as above, determining the proportion of individuals in the second sample that were included in the first sample (i.e. 'p').

By dividing the number of animals included in our first sample by the proportion 'p' (as above) we would estimate the total number of horses in our area of interest.

If we had several areas of interest, each corresponding to a separate sub-population of horses, we would separately carry out such calculations for each area, and then add the estimated numbers together to end up with an estimated total number for our entire area of interest.

Of course, just as in the marble example, there may be deviations from some of the simple assumptions. You should therefore not be surprised if scientific documents dealing with such capture-recapture methods for estimating horse population size seem more complicated.

This approach could give us estimates of horse numbers across different sub-populations within KNP, and hence across all of KNP.

Estimation of animal density

Suppose a friend (or fiend!) evenly scatters matchsticks across an area of your backyard lawn, telling you the boundaries of the area, but without telling you how many matches were scattered.

Suppose also that these matches could sense your presence, if nearby, and were mobile, by virtue of little legs.

Then imagine that your task is to estimate the density of matches and hence the total number of them within the demarcated area of lawn.

This would be conceptually analogous to first estimating horse density within a certain area and then extrapolating to estimate total horse abundance in this area.

In this situation, estimating match density is plagued by the following two fundamental problems: it is increasingly difficult for an observer to detect a match with increasing distance between match and observer; matches may respond to observer presence by moving towards (i.e. attraction) or away (i.e. repulsion). If you have tried observing birds, you should be well familiar with these phenomena!

The standard method for estimating match-stick density would be the transect-distance approach. In this case, one moves along a line (called the ‘transect line’), possibly while walking but probably better on one’s hands and knees, noting every match that is observed and estimating the distance between match and transect line. As explained below, statisticians have devised ways to convert such data into density estimates.

Important assumptions here are that close to the transect line (e.g. perhaps about 20 cm either side, if the observer is crawling along) any match is certain to be detected, and that the likelihood of detection declines with increasing distance between match and transect line. This latter relationship can either be assumed to fit a standard equation (e.g. Gaussian or Normal) or be estimated statistically.

Transect-distance data are converted into estimates of match density using computer software such as the program ‘*Distance*’.

This approach can be applied to estimating horse density through observations made either by people in helicopters or through recorded images from a drone. In either case, observations are made along transect lines with estimates of the distance between observed horse and the transect line. In either case, it must be assumed that horses relatively close to the transect line are certain to be detected and recorded.

Estimates of horse density can then be converted into estimates of total horse population size for the relevant area, just by multiplying estimated density by the size of the area involved.

This approach can provide estimates of horse numbers for different areas or horse sub-populations within KNP.

3. Reproductive Control: Background Information

Brief introduction to controlling population densities

Horse populations increase when horses are born or immigrate, and decrease when horses die or emigrate. This means that the population growth rate, i.e. the number of horses each year, is determined by the immigration, emigration, death, and birth rates.

We can control any population's growth rate by reducing the number of individuals entering the population each year by preventing immigration and/or reduce the birth rate or we can reduce the number of horses already in the population by relocating or culling individuals.

Reproductive control methods control population densities through altering the birth rate. Population traits that determine the birth rate include the number of females that produce offspring, i.e. the effective population size, and the number of offspring that each female produces, i.e. fecundity. The number of these offspring that are daughters and survive to sexual maturity, i.e. juvenile survival, will further influence the future birth rate. Thus, the birth rate is essentially the effective population size x fecundity x juvenile survival. To decrease the birth rate, we can reduce the effective population size (i.e. remove breeding females), reduce juvenile survival (i.e. remove foals) or reduce fecundity (i.e. reduce the number of offspring each female produces).

The method that works best (i.e. controlling immigration, emigration, deaths, or births) for **reducing** a population's density depends on aspects of the biology of the animal. Horses are long-lived with low fecundity (mares can only produce one foal per year), high juvenile survival, and low adult death rates. This means that even if all births are stopped, the population won't decrease in size any faster than the natural death rate. In the Alps, that is about 6% of adults who die each year (Dawson & Hone 2012). This means that reproductive control is not a good tool for quickly shrinking horse populations. In fact, reducing fecundity can increase adult survival since mares live longer when they are released from the stresses of having foals (Kirkpatrick & Turner 2008), making reproductive control even less effective at **reducing** population densities. The most efficient way to reduce the size of a horse population is to adjust the 'death/emigration' rate, i.e. removing individuals from the population (Barlow 1997; Dawson & Hone 2012; Fagerstone 2002).

While controlling reproduction is not an effective method for quickly reducing horse populations, it can be an effective way to maintain low densities and reduce the need for removal of horses. By making some mares infertile, we are reducing fecundity and shrinking the effective population size. The effective population size in horses is mostly determined by the number of mares, since only one stallion is needed to impregnate many mares. In terms of the population growth rate, every male is replaceable, and most are superfluous. This has two implications: firstly, reducing the effective population size means reducing the number of mares, i.e. controlling the number of stallions in the population is not an effective way of controlling birth rates. Secondly, reducing fecundity means reducing **mare** fecundity, i.e. controlling reproduction of stallions is not an effective way of reducing birth rates. Reducing reproductive rates via sterilisation of stallions would require nearly 100% of stallions to be treated (Bomford 1990; Eagle et al. 1993; Garrott & Siniff 1992). This also applies to the removal of horses to manage populations. Removing stallions does not slow the population growth rate since each band stallion you remove will be replaced by another and mares will continue to foal at the same rate.

In general, reproductive control will work best in closed populations of less than 300 horses for which all animals are identifiable, trackable, and relatively approachable/gatherable (e.g. Baker et al. 2018; Kirkpatrick & Turner 2008). This is because to administer any sort of control (vaccine, IUD, etc. see table below), we must be within touching (or darting) range of the mare. So, in general, horses need to be gathered and to some extent handled. If we are using immunocontraceptive vaccines, most need to be administered to the same mare in multiple years to be most effective, which means we need to be able to identify and recapture each treated mare. Furthermore, to ensure the treatment even worked, we need to follow each treated mare in the next season to see if she foaled and ensure no adverse welfare outcomes. This is all quite challenging to complete in large populations.

Controlling reproduction also works best in populations closed to immigration. When the population's density is reduced in an area, there is more food available, less competition, and the area becomes more attractive to dispersing individuals. In a population that we have lowered and then administer reproductive control to keep the population low, new mares will full fertility may immigrate into the lowered population, making the treatment less effective.

Initial steps for implementing a reproductive control method:

1) Identify the goal and the timeframe to achieve that objective.

We have to decide what the goal is. If it is to reduce a population from 500 to 250 within 5 years, controlling reproduction won't be effective. If it's to maintain 250 horses in an area at that density for the next 5 years, then reproductive control may be appropriate.

Because our goal is to reduce the negative impact of horses on the environment, this makes identifying the target population densities more difficult since we don't yet know at what density the negative impacts of horses are acceptable or mitigated. Thus, we don't know what our target population densities are yet and where using reproductive controls would be suitable.

2) Identify the available control method(s) that would be most suitable.

An updated summary of the available reproductive controls for mares is provided in Table 4 (above). In Australia, immunocontraceptives would have to be registered with the Australian Pesticides and Veterinary Medicines Authority (Humphrys & Lapidge 2008). Import of the vaccines might need to be sorted out with Australian Border Force. First, follow up in how achievable this is and in what timeframe before moving on. Other methods that may be useful and more quickly available: IUDs or surgical sterilisation of mares (see Appendix 6).

3) Assess whether the contraceptive could be delivered under field conditions in the chosen population.

Once we have chosen a method(s), we have to assess whether we could treat a large proportion of mares in our target population. If we are using vaccines, can we gather a sufficient number of mares and hand dart? Can we approach within 40 yards to be able to dart mares? We need to consider how will the treated mares be tracked and identified for booster vaccinations. Regardless of the methods used, we will need to follow each treated mare to determine a) if she gets pregnant after being treated and b) ensure there are no adverse welfare outcomes from being treated.

4) Determine whether the goal could be achieved in the field.

This requires modelling the effects of the control program on population dynamics (Barlow et al. 1997). Modelling can help inform what overall proportion of mares must be made infertile to achieve the goal, how frequently the treatment needs to be re-applied, and what effort would be required to achieve the target within a given timeframe. If modelling suggests that the goal can be achieved within the targeted time frame (and available budget), then we can move forward with implementing a program as informed by the modelling.

4. Humane Management of Wild Horses

The below information provides an outline of the ethical decision-making framework the SAP is recommending be followed for wild horse management in KNP. Below is a guide of decision making based on welfare outcomes of different wild horse management methods, and commonly asked Q&As regarding lethal vs. non-lethal control. This information is supplementary to the ethical decision making section in the main body of the report and Appendix 3.

Terminology

- ***Humane*** is a very subjective term and means different things to different people. Concerning population management methods, some use the word 'humane' to mean 'a method that does not cause significant animal suffering'. However, others use 'humane' to have a broader meaning, incorporating ethical values, which may include whether a method is lethal or non-lethal. Thus, some people may consider lethal control, by any method, even if there are good welfare outcomes (i.e. no suffering) not to be 'humane', but this is due to ethical values, rather than due to welfare impacts. The dictionary definition of 'humaneness' is 'characterised by tenderness, compassion and sympathy', so arguably for some people, no methods of population control would be considered humane using this definition.

    - It is important that we are clear about this, because when any of us object to a particular method of population control we need to consider whether it is because of our ethical values, or because of animal welfare impacts. These two issues should be addressed separately.
        - ***Ethical management***
        - ***Animal welfare impacts of management***

- The ITRG humaneness assessments from the 2016 draft plan (detailed in review of 2016 plan) are therefore more accurately considered as an ***'animal welfare impact assessment'***, although they did consider more than just animal welfare, by also considering whether the method was lethal or non-lethal. Lethal vs non-lethal is also problematic terminology since many methods considered non-lethal (e.g. fencing, trapping and removal, non-intervention) can also be lethal. This is further addressed below. It is also important to remember that whether a method is lethal or non-lethal doesn't necessarily impact on the animal welfare outcome; some non-lethal methods may have worse animal welfare outcomes (i.e. more suffering) than lethal methods. We will now use the term '***animal welfare impact'*** rather than 'humaneness' to clarify when we are discussing animal welfare aspects of a particular method, vs ethical views associated with a particular method. Other ethical aspects should be considered separately, but alongside, animal welfare.

- ***Ethics*** refers to the moral principles that guide our choices and behaviours to make 'the best' decisions.
    - There are many different ways of thinking about ethics, but one way is to think about ethics in terms of the consequences of a decision. 'The greatest good for the greatest number' is a maxim that is commonly recognised and accepted.
    - Sometimes the best decision may be very obvious, other times it can be really difficult to determine what the best decision is, and these situations are known as ***'ethical challenges'***.
    - Situations are often ethically challenging because the same decision that may lead to 'the best' outcome for one person, may lead to an undesirable outcome for another person, so the 'best decision' becomes less obvious.

- Thus, addressing ethically challenging situations involves weighing up the outcomes of a range of decisions for a range of ‘stakeholders’.
- Wild horse management is certainly an ethically challenging situation, and the ‘stakeholders’ that are affected by decisions are very broad including:
  - The horses themselves – as a species, population, and the welfare of individuals
  - Other animals sharing the same environment – as a species, population, and the welfare of individuals
  - The environment – as an ecosystem, and also individual aspects of the environment which might include certain features, specific plant species, soils, water systems, and other aspects such as aboriginal artefacts
  - People – the wide range of people in society with different value and views around wild horses is one of the reasons why wild horse management is so ethically challenging. In ethics, the outcome for all people needs to be considered and so even without considering the non-human stakeholders, the outcome with ‘the greatest good for the greatest number’ inevitably means that respect for different views and values is required and that compromises need to be reached to strike a balance between the range of views

Ethical decision-making in wild horse management

As many stakeholders are impacted by decisions, it is not a personal opinion that dictates what ‘the best’ decision is, but rather decision making should follow an ethical framework to ensure ‘the best’ decision is made to achieve ‘the greatest good for the greatest number’. It is recognised that the management of any wildlife can be ethically challenging for many of the same reasons highlighted above. As a consequence, a large panel of international wildlife, conservation and animal welfare experts got together to produce the ‘International Consensus Principles for Ethical Wildlife Control’, which was published in the peer-reviewed literature (Dubois et al 2017).

The SAP recommends that these principles are followed for wild horse management in KNP and following these principles form the main body of our advice. The principles are as follows:

1. ***Modifying human practices***

   Negative impacts were first evaluated as to whether the impact can be mitigated by modifying human behaviour.

2. ***Justification for control***

   Scientific evidence was evaluated to assess the population size, increase and the negative environmental impacts, as well as the horse welfare impacts of ineffective population control. Knowledge gaps were identified with recommendations for further research to define ‘sustainable populations’.

3. ***Clear and achievable outcome-based objectives***

   Adaptive management is recommended to each the end goal of environmentally sustainable populations of wild horses.

4. ***Animal welfare***

   Management methods used should cause the least harms to animal welfare to the least number of animals.

5. ***Social acceptability***

   Social acceptability is crucial, and is dependent on education, transparency and trust. Effective community engagement is required in development and execution of the management plan. Effective communication with the public and prevention of dissemination of misinformation is required.

6. ***Systematic planning***

   The recommended systematic planning comprises strategies for identifying an environmentally sustainable horse population, identifying management zones and horse heritage areas, monitoring of the horse population, monitoring environmental impacts and Aboriginal heritage, a process for decision making in choosing control methods for different zones and circumstances, and assessing the animal welfare impacts of control methods, with an adaptive management plan to prioritise the use of methods that have the least negative impact on animal welfare.

7. ***Decision making by specifics rather than labels***

   The SAP recommends an evidence-based management program in line with principles 1-6. The steps being followed in developing the management program would be the same for any wildlife species where management is required as based on scientific evidence. Management decisions are therefore not based on horses being an introduced species, or based on any particular group of people being biased against wild horses. The SAP recognises the importance of the horses to elements of the local and wider community. The SAP seeks to introduce a management plan that will achieve a balance, to reach an environmentally sustainable population of wild horses whilst also protecting and retaining other values of KNP.

'Fraser's practical ethic' is another ethical principle that is commonly applied to ethically challenging situations involving animals, in particular in a 'One Welfare' context, where it is sought to maximise the wellbeing of animals, the environment and people. ***'Conservation*** **Welfare**' is also a new defined discipline specifically applying animal welfare to conservation activities. These are both principles that the SAP recommend following in wild horse management, and are further detailed in Appendix 3.

Lethal vs ***non-lethal*** management

As eluded to in Appendix 3, some methods that are considered 'non-lethal', may ultimately be lethal and cause worse animal welfare impacts than other non-lethal methods. Examples here include fencing an area that may prevent access to food, water or con-specifics or result in getting trapped or injured, rehoming where the new home isn't successful and the horse may end up getting sent to a knackery, sometimes after a period of neglect or mistreatment, and may involve going through saleyards.

A non-lethal method that doesn't have severe impacts on animal welfare would always be considered preferential to a lethal method. However, there are situations where non-lethal management is not possible. The only non-lethal management options available are rehoming and reproductive control. However, there are limited rehoming opportunities compared to the number of horses that need to be removed from the park. Further, for rehoming to occur, the horses need to be in an area that is accessible for trucking out. There are many areas in KNP that are not accessible for trucking horses out. Reproductive control has the potential for reducing population increase but does not **reduce** the population in the short to medium term (see Supplementary Material 3). Therefore, where horse numbers need to be reduced to a higher degree than can be achieved with rehoming or reproductive control alone, then lethal methods are required. If used to reduce the population in the short term, the aim is then that eventually populations could be maintained with rehoming and reproductive control without the ongoing need for lethal control methods.

If lethal methods are not used, and non-lethal methods can not remove a sufficient number of horses the population of horses will continue to rise. This is not only detrimental to the environment and other animals sharing the environment, but it also becomes detrimental to the welfare of the horses themselves. With no natural predator of horses in KNP, the only thing that slows the population growth of wild horses is food availability. This means that without effective management large numbers of horses starve to death. This can occur very slowly over many months. This has much more severe welfare impacts than a quick death by shooting or lethal injection. Ineffective management is therefore unethical as many ‘stakeholders’ suffer including the environment, other animals and many individual horses themselves.

Therefore the ethics of lethal control is commonly assessed by balancing the other outcomes associated with killing or not killing the animal, i.e. animals should not be killed unnecessarily; there needs to be good justification of other positive outcomes resulting. In this case, potential positive outcomes would include environmental benefits, welfare and survival benefits to other species, and welfare benefits to remaining horses (increased resource availability and reduced competition).

There may be other ethical reasons against particular methods – for example, other concerns with shooting from the ground or air include the possibility of indiscriminately killing large numbers of animals in a short period of time, and concerns about carcasses being left in the environment. These are also very important concerns, but it is important that these are considered and addressed separately to the ethics of lethal control *per se*, and concerns about animal welfare impacts associated with the method.

When lethal control in certain circumstances is deemed justifiable, then we should ensure that the methods used, result in the least negative impacts on animal welfare (i.e. the least ‘suffering’, such as pain and fear) as possible.

Animal welfare impacts

Animal welfare is characterised as the animal’s perception of their mental state, in other words, what an animal is experiencing or feeling. It is conceptualised as the property of individuals, belonging to species considered to have the capacity for both pleasant (positive) and unpleasant (negative) mental experiences, a capacity known as sentience.

All interventions can have positive impacts on an animal’s mental experiences, or negative impacts. When we are talking about animal welfare impacts of methods of population control, we are generally talking about negative impacts on the individuals being removed from the population, although we should always be clear about that (e.g. some impacts could be positive; such as reducing a population can enable higher food availability for the remaining population, which can result in a positive animal welfare impact for the remaining population). Negative impacts associated with population management are negative mental experiences such as hunger, thirst, breathlessness, pain, fear, fatigue, weakness. These mental experiences are inferred using neurophysiological evidence and interpreting indicators of biological function and behaviour. There is a wealth of literature on this area of animal welfare science.

Animal welfare impacts are further evaluated based on the severity and duration of these negative mental experiences. On a population level, animal welfare impacts are also evaluated based on the proportion of animals experiencing negative impacts. For example, the proportion of animals experiencing injuries or dehydration. With lethal control methods, it may be the proportion of animals not experiencing instantaneous death.

Assessing animal welfare impacts, and reducing welfare impacts associated with different management options, including different methods of capturing horses, is detailed in Appendix 3. The following section aims to specifically answer some commonly asked questions surrounding lethal control methods.

Common Q&A's associated with lethal control methods

1. Why does lethal control have to be used at all, isn't this cruel?

There is sometimes confusion between 'ethics' and 'welfare impacts' when people consider cruelty. Animals should not be killed without justification (ethics) but following ethical decision making if it has been deemed justifiable that an animal should be killed (for example numbers need to be reduced and can not be done so with non-lethal methods), then it is the way in which the animal is killed that is important. If a method is 'cruel' it would suggest that it causes severe negative animal welfare impacts such as hunger, thirst, pain, fear. It is very important that when an animal is killed, whatever method is used, it is performed in a way that has the least negative impacts (or least harms) to animal welfare.

2. If lethal control has to be used what is the best method with the least animal welfare impacts?

The available ways for killing wild horses are capturing in yards and then tranquillizing before giving a lethal injection or shooting, or shooting without prior tranquilization. Alternatively, horses can be shot with a firearm without prior capture, either from the ground or from the air.
There are potential advantages and disadvantages of all these methods. Shooting *per se* is often considered by the public to be undesirable. However, when performed appropriately, it has the ability to cause instant unconsciousness and death without prior fear or pain, i.e. without significant negative welfare impacts. This can potentially have less welfare impacts than death by lethal injection.

The biggest concerns with shooting without capturing the horses first are that if a shot is inaccurate it could result in non-fatal injuries and that other horses may become anxious when one of their herd mates is shot. However, when performed under strict conditions (see Appendix 3) it would be expected that most animals would experience a very quick death, without having prior negative experiences of long durations, such as being removed from their natural environment through trapping or mustering, subjected to other steps such as being trapped and transported, or separated from their herds. Therefore there is a much lower risk of additional welfare impacts than other methods. Conversely, the main concerns with capturing horses first are that the additional step of capturing them has additional negative welfare impacts. Confining a wild horse can cause anxiety, fear, and, depending on the length of time they are confined, can impact their behaviour, ability to eat and drink, and risk injuries. Separating horses from their band mates prior to shooting may reduce the anxiety associated with being present when their herd mates are being shot, however separation from their herd could cause more anxiety. All these factors need to be carefully considered and balanced, and the method with the least harms to animal welfare chosen – this may vary between populations – sometimes better overall welfare outcomes may be achieved with capturing horses first, and in other situations, better outcomes may be achieved with shooting prior to capture.

There are many other factors that can influence the animal welfare impact of any method (detailed below), and what is important is that a carefully planned protocol is used to ensure that welfare impacts are minimized and that actual animal welfare impacts are assessed during control actions. If animal welfare impacts are found to be unacceptable then the method can be improved or discontinued. To date, no such trials have been performed with careful assessment of animal welfare impacts of these different methods to truly determined which will have the best animal welfare outcomes in different situations. Therefore, it is important that this is done to identify the methods with the best animal welfare outcomes and to continuously improve the outcomes.

3. What other factors influence the welfare outcomes other than the method used?

All methods, lethal and non-lethal, can cause mild to severe animal welfare impacts, depending on how that method is carried out and the situation that it is used in. There are many other potential variables that influence animal welfare impacts including band sizes, approachability of the horses, visibility in the environment, habitat and the terrain. Further, for example with any method of shooting, the shooter's expertise, techniques, firearm type and ammunition characteristics also all play a vitally important role.

4. When lethal control methods are used, how can it be ensured that there are good animal welfare outcomes (i.e. minimal suffering)?

Firstly, very strict Standard Operating Procedures would be in place. This provides directions for the circumstances in which the procedure can be carried out, and exactly how it is carried out. For example, a shot wouldn't be taken unless it was certain that rapid death could be achieved, and after a shot, it is always confirmed that the animal has died before moving on. NPWS shooters are highly trained professionals who are always using state of the art equipment and following the latest research in terms of the best firearms and ammunition types to ensure the best welfare outcomes.

Further, additional recommendations can be applied such as only using the most highly experienced shooters, only shooting in open areas and on flat terrain, and only small groups of horses. Finally, auditing of all control methods should be performed by animal welfare veterinarians to ensure that the best animal welfare outcomes are being achieved.

When lethal control methods are performed within the park, one big advantage is that good monitoring of animal welfare outcomes is possible, and NPWS have direct ability to determine practices that will increase the likelihood of the best animal welfare outcomes (e.g. by choosing skilled shooters, appropriate equipment and shooting configurations, and training), in addition to altering practices dependent on animal welfare outcomes. This is not possible for methods that involve removing the horses from the park, where it is then usually not possible for animal welfare outcomes to be monitored (e.g. at abattoirs) and severe animal welfare outcomes may occur without any knowledge to be able to address this. Slaughter off park would not be recommended unless processes and animal welfare outcomes can be audited by an animal welfare veterinarian.

5. Wouldn't the terrain in KNP make it difficult to shoot horses accurately without capturing them first?

KNP has extremely variable terrain from large open grasslands, to alpine heathlands, and mountainous forested areas. Habitats would need to be very closely assessed, and shooting without prior capture would only ever be advised where there would be high visibility of the horses, low risk of injury and low risk of non-fatal shots. All shooting should only be for a defined proportion/number of horses in a particular population are to be shot (i.e. indiscriminate use would not be recommended). This method is most likely to be recommended in regions where other methods are either not feasible, or more likely to be associated with higher animal welfare impacts (e.g. inaccessible locations or large numbers of horses in one area). Again, animal welfare outcomes would always be monitored, and results used for recommendations to further improve animal welfare outcomes or discontinue the method if acceptable animal welfare outcomes could not be achieved.

6. What about reports from members of the public describing horse carcasses with multiple bullet holes following shooting from the ground or air; how could these horses have died without suffering?

Standard Operating Procedures usually require that animals are shot at least twice in the cranium or thorax – this is to increase the chances of a quick death and does not mean that the first shot wasn't accurate. A shot is not taken if the shooter is not certain that the first shot will cause unconsciousness. Given the high level of training and experience of the shooters employed, it is very unusual for death to not occur with the first shot. However, it is possible to cause an animal to become unconscious with a first shot, and not dead. Once an animal is unconscious they have no feelings; they can't feel pain or fear, and so there are no impacts to their welfare once they are unconscious. However, an animal can regain consciousness if they are not dead and this would have severe animal welfare impacts. Therefore to ensure that this does not happen, it is standard operating policy to take further shots after the first one to ensure that the animal can not regain consciousness. This means that horse carcasses may have multiple bullet holes even if they experienced instantaneous death from the first shot. This is particularly common if horses are shot from the air, as part of the standard procedures is to fly back and repeat shoot all animals as an extra measure to be absolutely certain there are no horses left alive with non-fatal wounding. All deaths are therefore very rapid.

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Appendix 8

The unforeseen animal welfare impacts associated with bushfires

Andrea Harvey, feline specialist, animal welfare veterinarian and wild horse researcher tells of her experiences when the Green Wattle Creek fire hit her and her partner, Richard Malik's home and animal sanctuaries at Wombeyan caves. How were all the animals kept safe? What unforeseen animal welfare impacts arose? And importantly, what can we do as a profession to try and prevent these unforeseen animal welfare impacts arising in future bushfire events?

Andrea Harvey is a UK trained feline Specialist who spent her early career at the world-renowned University of Bristol Feline Centre before moving to Australia in 2011. After developing additional interests in animal welfare, and particularly equine welfare, she undertook ANZCVS memberships in Animal Welfare, and embarked on a PhD studying the welfare and ecology of wild horses, which she is currently writing up. During her PhD Andrea has also been involved in a lot of pro-bono veterinary work for brumbies undergoing domestication and rehoming, in addition to establishing her own animal sanctuary where she strives to exemplify gold-standard welfare.

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