Bruce Highway Upgrade Cooroy to Curra Section C (Traveston to Woondum)

EPBC Act Koala Research Program

Non-invasive monitoring of fragmented and rehabilitated koala habitats using detection dogs: maximising koala conservation outcomes from mitigation strategies (e.g. offsets)

Final Report

Prepared for the Queensland Department of Transport and Main Roads

Disclaimer

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GLOSSARY OF TERMS AND ABBREVIATIONS

Term	Description
Akaike Information Criterion (AIK)	A mathematical method for evaluating how well a model fits the data it was generated from.
Allele	A variant of a gene. For example, an allele may code for hair colour, but different variants may result in red or brown hair.
Anthropogenic	Environmental change caused or influenced by people either directly or indirectly.
ArcGIS	A geographical information system software that allows handling and analysing geographic information by visualising geographic statistics through layer building maps (e.g. climate data or trade flows).
Barrier effect	Roads and infrastructure developments, for example, can create a barrier effect whereby the landscape becomes less or impermeable to wildlife movement.
Covariate	An independent variable that can influence the outcome of a given statistical trial, but which not of direct interest.
DES	Queensland Department of Environment and Science
Dispersal	The action of process of distribution or spreading things or people over a wise area.
DNA	Deoxyribonucleic acid, a molecule carrying genetic information.
Effective population size (Ne)	One of the most important parameters in population genetics and conservation biology. This is because potential genetic issues are only indirectly linked to the census size of a population, instead they are directly dependent on the genetically effective population size. Effective population size translates the census size of a real population into the size of an idealised population showing the same rate of loss of genetic diversity, inbreeding, or genetic drift as the population under study. In the most basic of terms, it represents the number of individuals contributing to the gene pool of the target

	population. Current recommendations for the genetic conservation of species in the wild ((Mace *et al* 2008; IUCN 2012; Frankham *et al* 2014a) are that in order to (1) avoid inbreeding depression, effective population size needs to be between 500 – 1000 (Hoban *et al* 2021).
Environmental Impact Assessment (EIA)	A process of evaluating the likely environmental impacts of a proposed project or development.
EPBC Act	Environment Protection and Biodiversity Conservation Act, 1999
Evolutionary potential	The ability of a population to evolve to cope with environmental changes. Often simplistically equated with genetic diversity (especially for quantitative characters such as fitness), but it is also influenced by effective population size.
Fst, Gst and Mutual Information	Indices that measure of population differentiation due to genetic structure.
Gene flow	Movement of alleles between populations via migrants or gametes. Gene flow maintains genetic diversity and promotes evolution by spreading new genes and combinations of genes throughout a species' range, however it may also constrain evolution by preventing adaptation to local conditions (and therefore, animal translocations need to be carefully thought out).
Generalised linear model (GLM)	Is a statistical framework for comparing how several explanatory variables may impact a continuous response variable.
Genetic diversity	The extent of genetic variation in a population (or species, or across a group of species), and can be measured as heterozygosity or allelic diversity, for example.
Genetic drift	Changes in the genetic composition of a population due to random sampling in finite populations. In the most basic terms, it is evolution due to random chance events.
Genetic erosion	Inbreeding depression and loss of genetic diversity in small populations.

GIS mapping	Geographic information system mapping.
Ground truthing	The process of gathering the proper objective data.
Habitat resilience	The capacity of a habitat to respond to a perturbation or disturbance by resisting damage and recovering quickly.
Impact zone	Area defined as the EPBC Project Area Boundary for the Section C Project.
Inbreeding	Occurs when individuals are more likely to mate with relatives than with randomly chosen individuals in the population. Inbreeding increases the probability that offspring are homozygous, which can lead to lower fitness, a phenomenon commonly referred to as inbreeding depression.
Inbreeding depression	Reduction in fitness due to inbreeding.
IUCN	The International Union for Conservation of Nature is the global authority on the status of the natural world and the measures needed to safeguard it.
Koala Rapid Assessment Method (KRAM)	A standardised protocol for the sampling of koala scats that does not rely on the selection of koala focal trees (Woosnam-Merchez *et al.* 2012).
Landscape Connectivity	Is the extent to which the landscape facilitates or impedes movement among habitat patches (here, specifically for koalas).
Legally securing	An area that has been dedicated or declared by regulation for the purposes of the EPBC Act.
LGA	Local Government Area
MBR	Morton Bay Rail Link Project
Natural processes	For the purpose of this research, this term is defined as processes unrelated to the linear transport infrastructure developed as part of the Moreton Bay Rail (MBR) Koala Management Program. This includes, but is not limited to, koala mortality by dog attack, disease, and age, for example.

NSW	New South Wales
Non-remnant	An ecological community that has been significantly disturbed.
Protected areas (PAs)	A clearly defined geographical space, recognised, dedicated and managed, through legal or other effective means, to achieve the long-term conservation of nature with associated ecosystem services and cultural values.
Recolonisation	The process or action of colonising something again.
Rehabilitate	The action of restoring something that has been damaged to its former condition.
Relatedness	In genetics, defines the degree of consanguinity (i.e. kinship) between individuals. For example, offspring typically receive half of their DNA from each parent and have therefore a coefficient of relatedness of 0.5 with them.
Remnant habitat	An ecological community containing native flora and fauna that has not been significantly disturbed.
Research Project	Research proposal submitted as part of the EPBC Act approval entitled 'Non-invasive monitoring of fragmented and rehabilitated koala habitats using detection dogs: maximising koala conservation outcomes from mitigation strategies (e.g. offsets)'.
SAT	Spot Assessment Technique
Scat	Koala droppings/faeces.
Section C Project	Bruce Highway Cooroy to Curra (Section C: Traveston to Woondum) Project
SLATS	State-wide Landcover and Trees Study
SNP	Single Nucleotide Polymorphism is the most common type of genetic variation. Each SNP represents a difference in a single DNA building block, called a nucleotide (there are four nucleotides: A, C, T and G).

Stratification	The arrangement or classification of something into different groups.
TMR	Queensland Department of Transport and Main Roads
USC	University of the Sunshine Coast

EXECUTIVE SUMMARY

The University of the Sunshine Coast (USC) was contracted by the Department of Transport and Main Roads (TMR) to undertake the Koala Research Project (the Research Project) for the Bruce Highway Cooroy to Curra (Section C: Traveston to Woondum) Project (Section C Project) in accordance with Condition 7 of the *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act) approval EPBC 2014/7394. The Research Project is entitled 'Non-invasive monitoring of fragmented and rehabilitated koala habitats using detection dogs: maximising koala conservation outcomes from mitigation strategies (e.g. offsets)'. The Aims of the Research Project are twofold:

> **Aim 1** - Measure the long-term effects of habitat fragmentation on koala health dynamics and how these may be mitigated by the introduction of fauna connectivity structures (bridges, underground passages etc).
>
> **Aim 2** – Improve offsets for koalas by assessing the value and ecological characteristics of non-remnant areas for koalas and whether these would benefit from protection.[1]

The Research Project commenced in February 2016 and was initially to be conducted using koala samples collected in the Section C Project area (pre and post disturbance), however, following the low presence of koalas observed during the initial surveys conducted prior to the Section C Project construction, locations within Moreton Bay, Fraser Coast, Sunshine Coast and Redland City local government areas (LGAs) were also included in the research to ensure the scientific robustness of analyses.

This report provides the findings of Aim 1 and Aim 2 in the form of six Research Papers (three for Aim 1 and three for Aim 2) and a broader analysis of the combined results. The links to each Research Paper is provided in Appendix 1.

In addition to the six Research Papers which directly address the Aims, a further six Research Papers were produced as a result of this Research Project (see section 3.2 Additional Outcomes). These Research Papers are largely methodological and describe the advances developed by USC required to answer the Aims of this Research Project. See Appendix 2 for the links to these additional published papers. Additional genetic analysis were also undertaken on scats collected in the Gympie region. Appendix 3 provides the results, which show fine-scale population genetic

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[1] Originally, Aim 2 set out "to measure the long-term recolonisation patterns of koalas into rehabilitated landscape (including offsets) to assess whether rehabilitated landscapes can support sustainable populations of koalas". It became evident that the data would not be available, and with the agreement of TMR, USC collected and used 'one time point' large scale landscape surveys of koala scat presence/absence in order to assess the relevance and ecological characteristics of non-remnant areas for koala offsets. Non-remnant sites were chosen as proxies for rehabilitated sites.

structuring among koalas in the Gympie region (see section 1.2.4). Results and analysis contained within yet-to-be published papers may be updated to reflect feedback received via the scientific peer-review process.

Aim 1

Under this Aim, research was undertaken to 1) help understand the impacts of fragmentation by linear transport infrastructure and, 2) identify measures which may assist in the management of koala populations impacted by habitat loss and fragmentation. This research is presented in the form of three papers (one published, two prepared) which are provided in Appendices 1, 2 and 3 as follows:

Research Paper 1: Schultz, A. J., Cristescu, R. H., Hanger, J., Loader, J., de Villiers, D., & Frère, C. H. (2020). Inbreeding and disease avoidance in a free-ranging koala population. *Molecular Ecology*, 29(13), 2416-2430.

This research investigated whether koalas in a free-ranging population can avoid mating with close relatives or with diseased individuals infected with Chlamydia (*C. pecorum*). It drew on data from the TMR Moreton Bay Rail Project (MBR) Koala Management Programme in Southeast Queensland, in which 452 free-ranging koalas were monitored between 2013 and 2017. The data included long-term VHF tracking data, veterinary examination data, and parentage assignment analyses from a wild peri-urban population to test whether free-ranging female koalas avoid mating with (a) more closely related males; and (b) males infected with *C. pecorum.*

Key Finding/s:
This study found that female koalas did not exhibit any active mechanisms to avoid mating with close relatives (i.e. inbreeding) or to avoid breeding with koalas infected with Chlamydia. Moreover, this study identified that only 30 percent of males contribute to the next generation.

These findings highlight the underlying long-term risk of inbreeding for fragmented and isolated koala populations where dispersal is limited. They also highlight the importance of appropriate mitigation strategies to the planning of linear infrastructure. Mitigation strategies should aim to limit the risks of inbreeding depression and disease transmission to reduce the risks of local extinction to impacted koala populations over time. This study also highlights the importance of understanding and monitoring the genetic and disease status of impacted koala populations in the development of infrastructure projects.

Research Paper 2: Frère, C.H., O'Reilly, G.D., Strickland, K., Schultz, A., Hohwieler, K., Hanger, J., de Villers, D., Cristescu, R., Powell, D. and Sherwin, W.

(2023). Evaluating the genetic consequences of population subdivision as it unfolds and how to best mitigate them: A rare story about koalas. *Molecular Ecology*, 00, 1-12.

This research investigated the effect of habitat fragmentation caused by the construction of linear infrastructure on the genetic diversity of an impacted koala population and identified the amount of dispersal across infrastructure required to mitigate the observed loss of genetic diversity. It used data from the TMR MBR Koala Management Programme in Southeast Queensland.

Key Finding/s:
This research found that, as a result of the construction of the rail line project, the koala populations were fragmented and experienced an immediate loss of genetic diversity. Further, models revealed a predicted long-term increased rate of genetic diversity loss over time as a consequence of genetic drift. Last, and using forward migration simulations, it identified that at least eight koalas would need to disperse from each side of the subdivision (southwest and the northwest rail line) per generation to maintain genetic connectivity close to zero but that 16 koalas would ensure that both genetic connectivity and diversity remained unchanged. These results have important consequences for the genetic management.

Research Paper 3: **Hohwieler, K., de Villers, D., Cristescu, R. and Frère, C.H. (2022). Genetic erosion detected in a specialist mammal living in a fast-developing environment. *Conservation Science and Practice*, 2(7), e12738.**

This research investigated the cumulative impact of anthropogenic pressures on the genetic integrity of koalas over a 12-year period. This study examined if signals of rapid genetic erosion could be detected within as few as two koala generations in an urban koala population which experienced increased anthropogenic pressures during this time due to urban expension. This study used data collected in the Redland City Council region (mainland) in Southeast Queensland. The population was sampled at two time points 2006 - 2007 and 2018.

Key Finding/s:
This study demonstrated that an increase in traffic, human population growth, and landscape fragmentation through land clearing have occurred in parallel with the rapid genetic erosion of koala populations in as few as two generations. Together, the results show how the cumulative impact of human induced landscape coinsided with the rapid loss of genetic diversity over a short period of time. Results indicate an increased risk of inbreeding depression, but an unlikely ability to retain evolutionary potential and as such decrease the koala's ability to adapt to future environmental disturbances (Frankham *et al*, 2017; Franklin, 1980).

Recommendations arising from Aim 1

The body of research presented in Aim 1 highlights the critical need to understand and monitor the genetic and disease status of impacted koala populations to mitigate the consequences of linear infrastructure projects on koalas. This will provide evidence regarding the level of vulnerability of the impacted population (e.g. how at risk is the population to inbreeding depression, disease prevalence and/or in the longer-term, genetic erosion), which can then inform the design of mitigation measures to be implemented during and after construction. Furthermore, these studies indicate that mitigation measures should adequately address the cumulative impact of human induced landscape change on this species and not just the impact of an individual project.

To assist in the management of the risks posed to koala populations by future linear infrastructure projects, it is recommended that the following staged approach be adopted with the aim of avoiding and minimising any impact to koala populations or habitat:

- **Stage 1 Population assessment:** (Options Analysis and Business Case Phases) - Determine if koalas are present within the study area by undertaking a desk-top and presence/ absence field assessment.
- **Stage 2 Vulnerability assessment:** (Business Case Phase) – If koalas are present undertake a vulnerability assessment of the population (to determine population size, sex make-up, genetic diversity and inbreeding risk, presence of disease, and microbiome health).
- **Stage 3 Targeted surveys:** (Business Case Phase) - Complete a targeted survey of the koala population to obtain information on the fine scale movement of the impacted koala population.
- **Stage 4 Identify avoidance and mitigation measures:** (Preliminary and Detailed Design Phase) - Design management and mitigation measures (e.g. fauna crossings) that are specific and relevant to the population and which minimise or mitigate habitat fragmentation and support landscape connectivity.
- **Stage 5 Monitor during construction:** (Construction Phase) – If koalas are present in the impact zone - monitor koalas during construction.
- **Stage 6 Post construction koala management:** (Post Construction Phase) – post construction monitoring of koalas to monitor the genetic health of the impacted population (monitoring of underpasses, predictive modelling and/or genetic rescue could be considered).

USC

The recommended measures will show the immediate (e.g. how many individuals will be impacted (including by sex), how diseased is it, how inbred is it, how connected it is to other populations, how much habitat it is going to lose) and long-term (e.g. genetic erosion) vulnerability of the population.

Aim 2

Under this Aim, research was conducted to assess the value and ecological characteristics of non-remnant areas for koalas and whether these would benefit from protection, with the aim of improving koala conservation. This research is presented in the form of three papers (one published (Research Paper 4), two in final preparation) which are provided in Appendices 4, 5 and 6 as follows:

Research Paper 4: Cristescu R H, Scales K L, Schultz AJ, Miller RL, Schoeman DS, Dique D & Frère CH (2019) Environmental impact assessments can misrepresent species distributions: a case study of koalas in Queensland, Australia. *Animal Conservation*. 22 (4), 314-323. https://doi.org/10.1111/acv.12455

This research aimed to test the assumption that environmental impact assessment (EIA) guidelines are successful in determining koala occurrence, assessing koala presence/absence across areas in Noosa, Gympie and Fraser Coast earmarked for three future linear infrastructure developments. This resulted in a total of 400 scat surveys conducted. A generalised linear model (GLM) was used to estimate whether habitat quality and survey design (as currently defined by governmental survey guidelines) explained a significant proportion of observed koala presence.

Key Finding/s:
Research Paper 4 demonstrates that current recommendations for EIA recommend focusing survey efforts on what is perceived as known or high-quality koala habitat may misrepresent the true occurrence of koalas. The results of this research show that across both individual development projects and at a greater landscape scale, koalas were equally as likely to be present in what is perceived as low-quality koala habitat (including non-remnant habitat) to that perceived as high-quality koala habitat.

Research Paper 5: Gardiner R, Terraube, J, Frère, CH and Cristescu, R (2021) Roads and water availability influence the occurrence of koalas (*Phascolarctos cinereus)* in secondary habitat: a multiscale approach. *Biodiversity and Conservation,* 32, 163–180 (2023).

This research investigated whether non-remnant habitat could be used as an effective tool to protect koalas. This study utilised scat detection dogs to assess koala presence/absence across seven local government areas (LGAs) in Southeast Queensland between 2015 and 2020 (Sunshine Coast, Noosa, Gympie, Toowoomba, Moreton Bay, Redlands City Council and Fraser Coast). In total, 2230 scat surveys were conducted with 959 surveys used for analysis. Koala presence was detected in 575 surveys, with 384 surveys being negative for koala occurrence.

Key Finding/s:
This study found that koala occurrence was always lower in non-remnant than in remnant forests, however the use of non-remnant habitat increased with distance from roads and with increased water availability.

Research Paper 6: Terraube, J, Gardiner, R, Hohwieler K, Frère CH. and Cristescu R. (2023). Forest protection has a minor effect on koala occurrence in Eastern Australia. *Biodiversity and Conservation,* 292, 4 (2023).

This paper investigated whether protected areas were benefiting koalas. This study utilised scat detection dogs to assess koala presence/absence across seven LGAs in Southeast Queensland between 2015 and 2021 (Sunshine Coast, Noosa shire, Gympie, Toowoomba, Moreton Bay, Redlands City Council and Fraser Coast). In total 2230 scat surveys were conducted with 1463 surveys used for analysis. Koala presence was detected in 861 surveys with 602 surveys being negative for koala occurrence.

A total of eight different models were designed, including two estimates of percentage of protected forests, i) for all protected area categories (IUCN categories I-VI) and ii) only for strictly protected areas (IUCN categories I-IV), for each buffer size (1km and 3km), with and without the additional 'habitat quality' covariates.

Key Finding/s:
This study found that protected habitats had a minor positive effect on koala occurrence highlighting the importance of strategically selecting offsets that are connected to protected habitat.

Recommendations arising from Aim 2

The body of research presented in Aim 2 highlights the need to:

- use field verification over presumed or mapped high quality koala habitat to determine koala occurrence for environmental impact assessments (Proposed survey design Figure 2 of Aim 2 recommendations).
- improve management of data availability relating to offsets and rehabilitation (e.g. location and number of land parcels, size, age and type of rehabilitation so that the presence or absence of koalas across these landscape units can be assessed more easily and effectively).
- confirm koala use of offsets via scat surveys to ensure direct benefits to koalas.

- consider non-remnant areas as important areas for offsets to support koala conservation.
- ensure legal protection of future offsets.

Analysis of results and conclusions from Aims 1 and 2

This Research Project has demonstrated that koalas occupying disconnected islands of vegetation, where dispersal capabilities are reduced, are more likely to be at significant risk of inbreeding depression, genetic erosion and disease prevalence. This can be mitigated in two ways. First, fauna underpasses will be critical, but their locations must be strategically placed to correspond with data on koala crossings of the proposed infrastructure prior to construction so as to increase their likely use and thus support sufficient dispersal to maintain genetic connectivity and minimise loss of genetic diversity. Second, offsets should be selected to restore landscape connectivity between isolated koala populations to minimise genetic erosion over time. This is because retaining and restoring genetic connectivity, through habitat corridors, can allow exchange of genetic variants between populations and slow down the loss of genetic diversity.

This research shows that this can be achieved through the selection of both remnant and non-remnant habitat (Aim 2, Research Papers 4 and 5). Aim 2 Research Paper 5, for instance, shows that 40 - 60 percent of non-remnant sites surveyed (had koala scats present, demonstrating that non-remnant areas should not be discounted in the selection of potential offset parcels (as long as efforts are invested to assure habitat quality over the long term). This is especially true if these sites have confirmed koala presence (e.g. scats), provide koala habitat connectivity in the landscape, and meet the criteria outlined in Research Paper 5.

Restoring landscape connectivity through offsets will be best achieved through a coordinated approach between all levels of government (Commonwealth, state and local). This is because offset locations should be prioritised in relation to their relationship to the greater landscape (e.g. whether they abut, or provide new landscape connectivity between, existing offsets or other protected areas e.g. state or national parks). Such a coordinated approach by all governments will help mitigate the cumulative impact of anthropogenic development on the species.

Finally, whether sites with remnant or non-remnant vegetation are identified as potential offset sites, koala use of these sites should be confirmed through ecological surveys rather than relying on vegetation quality and/or type as an indicator of koala occurrence as demonstrated in Aim 2, Research Paper 4. For non-remnant habitat offsets (see ecological characteristics which increase probability of koala occurrence in Aim 2 Recommendation), efforts should be placed on improving the quality of the habitat for koalas by, for instance, planting koala food trees. Either way, once offsets are secured, these should be legally secured.

In this regard key questions are outlined below (in order of priority) for the selection of offset parcels for koalas:

1) Does the land parcel provide connectivity?
2) Are any of the adjoining land parcels protected?
3) Does the offset land parcel(s) contain remnant or non-remnant vegetation?
4) Does the adjoining land parcel(s) contain remnant or non-remnant vegetation?
5) Is the offset land parcel 18kms or more from a major road?
6) Is the offset land parcel near water?

Together, these questions should be used as guiding criteria for the consideration of any future offsets.

1 INTRODUCTION

1.1 Background

Koala numbers are declining across much of the remaining free-living populations in Queensland and are now listed as vulnerable under the EPBC Act. It is well known that koalas struggle alongside urban expansion and factors such as habitat destruction, habitat fragmentation, Chlamydial disease and additional anthropogenic mediated threats (vehicle collision and canine attacks) are causing major population decline (Preece 2007; Cristescu *et al* 2011). Adding to this, climate change is predicted to force a contraction of the koalas' natural range toward cooler coastal areas where anthropogenic landscape change from urban development is predicted to increase most significantly (DESA 2012). Minimising the impact of this anthropogenic development on koalas will therefore require an understanding of how to best to mitigate and offset such anthropogenic pressures.

Whilst anthropogenic development is inevitable to accommodate the current and projected human population growth, the use of offsets provides a mechanism to protect species impacted by clearing of habitat. Offset impacts are a hotly debated biodiversity conservation topic, with suggestions that they can produce perverse incentives and exacerbate biodiversity decline (Walker *et al* 2009; Gordon *et al* 2015). Conversely, others see offsets as a holistic approach incorporating loss and gain of habitat in an effort to provide the best conservation outcome (Madsen 2011). Notwithstanding this debate, it is agreed that if offsets are to be undertaken, their design is critical to achieving the most efficient conservation outcomes for the impacted species (Quétier & Lavorel 2011). To best support the future of koala conservation, it is therefore critical that the selection of offsets is continuously optimised to ensure direct benefits to koalas. This, however, may become increasingly difficult as areas identified as suitable under the Queensland Department of Environment and Science (DES) guidelines (https://environment.des.qld.gov.au/__data/assets/pdf_file/0016/102850/koala-conservation-offsets.pdf) will become increasingly threatened by deforestation and land clearing due to projected increased anthropogenic demands (Ward *et al* 2019).

Furthermore, there is still much to understand about how the fragmentation of koala habitat by anthropogenic development, such as linear transport infrastructure, impacts the fine-scale population dynamics of koalas (e.g. genetics, disease and health). The International Union for Conservation of Nature (IUCN) recognizes the need to conserve genetic diversity as one of three global conservation priorities (McNeely *et al* 1990). This is because populations that can retain high levels of genetic diversity have increased potential for adaptation to changes in habitat, climate change or pathogens

(Reed & Frankham 2003; Frankham 2005). While councils and government departments are trying their best to mitigate their impact on koala populations, there is little understanding of the impact that developments have on the health dynamics (i.e. genetics and disease) of the immediate and surrounding koala population and if the implementation of mitigation strategies, such as corridors, are effective to address this impact. Increasing understanding how fragmentation, connectivity and offsets impact fine scale koala population dynamics over time will help ensure the most effective strategies to deliver an ecologically defensible mechanism to balance conservation and development (Gardner *et al* 2013).

In this context, the University of the Sunshine Coast (USC) was contracted by the Department of Transport and Main Roads (TMR) to undertake the Koala Research Project (the Research Project) for the Bruce Highway Cooroy to Curra (Section C: Traveston to Woondum) Project (Section C Project) in accordance with Condition 7 of the *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act) approval EPBC 2014/7394. The Research Project is entitled 'Non-invasive monitoring of fragmented and rehabilitated koala habitats using detection dogs: maximising koala conservation outcomes from mitigation strategies (e.g. offsets)'. The Aims of the Research Project were twofold:

> **Aim 1** - Measure the long-term effects of habitat fragmentation on koala health dynamics and how these may be mitigated by the introduction of fauna connectivity structures (bridges, underground passages etc).
>
> **Aim 2** – Improve offsets for koalas by assessing the value and ecological characteristics of non-remnant areas for koalas and whether these would benefit from protection.[2]

The Research Project commenced in February 2016 and was initially to be conducted using koala samples collected in the Section C Project area (pre and post disturbance) (Figure 1). This area was defined as the 'impact zone'. Within the Section C Project area, surveys were also conducted within 200m, 2km and 10 km buffer zones (refer Figure 1). Field surveys were undertaken using fully trained koala detection dogs. TMR provided funding for the training and maintenance of two koala detection dogs as part of the offset proposal for the Section C Project. Following the low presence of koalas observed during the initial surveys conducted prior to the Section C Project construction, locations within Moreton Bay, Fraser Coast, Sunshine Coast and Redland City local government areas (LGAs) were also included in the research to ensure the

[2] Originally, Aim 2 set out "to measure the long-term recolonisation patterns of koalas into rehabilitated landscape (including offsets) to assess whether rehabilitated landscapes can support sustainable populations of koalas". It became evident that the data would not be available, and with the agreement of TMR, USC collected and used 'one time point' large scale landscape surveys of koala scat presence/absence in order to assess the relevance and ecological characteristics of non-remnant areas for koala offsets. Non-remnant sites were chosen as proxies for rehabilitated sites.

scientific robustness of analyses. In addition, data collected also aimed to increase knowledge about koalas in the Gympie region (see Appendix 13).

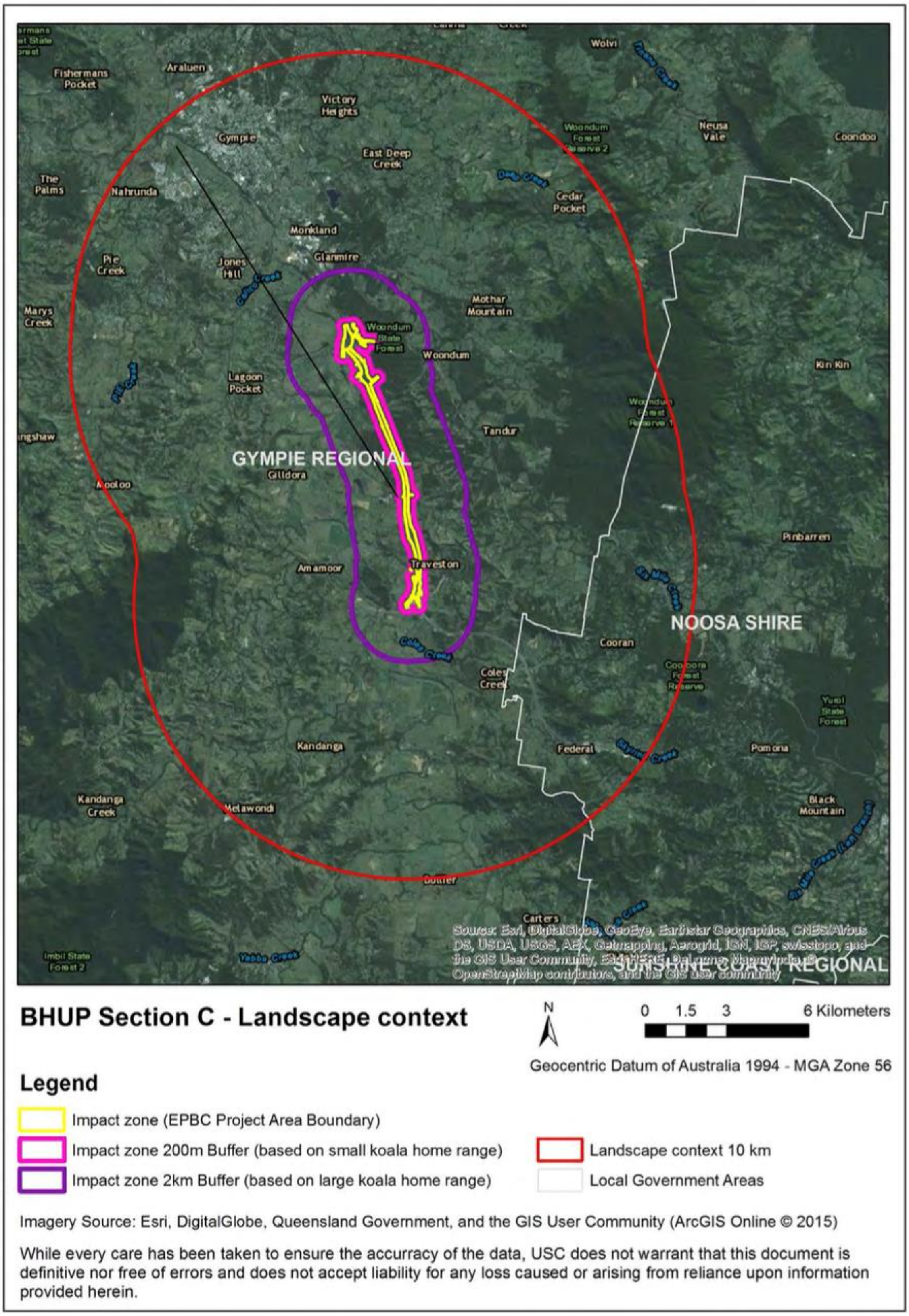

Figure 1. Map of the Impact Zone and Associated Survey Areas

In the preparation of the Research Proposal, the following were identified:

1. Data on the species was limited to a small number of records on relevant database searches, anecdotal records from local residents and field investigation results. The presence of koala and population dynamics within the Section C Project area and immediate surrounds were relatively unknown.
2. Direct sightings of koalas were not reported in any of the surveys undertaken for the Section C Project.
3. There was limited data available to inform the selection of suitable offset sites for the protection and rehabilitation of koala populations.
4. There was limited published information available to identify if koala populations re-colonised fragmented habitat.
5. There was limited published information about the immediate and longer-term genetic consequences of habitat fragmentation by linear infrastructure development on koalas.

This report provides the findings of Aim 1 and Aim 2 in the form of six Research Papers (three for Aim 1 and three for Aim 2) and a broader analysis of the combined results. These Research Papers are provided in Appendices 1 to 6 and are listed below:

Under Aim 1, research was undertaken to 1) help understand the impacts of fragmentation by linear transport infrastructure and, 2) identify measures which may assist in the management of koala populations impacted by habitat loss and fragmentation. This research is presented in the form of three papers (one published, two prepared) which are provided in Appendices 1, 2 and 3 as follows:

1. Schultz, A. J., Cristescu, R. H., Hanger, J., Loader, J., de Villiers, D., & Frère, C. H. (2020). Inbreeding and disease avoidance in a free-ranging koala population. *Molecular Ecology*, 29(13), 2416-2430. https://doi.org/10.1111/mec.15488
2. Frère, C.H., O'Reilly, G.D., Strickland, K., Schultz, A., Hohwieler, K., Hanger, J., de Villers, D., Cristescu, R., Powell, D. and Sherwin, W. (2023). Evaluating the genetic consequences of population subdivision as it unfolds and how to best mitigate them: A rare story about koalas. *Molecular Ecology*, 00, 1-12.
3. Hohwieler, K., de Villers, D., Cristescu, R. and Frère, C.H. (2021). Genetic erosion detected in a specialist mammal living in a fast- developing environment. *Conservation Science and Practice*, 2(7), e12738.

Under Aim 2, research was conducted to assess the value and ecological characteristics of non-remnant areas for koalas and whether these would benefit from protection, with the aim of improving koala conservation. This research is presented in the form of three papers (one published (Research Paper 4), two in final preparation) which are provided in Appendices 4, 5 and 6 as follows:

4. Cristescu R H, Scales K L, Schultz AJ, Miller RL, Schoeman DS, Dique D & Frère CH (2019) The impact of misrepresentation of species distributions in Environmental Impact Assessments: A case study of koalas in Queensland, Australia. *Animal Conservation*. 22 (4), 314-323. https://doi.org/10.1111/acv.12455
5. Gardiner R, Terraube, J, Frère, CH and Cristescu, R (2021) Roads and water availability influence the occurrence of koalas (*Phascolarctos cinereus)* in secondary habitat: a multiscale approach. *Biodiversity and Conservation,* 32, 163–180 (2023).
6. Terraube, J, Gardiner, R, Hohwieler K, Frère CH. and Cristescu R. (2023). Forest protection has a minor effect on koala occurrence in Eastern Australia. *Biodiversity and Conservation,* 292, 4 (2023).

Results and analysis contained within yet to be published papers may be updated to reflect feedback received via the scientific peer-review process.

1.2 Additional Outcomes

In addition to the six Research Papers which directly address the Aims, a further six Research Papers were produced as a result of this Research Project.

These Research Papers are largely methodological and describe the advances developed by USC required to answer the Aims of this Research Project and links to them can be found in Appendix 2.

1.2.1 Advancement and testing of genetic tools for koala conservation

In order to address the Aims set out in this Research Project, significant methodological advances in genetic tools were developed by USC. These are described in six additional peer-reviewed Research Papers which can be accessed from the links in Appendix 2:

1. Schultz AJ, Cristescu RH, Littleford-Colquhoun BL, Jaccoud D, Frère CH. (2018) Fresh is best: Accurate SNP genotyping from koala scats. *Ecology and Evolution*. 8: 3139– 3151.

This Research Paper describes the development of new genetic molecular markers for improved measures of genetic diversity in koalas.

2. Cristescu RH, Miller R, Schultz AJ, Hulse L, Jaccoud D, Johnston S, Hanger J, Booth R, Frère CH (2019). Developing non-invasive methodologies to assess koala

population health through detecting Chlamydia from scats. *Molecular Ecology Resources*.

This Research Paper describes methods for the improvement of the detection of Chlamydia from non-invasive koala scat samples.

3. Schultz AJ, Strickland K, Cristescu RH, Hanger J, De Villiers D, Frère CH. (2022) Testing the effectiveness of genetic monitoring using genetic non-invasive sampling. *Ecology and Evolution.* 12: e8459.

This Research Paper describes testing the robustness of genetic monitoring using koala scats.

4. Cristescu, R. H., Strickland, K., Schultz, A. J., Kruuk, L. E. B., de Villiers, D., & Frère, C. H. (2022). Susceptibility to a sexually transmitted disease in a wild koala population shows heritable genetic variance but no inbreeding depression. *Molecular Ecology*, 31, 5455– 5467.

This Research Paper shows that susceptibility of Chlamydial disease in koalas is partly genetically heritable.

1.2.2 Use of detection dogs for koala conservation

A further two additional peer-reviewed Research Papers were generated under this Research Project to disseminate research and information on the usefulness of detection dogs for koala conservation. This research is described in the following and can be accessed from the links in Appendix 2:

5. Cristescu RH, Miller RL, Frère CH. Sniffing out solutions to enhance conservation: How detection dogs can maximise research and management outcomes, through the example of koalas (2020) *Australian Zoologist.* 40 (3): 416–432.

This Research Paper details the utility of trained detection dogs for koala conservation.

6. Cristescu CH, Scales K, Schultz AJ, Miller RL, Schoeman DS, Dique D, Frère CH. Robust science underpinning legislation can create better outcomes for threatened species impacted by infrastructure project through the example of koalas (2019) *Animal Conservation.* 22: 328-330.

This Research Paper discusses ways of improving the scientific rigour of environmental assessments for koalas.

1.2.3 Training of Detection Dogs

To address both Aims 1 and 2 of the Research Project, three koala scat detection dogs were trained, tested and then deployed for data collection. Two dogs (Baxter and Billie-

Jean) were funded by TMR as part of the Research Project and the third dog (Charlie) provided in-kind by USC. The detection dog Baxter located koala scats regardless of age, Billie-Jean located fresh koala scats only (necessary for genetic analyses), and Charlie identified Chlamydia in koala scats. Together Baxter, Billie-Jean and Charlie have conducted a total of 2242 surveys both on this Research Project and others, as summarised in Table 1. Each of these three dogs plays a particular role in fieldwork and therefore not all will produce similar outputs. For example, Charlie the Chlamydia detection dog, was only deployed in a few circumstances, but still made important contributions to research outcomes. Billie-Jean is still being deployed, whilst Charlie and Baxter have since been retired.

Table 1. Survey effort by USC trained detection dogs. These include surveys conducted within and external to the Research Project and the data contribution to published research.

Dog	Detection Type	Number of Surveys	Research contribution
Baxter	All koala scats	1480	Cristescu RH, Miller RL, Frère CH. (2020) Sniffing out solutions to enhance conservation: How detection dogs can maximise research and management outcomes, through the example of koalas. *Australian Zoologist.* Cristescu, RH, Schultz AJ, Schoeman D, Scales K, Frère, CH. (2019) Environmental impact assessments can misrepresent species distributions: a case study of koalas in Queensland, Australia. *Animal Conservation*.
Billie-Jean	Fresh koala scats (for genetics)	735	Cristescu, RH, Miller RL, Frère, CH. (2020) Sniffing out solutions to enhance conservation: How detection dogs can maximise research and management outcomes, through the example of koalas. *Australian Zoologist.* Cristescu, RH, Schultz AJ, Schoeman D, Scales K, Frère, CH. (2018) The impact of misrepresentation of species distributions in Environmental Impact Assessments: A case study of koalas in Queensland, Australia. *Animal Conservation*.
Charlie	Identification of Chlamydia in koala scats	27	Cristescu, RH, Miller R, Schultz AJ, Hulse L, Jaccoud D, Johnston S, Hanger J, Booth R, Frère CH (2019). Developing non-invasive methodologies to assess koala population health through detecting *Chlamydia* from scats. *Molecular Ecology Resources.* Cristescu, RH, Miller RL, Frère, CH. (2020) Sniffing out solutions to enhance conservation: How detection dogs can maximise research and management outcomes, through the example of koalas. *Australian Zoologist*

1.2.4 Genetic analysis

Additional genetic analysis were also undertaken on scats collected in the Gympie region. Appendix 3 provides the results, which show fine-scale population genetic structuring among koalas in the Gympie region. Koalas around the Kybong/Traveston area showed some level of genetic differentiation from koalas in Goomboorian and to the far west and south. There is, however, evidence of genetic flow across these regions. However, both set of analyses indicated that koalas within the impacted zone (2 Km buffer, see Figure 1) showed some extent of genetic differentiation from the rest of the koalas in the Gympie Region, suggesting that these may be more vulnerable to long-term genetic erosion if connectivity between both sides of the highway is not adequately maintained. For now, our genetic analyses showed that koalas both sides of the highway within the impacted zone still form one connected gene pool.

2 AIM 1

To address Aim 1 of this Research Project an investigation into the long-term effects of habitat fragmentation on koala health and how these may be mitigated by the introduction of fauna connectivity structures was conducted. For the purposes of Aim 1, koala health was measured through genetic diversity and/or the presence/absence of Chlamydial disease.

2.1 Background

It is known that habitat loss, degradation and fragmentation are challenges to wildlife worldwide and that it results in small, isolated populations. Such widespread fragmentation patterns negatively affect migration and gene flow, which can result in the decrease of genetic diversity through increased inbreeding, genetic drift and decrease in effective population size (N_e) (Frankham *et al* 2017).

Any decrease in genetic diversity can impact the viability of a population in two primary ways. First, it can impact the fitness of individuals (survival and reproduction) within a population, as a result of inbreeding (mating between close relatives (Frankham *et al* 2017). Second, loss of genetic diversity can impact a population's ability to respond to disturbances and environmental changes (e.g. infectious diseases; (Frankham *et al* 2017)). In the case of koalas, any additional negative impact on the viability of their populations across Southeast Queensland is of concern, given it is known that many are experiencing sharp declines (approximately 50-80 percent in recent decades (McAlpine *et al* 2015) due to threats such as habitat loss, disease, dog attacks and vehicle collisions (Rhodes *et al* 2011). Nationally, koalas have been identified as 'vulnerable' under the EPBC Act. For koalas, the added threats associated with linear transport infrastructure should then be mitigated with this existing vulnerability in mind.

2.2 Surveys and data collection

For the purpose of genetic sampling, USC conducted a total of 608 detection dog surveys from the start of the Research Project which identified scats that were fresh enough for downstream DNA extractions. However, it is important to note that not all koala DNA extracted from scats resulted in accurate genotypes for downstream genetic analyses due to the degraded nature of scat DNA.

Initial survey design focused on the Section C Project Area impact zone so as to monitor genetic and health changes before, during and after construction for the impacted koala population. Detection dog surveys undertaken during this Research Project yielded too few fresh scats pre/during/post construction to undertake any robust downstream genetic analyses, so to counter this limitation:

1) Surveys undertaken in Fraser Coast and wider Gympie area were used to increase knowledge about koalas in the Wide Bay region. Genetic analyses of koala scats in the Gympie Region can be found in Appendix 13, a deliverable sought by the Gympie Koala Action Group.
2) Blood/tissue samples for koalas that were monitored by Endeavour Veterinary Ecology during the Moreton Bay Rail (MBR) Project. This dataset was extensive enough to robustly answer questions under Aim 1.
3) Surveys were also included from the Redland City Council area. These were undertaken by a PhD student funded under Section C and in partnership with Redland City Council. This location, instead of Gympie, was selected because of the availability of a historical koala DNA dataset dating from 2006. This offered the opportunity to compare levels of genetic diversity across a 12-year period and in doing so identifying the cumulative effects of anthropogenic developments on the genetic diversity of koalas.

A summary of scats collected during this research is presented in Table 2.

Table 2. Number of samples collected during this Research Project for genetic analyses in Aim 1

Location (Local Government Area (LGA))	**Sample type**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**	**Total no. samples**
Fraser Coast	Scat	16	41	22	-	-	-	-	79
Gympie	Scat	-	55	166	42	7	41	-	322
Section C Impact zone	Scat	-	-	-	-	-	-	-	*50*
Redland City	Scat	-	-	-	561	137	64	181	555
Moreton Bay	Blood/ Tissue	-	-	-	-	-	-	-	452

2.3 Research Paper 1

Schultz AJ, Cristescu RH, Hanger J, Loader J, de Villiers D, & Frère CH. (2020) Inbreeding and disease avoidance in a free-ranging koala population. *Molecular Ecology*, 29(13), 2416 – 2430.

2.3.1 Background

Inbreeding can influence population viability negatively (Armbruster & Reed 2005; Harrisson *et al* 2019) as it can reduce an individual's reproductive success, reproductive ability, and survival (Frankham *et al* 2017). This is because as individuals breed with close relatives, it allows harmful dormant recessive alleles present in the population to become expressed, often resulting in the rise of detrimental genetic conditions, such as, sickle cell anaemia and cystic fibrosis in humans (Antonarakis 2019). When inbreeding causes fitness consequences, such as the two human examples given prior, it is known as inbreeding depression (Charlesworth & Charlesworth 1987). As such, the ability of species to avoid breeding with close relatives will be key to their persistence in anthropogenically-altered landscapes (e.g. fragmented landscapes), where close relatives may not be able to disperse freely.

Inbreeding avoidance mechanisms can vary between passive and active avoidance (Pusey & Wolf 1996). Passive inbreeding avoidance typically involves juvenile sex-biased dispersal from the natal home range. For example, juvenile male American black bears (*Ursus americanus*) disperse far enough from their natal home ranges that most females close enough to be potential mates are unrelated to them (Costello *et al* 2008). Active inbreeding avoidance behaviour relies on a combination of active mate choice and kin recognition, for example, cooperatively breeding southern pied babblers (*Turdoides bicolor*) will only inherit a dominant position in their natal group if an unrelated breeding partner is available (Nelson-Flower *et al* 2012). African elephants' active avoidance of inbreeding is thought to rely on kin recognition, with males avoiding sexual behaviour leading to reproduction with both maternal and paternal kin (members of natal family) (Archie *et al* 2007).

Although studies have shown that koalas do exhibit male-biased juvenile dispersal (Dique *et al* 2004), movement barriers created by habitat fragmentation will reduce the viability of dispersal as an inbreeding avoidance tactic. There is evidence of some female mate choice in koalas, based on male bellows (a cue to male body size) (Charlton *et al* 2013), although given the coercive nature of koala mating (Martin & Handasyde 1999) little is known about whether females are able to actively avoid mating with close relatives. This research addresses this gap and helps us to understand whether koalas in small, isolated populations with limited dispersal are at a greater risk of inbreeding and consequently vulnerable to local extinction.

Infectious diseases pose further complications for small, isolated populations (Cunningham *et al* 2017). Some species are known to actively avoid diseased conspecifics, such as the spiny lobster (*Panulirus argus*) which avoids sharing shelters with diseased conspecific (Behringer & Butler 2010), while others cannot (e.g. Olive baboon, *Papio anubis* (Paciência *et al* 2019)).

For koalas, increased risk of Chlamydial disease transmission is an important consideration for the long-term conservation of the species. Chlamydial disease caused by *C. pecorum* is an obligate intracellular parasitic bacterium that is sexually transmitted, although mother-offspring transmission may occur (see Nyari *et al* 2017).

Chlamydial disease is an increasing cause of koala mortality (Polkinghorne *et al* 2013) and is present across the koala's geographic range (Kollipara *et al*., 2012). Chlamydial disease in koalas can manifest as cystitis (inflammation of the bladder), keratoconjunctivitis which may lead to blindness, and reproductive cysts which are associated with infertility in females (Polkinghorne *et al*, 2013). This suggests that female koalas may suffer greater fitness losses from *C. pecorum* infections, although some research links Chlamydial disease to infertility in males (Deif 2011). Further, subdivided populations with limited dispersal may experience an increased risk of disease transmission. As impacted populations are forced into smaller patches of habitat, individuals may become clumped across the landscape (McAlpine *et al* 2017). This means that encounters with diseased individuals may increase (e.g. multiple males mating with an infected female) facilitating rapid disease transmission through the impacted population. Whilst some theories have suggested that habitat fragmentation may be beneficial by isolating sick animals from healthy populations, empirical published evidence has consistently shown the contrary (White *et al* 2018; Gao *et al* 2019).

Avoiding infection with *C. pecorum* is comparable to actively avoiding inbreeding with relatives, in that it requires a koala to identify an undesirable mate (i.e. closely-related or diseased) and then act to avoid mating with that individual. Prior to this research, it was unknown whether koalas are able to actively identify and avoid mating with either closely related or Chlamydial-diseased conspecifics. Furthermore, if koalas are unable to behaviourally avoid inbreeding or sexually transmitted disease transmission, this may impact the viability of populations into the future and require widespread health and genetic management of at-risk populations. This was investigated as part of this research.

2.3.2 Methodology

Research Paper 1 drew on data from the MBR Koala Management Programme in Southeast Queensland, in which 452 free-ranging koalas were monitored between 2013 and 2017. The data included long-term VHF tracking data, veterinary examination data, and parentage assignment analyses from a wild peri-urban population to test whether free-ranging female koalas avoid mating with (a) more closely related males; and (b) males infected with *C. pecorum*. The methods outlined below were used.

2.3.2.1 Genetic Sampling and analysis

Assigned mothers and fathers of koalas in the population were identified using parentage assignment using single nucleotide polymorphism (SNP) genotyping data from blood or tissue samples, collected during routine veterinary examinations. SNP genotyping was used to calculate pairwise relatedness, but also heterozygosity as a measure of internal relatedness.

2.3.2.2 Koala tracking points and spatial estimates

Home range estimates for males and females were calculated using VHF tracking data to assess which candidate's father were in geographic proximity to each koala mother, and thus available as potential mates. For both male and female koalas, VHF tracking data in this study was limited to before habitat clearing for the infrastructure project began (data collected between March 2013 and March 2014 inclusive), in order to avoid including koala movement driven by active removal of habitat or short-distance relocations from dangerous areas.

2.3.2.3 Disease presence

As part of regular veterinary examinations of koalas monitored during the monitoring period, koalas were tested for *C. pecorum* infection. To assess whether female koalas were avoiding mating with infected candidate fathers, a male was classified as Chlamydia-positive if they tested positive for *C. pecorum* (by any testing protocol) at any time during the 12-month conception range of the offspring for which they were a candidate father.

2.3.2.4 Statistical analysis

A total of 59 mother-father-offspring trios and candidate fathers (potential mates available to the mother) were used to determine if females exhibited active avoidance for genetically related males. Further, a total of 29 mother-father-offspring trios and associated candidate fathers which had *C. pecorum* testing data and weight data were used to determine if females exhibited active avoidance for uninfected males.

The model was designed to test whether the relative relatedness, relative weight, or relative heterozygosity of candidate males available to each female influenced siring success, or whether absolute disease presence influenced siring success, thus informing whether inbreeding or disease avoidance occurred in our study population.

2.3.3 Key Findings

Research Paper 1 (Appendix 1) identified that female koalas did not avoid mating with closely related individuals. For example, it found that the higher number of close male relatives found within a female's home range, the more likely she is to mate with them (e.g. mating with parents, offspring, half-siblings and cousins). Moreover, this study identified that only 30 percent of males contribute to the next generation. In species already under pressure from multiple anthropogenic impacts, such as the koala, the lack of apparent mechanisms to avoid inbreeding is concerning.

This has direct planning implications for linear transport infrastructure given its impact on the environment often results in the creation of smaller and disconnected habitat patches. For koalas that inhabit such fragmented landscapes, this often results in the formation of smaller isolated populations with limited dispersal abilities where the risk of breeding with close relatives and/or diseased individuals increases. Without the ability to avoid mating with related individuals, these impacted populations will experience greater risk of inbreeding depression, which is known to negatively impact survival, reproduction, and disease resistance (Frankham *et al* 2017)). This is then further compounded by a greater risk of disease transmission, and thus population level disease prevalence - a known driver of population decline in koalas (Beyer *et al* 2018; Taylor-Brown *et al* 2019). Together, the risk of inbreeding depression coupled with higher disease prevalence will increase the risk of local extinction for koala populations over time.

2.3.4 Conclusion

This study highlights the importance of understanding and monitoring the genetic and disease status of impacted koala populations to mitigate the consequences of linear infrastructure on their viability into the future.

2.4 Research Paper 2

Frère, C.H., O'Reilly, G.D., Strickland, K., Schultz, A., Hohwieler, K., Hanger, J., de Villers, D., Cristescu, R., Powell, D. and Sherwin, W. (2023). Evaluating the genetic consequences of population subdivision as it unfolds and how to best mitigate them: A rare story about koalas. *Molecular Ecology*, 00, 1-12.

2.4.1 Background

The implementation of mitigation measures to alleviate the barrier effect produced by linear transport infrastructure on local fauna is not new (Glista *et al* 2009). There are now many types of purpose-built fauna crossing structures used across the world (i.e. underpasses and overpasses) with one common goal, facilitating animal movement across linear transport infrastructure in the hope they will improve/restore habitat connectivity/permeability across road and rail networks.

While research has shown that animals can and do use these purpose-built fauna crossing structures (Lesbarreres & Fahrig 2012), it is also now known that their use is heavily influenced by their design (Cain *et al* 2003; Clevenger & Waltho 2005), their placement (Rodriguez *et al* 1996; Clevenger & Waltho 2000) and the biology of the target species (Dexter *et al* 2016). An understanding of their effectiveness in preventing the longer-term impacts of linear transport infrastructure on habitat connectivity via gene flow, however, remains poorly understood (Glista *et al* 2009; Lesbarreres & Fahrig 2012; Sawaya *et al* 2014; Soanes *et al* 2018). While animals may use these purpose-built fauna crossing structures, it is yet to be understood as to whether these crossing events occur at a frequency that is high enough to maintain/restore genetic connectivity. This was investigated as part of this research through the example of the koala. This research addressed three key questions: (1) what are the immediate and (2) predicted long-term genetic consequences of linear infrastructure on an impacted koala population and (3) using simulations, what extent of koala movement between habitat fragments post-linear infrastructure construction is required to maintain genetic connectivity.

2.4.2 Methodology

This study used data from the MBR Koala Management Programme in Southeast Queensland, in which 452 free-ranging koalas were monitored between 2013 and 2017. TMR installed fencing alongside the rail infrastructure project and strategic underpasses were built at different locations throughout the rail line (Hanger *et al* 2017). Because all koalas were VHF and/or GPS tracked during all phases of construction (pre, during and post), it afforded a detailed knowledge of how and why the impacted koala population's size varied throughout the rail line construction phases: (1) all deaths and births were recorded and causes of death identified (Hanger *et al* 2017; Beyer *et al* 2018), (2) which koalas were translocated as a result of the rail line infrastructure project, (3) their locations pre and post-construction (whether koalas occupied habitat on one side or the other of the rail line), and (4) rail crossing events by koalas after establishment of the rail barrier using both dedicated fauna and hydrology culverts (Dexter *et al* 2017).

2.4.2.1 Genotyping and final genetic dataset

A total of 367 individual koalas were genotyped for 3655 SNP (see Appendix 1, Research Paper 2 for details).

These koalas were organised into three genetic datasets:

- Dataset 1 (n = 270) contained all successfully genotyped koalas monitored alongside the rail line footprint during the 4 years.
- Dataset 2 (n = 114) contained all successfully genotyped koalas from Dataset 1 minus those that died of 'natural' processes.
- Dataset 3 contains all successfully genotyped koalas from Dataset 2 minus those that were translocated as a result of the rail line infrastructure project.

Dataset 3 was further subdivided into northwest (n = 27) and southwest (n = 75) of the rail line.

By organising genetic data into these three subsets, it was then possible to document and distinguish between the genetic consequences of:

(1) genetic loss caused by 'natural' processes (i.e. death by predation, trauma, disease and unknown – death not directly caused by the immediate infrastructure project) [Dataset 1 vs Dataset 2]; and

(2) disturbance caused by the linear transport infrastructure project (i.e. population size reduction by translocation and population subdivision) [Dataset 2 vs Dataset 3].

2.4.2.2 Genetic analyses

This study estimated the immediate genetic consequences of linear transport infrastructure by measuring differences in genetic diversity using multiple indices (see Appendix 1, Research Paper 2), effective population size (i.e. the number of individuals contributing to the gene pool) and genetic differentiation between the three datasets (see Appendix 1, , Research Paper 2).

This research modelled the predicted loss of genetic diversity over time for the two post-construction fragmented koala populations (northwest, southwest) to estimate what the longer-term genetic consequences of linear transport infrastructure could be if genetic connectivity was not restored across the rail line.

This study used forward time simulations to estimate the extent of dispersal post-construction required to maintain genetic connectivity between the northwest and southwest fragmented koala populations (see Appendix 1, Research Paper 2).

2.4.3 Key Findings

The results of this study revealed several insights about the genetic consequences of habitat fragmentation by linear infrastructure.

First, this study shows how population decline caused by the linear transport infrastructure project resulted in an immediate decrease in genetic diversity and effective population size (see Dataset 2 vs 3 in Table 1, Appendix 1, Research Paper 2).

Second, models predicted that the post-construction koala populations may, over time, experience an increased rate of genetic diversity loss if genetic connectivity is not maintained between the northwest and southwest rail line koala populations (Figure 2).

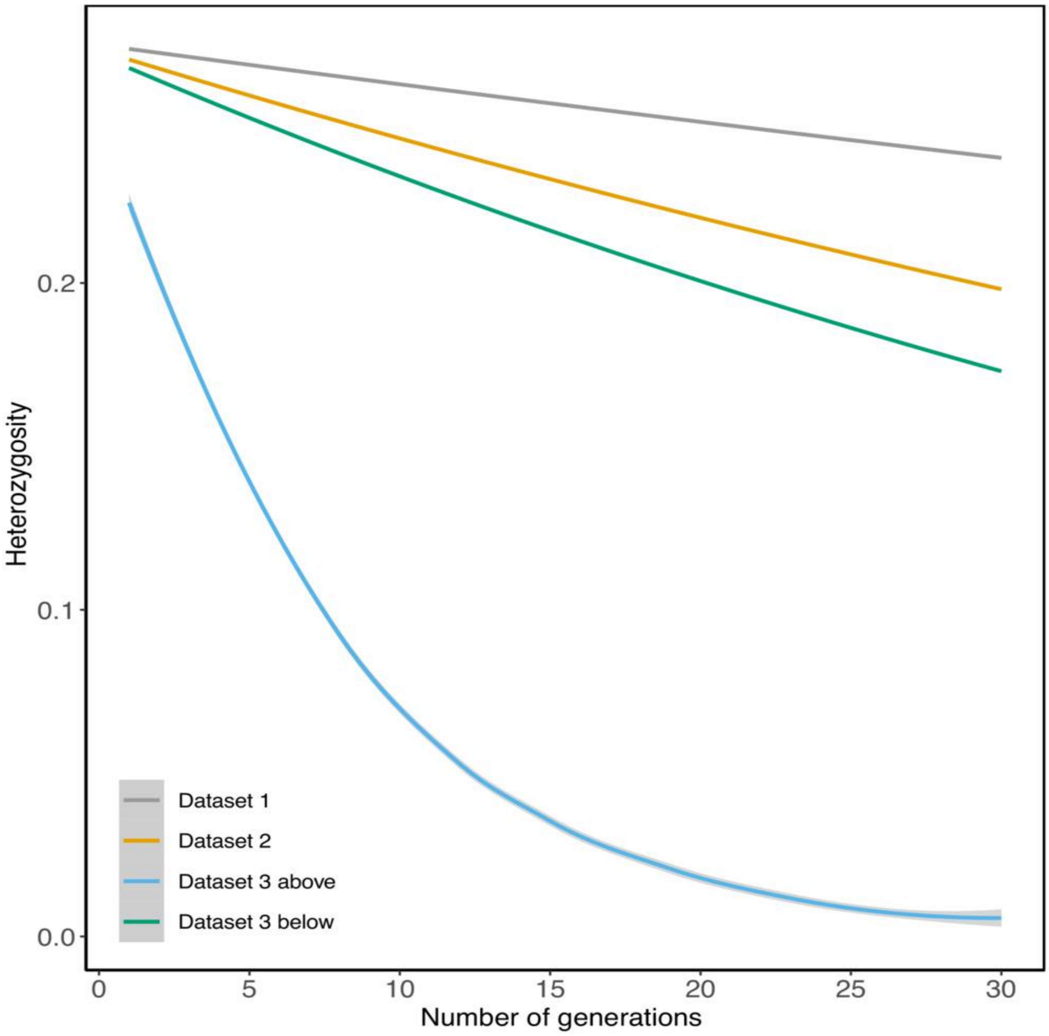

Figure 1. Predicted genetic erosion for each genetic dataset. *Heterozygosity is used as a measure of genetic diversity to demonstrate the predicted genetic erosion. Dataset 1 (pre) represented in blue is the predicted genetic erosion patterns for the resident koala population without any natural or linear transport infrastructure impacts. Dataset 2 (post) represented in green is the predicted genetic erosion patterns for the resident koala population impacted by 'natural' mortality events. Dataset 3 (southwest) represented in orange is the patterns of genetic erosion for the resident koala impacted by the linear transport infrastructure located southwest of the rail line. Dataset 3 (northwest) represented in grey is the patterns of genetic erosion for the resident koala impacted by the linear transport infrastructure located northwest of the rail line.*

Last, and using forward migration simulations (Figure 2 & 3), this study estimated that a minimum of eight koalas would need to disperse from each side of the subdivision per generation to maintain genetic connectivity close to zero but that 16 koalas would ensure that both genetic connectivity and diversity remained unchanged. Eight dispersing koalas would, however, still result in an average loss in genetic diversity (HE) of more than 15% (above) and 8% (below) when compared to the genetic diversity (HE) prior to the population subdivision (Figure 3). Sixteen dispersing koalas would require 59.26% and 21.33% of koalas dispersing from the above and below population respectively.

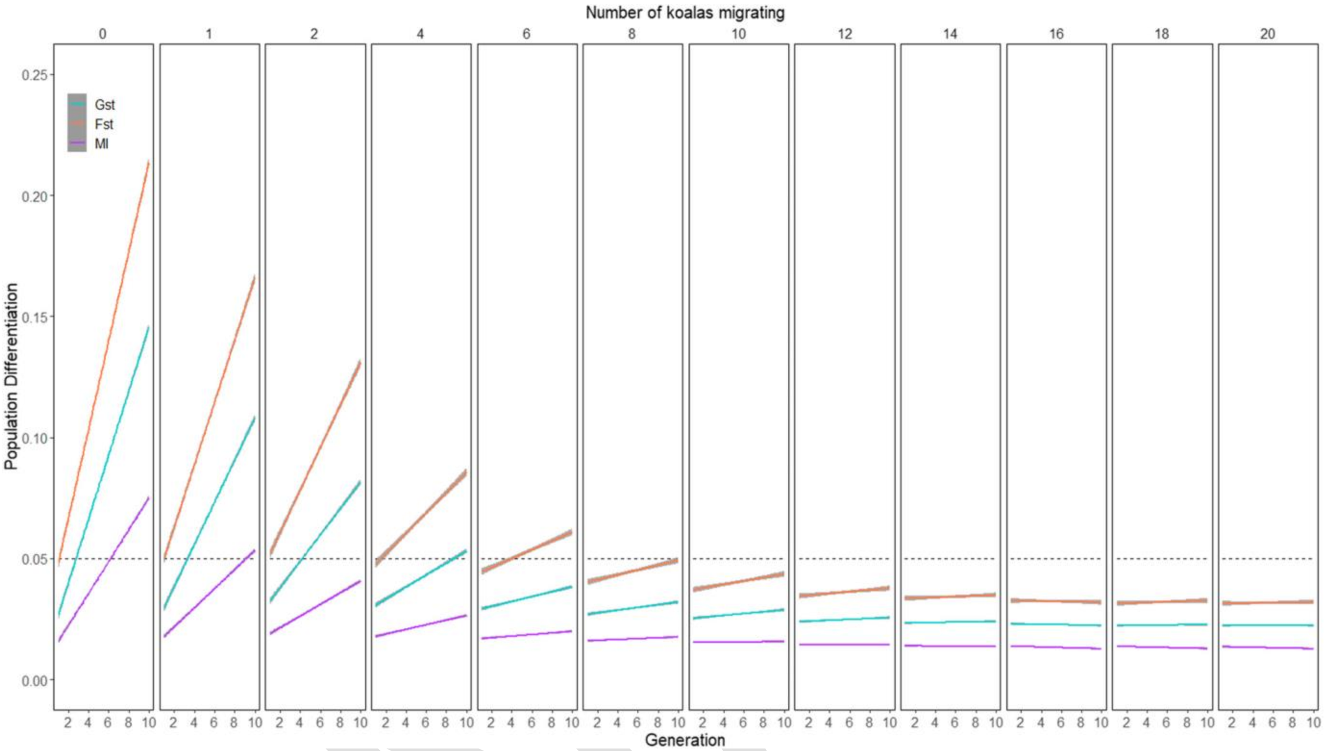

Figure 2. Predicted effect of varying dispersal rate on the genetic connectivity of the fragmented koala populations located on either side of the newly built rail line. *Genetic connectivity was measured using Fst, Gst and Mutual Information (MI).*

Together, this study demonstrates how existing threatening processes (e.g. predation and disease) and the impact of the rail line construction (e.g. translocation and population subdivision) resulted in two subdivided koala populations with severely reduced census population sizes, decreased genetic diversity and effective population size. However, intensive koala monitoring and threat management prior to and during construction (Beyer *et al* 2018), increased rail line permeability demonstrated by a higher koala use of culverts toward the end of the construction (Dexter *et al.* 2017) and the likely gene flow from adjacent koala populations may help mitigate these potential genetic impacts in the long-term. Further research would be needed to ascertain whether this is the case. Nevertheless, research has shown that genetic erosion, combined with small population size, are significant contributors to population extinction risk (Templeton *et al* 2001). As such this study has important conservation implications for mitigation on future linear infrastructure development given that mitigations to minimise these costs rarely integrate its long-term genetic impact.

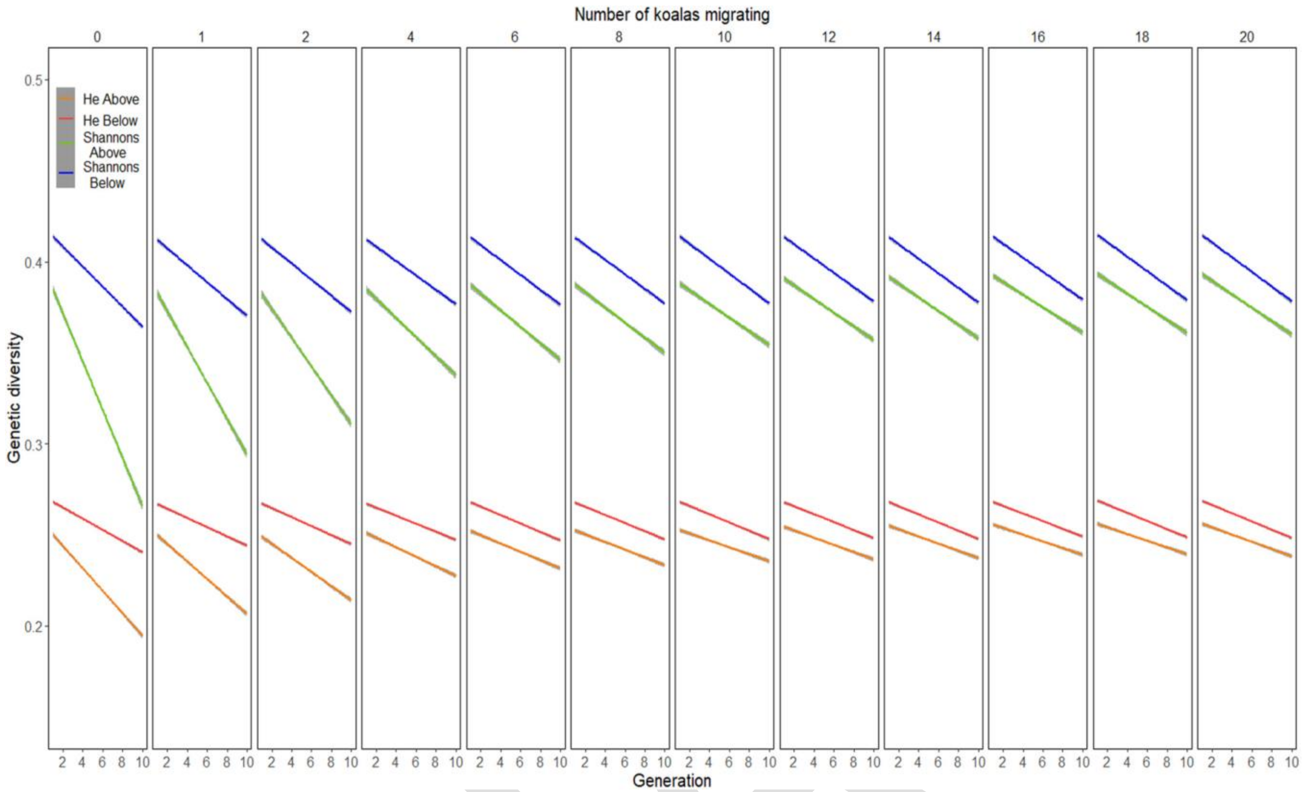

Figure 3. Forward dispersal simulations showing the effect of the number of dispersing koalas on the extent of genetic diversity from one to 10 generations after the subdivision for the koala populations located on either side of the linear transport infrastructure. Genetic diversity was measured using expected heterozygosity (H_E) and the Shannon's information ($\ln(^1D)$). The forward simulations imported the genetic data from the two post-construction data sets (Dataset 3_{above} = 27 koalas and Dataset 3_{below} = 75 koalas) as a starting point. The starting point of these genetic diversity measures at generation 0 were H_E_above = 0.267, H_E_below = 0.270, $\ln(^1D$_above) = 0.435 L $\ln(^1D$ _below) = 0.440.

2.4.4 Conclusion

To conclude, this research study provides important empirical evidence for the immediate and long-term genetic consequences of habitat fragmentation by linear transport infrastructure modelled on the absence of suitable mitigation. This research shows the importance of understanding existing threatening processes and the relative vulnerability of the impacted species prior to construction given that these will influence (1) the extent of the impact of the linear infrastructure construction and importantly (2) how these could be mitigated. The results of this research, for instance, show that while laudable, the attempts to preserve connectivity would need to result in 16 koalas (59.26% and 21.33% of koalas dispersing from the above and below population respectively) to disperse per generation to avoid long-term genetic drift.

2.5 Research Paper 3

Hohwieler, K., de Villers, D., Cristescu, R. and Frère, C.H. (2022). Genetic erosion detected in a specialist mammal living in a fast-developing environment. *Conservation Science and Practice*, 2(7), e12738.

2.5.1 Background

Linear infrastructure fragments landscapes hindering the ability of animals to disperse safely (Ascensão *et al* 2019). Research Papers 1 and 2 have shown that the formation of small, isolated populations of koalas can result in an increased risk of inbreeding and an immediate loss of genetic diversity. Genetic erosion models from Research Paper 2 further predict a rapid decline in genetic diversity caused by genetic drift, whereby genetic variants will change in freqeuncey based on random chance events (Appendix 1, Research Paper 3). Despite this, little is known about the longer-term consequences of increased urbanisation on the genetics of koala populations.

To date, the impact of linear transport infrastructure on species is assessed on a case-by-case basis and managed accordingly (Hawke 2010; Tulloch *et al* 2016). However, degradation of a landscape and its consequent impact on species occurs over time through the cumulative impact of multiple anthropogenic demands such as increased number of roads, traffic, and habitat loss (Dales 2011). As such, treating each construction project as a separate entity is hindering our ability to protect vulnerable koala populations.

This study (Appendix 1, Research Paper 3) examined if signals of rapid genetic erosion could be detected within as few as two koala generations in an urban koala population which experienced increased anthropogenic pressures during this time due to urban expension. Together, the results show how the cumulative impact of human induced landscape change can drive the rapid loss of genetic diversity over a short period of time.

2.5.2 Methodology

This study used data collected in the Redland City Council region (mainland) in Southeast Queensland. The population was sampled at two time points. From 2006-2007, 261 koala ear biopsies were collected from sick and injured koalas at two major wildlife hospitals in the area. Twelve years later, as part of the Redland City Council koala population assessment, 345 koala scats were sampled across the council area using koala scat detection dogs. Both koala tissue and scats from the two time points were genotyped using SNPs.

2.5.3 Genetic analysis

SNP genotyping was used to assess the population for signals of genetic erosion using the following measures:

- **Genetic diversity loss**

This was measured by comparing population level genetic diversity in 2006 to 2018.

- **Population structure and subdivision**

This was investigated by measuring the amount of population differentiation between 2006 and 2018. Further, while investigating more cryptic population genetic patterns, it was found a potential barrier to gene flow imposed by State Route 21. Therefore, it was investigated if this pattern had strengthened over time.

- **Effective population size**

This was estimated for both 2006 and 2018. Effective population size estimates can provide an indication of a genetic bottleneck, whereby a population has experienced a dramatic loss in the number of individuals at some point in time (Potvin *et al* 2017).

- **Inbreeding risk**

Current inbreeding risk was assessed by investigating population level inbreeding values for 2006 and 2018 and by looking at the distribution of related individuals across the landscape in both 2006 and 2018 datasets.

2.5.4 Environmental variables

To understand potential observed genetic trends in context, changes in physical environment were investigated. This included:

- **Woody extent**

LandSAT imagery was used to calculate the total area of forest (minimum 20 percent canopy cover for a minimum area of 0.2 hectares), sparse woody (canopy cover of 5-19 percent) and non-woody land cover (0-4 percent canopy cover) for 2006 and 2018. Further, the amount of vegetation cleared between these two time points was calculated using State-wide Landcover and Trees Study (SLATS).

- **Human population growth**

Estimated human population size in Redland City Council was derived from the Australian Bureau of Statistics.

- **Traffic**

The average amount of vehicle traffic was estimated for 2006 and 2018, which was used to calculate the change in traffic between the two time points.

- **Vehicle collision trends**

To understand trends in mortality that may be associated with an increase in road infrastructure or density koala-vehicle collision data from 1997 to 2019 was obtained through the Open Data Portal of the Queensland Government.

2.5.5 Key findings

Here, Research Paper 3 provides evidence of rapid genetic erosion in an urban population of koalas. Within only two generations, the population experienced a dramatic loss of genetic diversity, increased levels of inbreeding and genetic sub-structuring, higher average relatedness, and a three-quarter reduction in effective population size (Table 1). Together, these signals of rapid genetic erosion present an alarming trend, indicating not only an increased risk of inbreeding depression, but an unlikely ability to retain evolutionary potential and as such decrease the koala's ability to adapt to future environmental disturbances (Franklin 1980; Frankham *et al* 2017).

Table 1. Summary of genetic trends observed between 2006 and 2018 in Redland City koala population.

Measurement	2006	2018	Pattern observed
Genetic diversity (measured as observed heterozygosity)	0.356	0.276	22.5 % decrease
Inbreeding risk (measured as internal relatedness (Höglund, 2009))	-0.071	0.076	207.04 % increase
Average relatedness across landscape	0.007	0.124	1671.43 % increase
Effective population size (Ne) using the LD method	372.42	117.1	68.55 % decrease

Further, it was found that this population of koalas experienced an increase in anthropogenic threats alongside strong evidence of a sharp decline in population size between 2006 and 2018. For instance, 2145 hectares of vegetated land have been lost or degraded. Simultaneously, the human population increased by 19.9 percent from 2006 to 2018. That is, an increase of 26065 inhabitants. This increase in population was also reflected in a 16.6 percent increase in vehicle traffic between the two time points. These patterns suggest that the rapid human induced landscape changes may be inherently linked to the dramatic population decline and rapid genetic erosion observed in the local koala population.

Across the 12-year period investigated, and despite an increase in traffic, a steady decline in the incidences of koala-vehicle collisions was observed, with fewer collisions each year. While this result could have multiple possible interpretations, for example, improved mitigation measures (Rytwinski *et al* 2016) or reduced movement (Tucker *et* *al* 2018; Ascensão *et al* 2019), the most likely explanation is a decrease in koala population size. This is because it has frequently been shown that roadkill data can be accurate in inferring population trends (Baker *et al* 2004) and it is known that koalas have decreased in population size by 51 percent within only three years in the northern part of its range (Dique *et al* 2004; Lee *et al* 2010).

By linking vehicle mortality trends, anthropogenic landscape change and genetic trends, this research highlights that continued anthropogenic landscape development (e.g. over 12 years) can result in a significant cumulative impact on the affected koala population as a whole. This was demonstrated in the rapid nature of genetic erosion and extensive landscape change over this time. Hence, without considering the contribution that an individual development project may have to a broad scale pattern of landscape change (i.e. a cumulative impact), mitigation strategies may not provide acceptable outcomes for koalas post construction.

2.5.6 Conclusion

The results of this study showed how the cumulative effect of human landscape changes (e.g. urbanisation, roads and traffic) have coincided with the rapid genetic erosion (in just two generations; see Table 1) for koalas. In the context of linear transport infrastructure development project this study underlines the importance of developing mitigation strategies which incorporate both historical and predicted future human induced landscape change (i.e. not considering the consequence of many projects taking place over time and space) as it is the cumulative impact of these which pose greatest threat to the viability of koala populations into the future.

2.6 Recommendations arising from Aim 1

The body of research presented in Aim 1 highlights the critical need to understand and monitor the genetic and disease status of impacted koala populations to mitigate the consequences of linear infrastructure projects on koalas. Furthermore, it was shown how mitigation measures should adequately address cumulative impact of human induced landscape change on species. To assist in the management of the risks posed to koala populations by future linear infrastructure projects, it is recommended the following staged approach be adopted with the aim of avoiding any impact to koala populations or habitat:

- **Stage 1 Population assessment:**

  (Options Analysis and Business Case Phases) - Determine if koalas are present within the study area.

- **Stage 2 Vulnerability assessment:**

(Business Case Phase) – If koalas are present undertake a vulnerability assessment of the population.

- **Stage 3 Targeted surveys:**

  (Business Case Phase) - Complete a targeted survey of the koala population to inform strategic mitigation measures.

- **Stage 4 Identify avoidance and mitigation measures:**

  (Preliminary and Detailed Design Phase) - Design avoidance, management and mitigation measures that are specific and relevant to the population. Design any offsets that may be required, taking consideration of offset design measures described in the results of the Aim 2 research.

- **Stage 5 Monitor during construction:**

  (Construction Phase) – If koalas are present in the impact zone - monitor koalas during construction.

- **Stage 6 Post-construction koala management:**

  (Post Construction Phase) – post construction monitoring of koalas to mitigate the long-term impacts of development.

Each of these stages have been discussed in more detail below.

2.6.1 Stage 1: Population Assessment

Based on the research presented herein, it is recommend that TMR determine the presence or absence of koalas within and adjacent to the study area and, if present, undertake an assessment of the impacted population. This should include the following:

2.6.1.1 Desk-top assessment

This can be undertaken using publicly available databases to determine the characteristics of the study and buffer areas (approximately 1km radius as an example). The assessment should consider the following:

- Habitat type and presence of mapped koala habitat, remnant and non-remnant vegetation
- Presence of wildlife corridors or ecological linkages to the broader landscape
- Location of any protected areas
- Previous records of koalas.

2.6.1.2 Field verification

Field verification of the mapped characteristics of the study and buffer areas identified in the desk-top assessment should be undertaken by a suitably qualified ecologist. Preferably, this should be done through multiple surveys at a minimum once during the breeding season and once during non-breeding season. Potential survey methods include:

2.6.1.3 Scat surveys (e.g. human field surveys, koala scat detection dogs using the casual survey method).

The casual survey method is an efficient way to survey a larger area. In casual surveys the dog is not constrained by the handler to check 30 trees (i.e. the Spot Assessment Technique (SAT) method) and can freely follow its nose. The detection dog is motivated by a tennis ball and given the command to search. If a koala scat is found, the location, number of scats found, their age and whether they come from one or different individual koalas (when possible, to establish from sizes) are recorded. The search resumes until the entire survey area is searched. Fresh scats are collected opportunistically during this survey.

2.6.1.4 Thermal imaging surveys using drones.

The use of thermal imagery and drones is an efficient way to locate koalas over large areas.

2.6.1.5 Determine if koalas are present within a potential impact zone:

Based on the results of the desk-top and field assessments determine the presence/absence of koalas within the study and buffer areas. This data can be used to inform the design of the corridor alignment and forms the basis of the ongoing koala assessment.

2.6.2 Stage 2: Vulnerability assessment

If koalas are present witihn the study and buffer areas a vulnerability assessment of the impacted population should be undertaken to determine appropriate mitigation strategies. It should comprise the following:

2.6.2.1 Landscape connectivity assessment through GIS analysis and field verifications and collection of scats for genetic analyses.

Undertake GIS landscape analysis to map vegetation corridors across the landscape. Use field verifications to identify whether these corridors are used by koalas. During these field verifications koala scats should be collected to assess the extent of genetic connectivity across the landscape. This will assist in determining whether an impacted koala population is already geographically isolated and/or at risk of becoming isolated because of the infrastructure development. If so, this would increase the risk of inbreeding depression, genetic erosion and disease prevalence over time and increase the risk of local extinction.

2.6.2.2 Analysis of scats to determine the vulnerability of the impacted population

Undertake systematic surveys of the impacted population to collect fresh koala scats. From these, DNA is extracted from fresh scats collected during the field surveys (casual and systematic) and analysed for the following:

Analysis 1 (baseline)

- number of koalas
- number of males/females
- genetic diversity and inbreeding risk (e.g. whether the population comprises close relatives).

Analysis 2

- Chlamydia and retro-virus (presence/absence and genotyping).

Analysis 3

- health assessment through gut microbiome analyses.

Analysis 4

- landscape genetic connectivity – the results of the genetic analysis can be used to understand the extent of gene flow between the impacted koala population and the greater landscape. This will inform the risk of genetic erosion post-construction.

Depending on the scope of work and available time and budget, ‘Analysis 1 (baseline)’ or more could be selected. These four analyses, however, are strongly recommended as they will inform the management of the impacted koala population during and after construction.

2.6.3 Stage 3: Targeted Surveys

If avoidance of koala habitat cannot be achieved and fauna connectivity structures are required, targeted surveys are to be undertaken to inform the design of these measures. The assessments should comprise targeted fine scale systematic surveys along the alignment to identify areas of high koala movement or presence. There are two methods that can be used for these surveys:

- non-invasive, fine scale koala scat surveys (e.g. human surveys or koala scat dogs) within the proposed alignment and a 100 - 500 meters buffer zone.
- catching and fitting koalas with GPS and VHF tracking collars within the potential impact zone to provide fine scale movement of the existing koalas and where they may be moving through habitat or impacted zones.

Data collected can be used to:

- inform the location, number, and type of fauna connectivity structures.
- inform planning for the location of interchanges (which significantly limit the movement of koala populations) for which current mitigation measures are limited.
- allow for management of individual koalas as they move through the landscape during clearing and construction (intensive collaring and catching only, not scat surveys).

2.6.4 Stage 4: Identify avoidance and mitigation measures

Data collected in Stages 1 and 2 will allow the development of avoidance and mitigation strategies during and post-construction to minimise impact to koalas. These could include:

- informing the design of the location of the road/rail alignment to avoid or minimise any habitat fragmentation and impact on the population.
- maintaining connectivity using evidence-based placement of koala crossings.
- inform the selection of offsets to restore landscape connectivity with the broader landscape/koala population.
- the incorporation of disease management programs in the stages of design and planning through veterinary examination and/or the roll-out of vaccination programs.

2.6.5 Stage 5: Monitor during construction

Koalas should be monitored throughout the construction phase, using a combination of techniques currently available including GPS and VHF tracking, thermal drone surveys and fauna spotter catchers.

2.6.6 Stage 6: Post construction koala management

Follow-up the impacted population

A key consideration of linear transport development and its impact on koalas is the genetic health and connectivity of the impacted population. This includes ensuring that after the construction phase the impacted population has adequate genetic connectivity, levels of genetic diversity and low risk of inbreeding. This can include:

- Using predictive modelling (as in Research Paper 2) to determine the necessary dispersal to maintain connectivity.
- Monitoring the use of underpasses to ensure sufficient dispersal to maintain genetic connectivity and mitigate usage by feral animals, which may threaten koala persistence (e.g. dogs).
- Long-term genetic monitoring to ensure genetic erosion can be aptly identified and mitigation measures, such as genetic rescue can be applied early to maximise their effectiveness. The temporal design of long-term monitoring should be based on the vulnerability assessment and use of underpasses post construction. This should include forward dispersal simulations, predictive genetic erosion models, effective population size (<100 is cause for great concern (Hoban *et al* 2021) and use of underpasses (Aim 1, Research Paper 2):

High risk populations would be defined by low effective population size Ne < 100, rapid predicted loss of genetic diversity (Frankham *et al* 2014a; Hoban *et al* 2021) (< 6% over 2-5 generations; this is based on the average of genetic diversity loss over the last century of 91 (Leigh *et al* 2019) and insufficient rate of dispersal across underpasses post-construction. Genetic monitoring should be undertaken every 2 years for the life of the offset.

Medium risk populations would be defined by low-moderate effective population size 100 < Ne < 500, rapid predicted loss of genetic diversity (Frankham *et al* 2014a; Hoban *et al* 2021) (> 6% in 2-5 generations) and sufficient rate of dispersal across underpasses post-construction. Genetic monitoring should be undertaken every 4 years for the life of the offset.

Low risk populations would be defined by effective population size Ne > 1000, slow predicted loss of genetic diversity (< 6% over 20-30 generations) (Frankham *et al* 2014b). No need for genetic monitoring.

In cases where loss of genetic diversity cannot be stopped, the translocation of individuals into the population or artificial insemination programs (Hogg *et al* 2006; Hedrick & Fredrickson 2010) may need to be considered to restore adaptive potential.

3 AIM 2

Research was conducted under Aim 2 to assess the value and ecological characteristics of non-remnant areas for koalas and whether these would benefit from legal protection, with the aim of improving koala conservation.

3.1 Background

In recent decades, conservation efforts have accelerated in an attempt to reverse negative trends in wildlife populations rand. Most efforts are placed on protecting large contiguous core habitat (Tulloch *et al* 2016; Wintle *et al* 2019), which is considered the gold standard for supporting population viability. However, with escalating threats and anthropogenic activities (e.g. infrastructure development), these optimal habitats have become increasingly limited and isolated. To compensate for habitat loss, efforts have focused on offsetting impacts by procuring and protecting habitat or adding habitat by restoring and rehabilitating degraded landscapes. However, habitat is continuously being cleared, achieving offsets with clear and direct benefits to koalas has become increasingly challenging.

Under the initial design of Aim 2, a comparison was to be undertaken between ten rehabilitated and ten control sites, using a genetic analysis of koala scats from both sites to understand whether rehabilitated offsets would yield direct and long-term benefits to koalas. However, a number of challenges associated with the availability of data prevented USC from taking this approach, as follows:

1) There were too few rehabilitation sites of size large enough that we could identify that represented a substantial proportion of a koala home range even after extending the search to a 500 km radius (from Fraser Coast all the way down to Moreton Bay). This would limit statistical power to detect signals.

2) The identified rehabilitation sites were too small in size and as such would have not contained enough koalas to link rehabilitation with key health indicators.

As such, Aim 2 was revised in 2018-19 when USC proposed an analysis of data collected by TMR under the MBR project which monitored koalas at the Griffin site, a site that comprised a significant area of rehabilitated land utilised by koalas. Unfortunately, this dataset was likewise too limited to answer any meaningful questions (8 koalas used the rehabilitation with a total of 13 VHF tracking points).

A review of 76 EPBC Act referrals was undertaken with the aim to seek data and information about recolonisation of koalas in offsets. 68 met the selection criteria (submitted for approval between 2012 and 2018 and deemed a controlled action). Of the 68 referrals, 40 were randomly selected to be reviewed. Further, a search was conducted, utilising publicly available information, to locate associated documents for each of the selected 40 referrals including: offset management plans, koala management plans, biodiversity offset strategies, and/or compliance reports, audits, monitoring programs (if available). As a result, a total of 472 documents were reviewed. Most of these referrals unfortunately contained limited publicly available information to determine if and what measures were successful for the long term recolonisation patterns of koalas in offset areas. To enable future research, TMR could develop a database of the collation of their own data to inform offsets into the future.

Given these challenges, USC proposed to undertake a 'one time point' large scale landscape surveys of koala scat presence/absence which would allow for the classification of each as either 'rehabilitated' or remnant sites with the idea of aging rehabilitated sites for further scat surveys and analyses. This was done in collaboration with TERN but unfortunately aging through satellite imagery gave inconsistent results.

To counter the limitations around the aging of sites, USC proposed the use of non-remnant sites as a proxy for rehabilitated sites. USC then investigated the relevance and ecological characteristics of non-remnant areas for koala conservation. This approach was successfully applied and fed into Research Papers 4, 5 and 6. The links to those Research Papers can be found in Appendix 1.

3.2 Surveys and data collection

During this research project, USC undertook a total of 3425 koala scat presence/absence surveys as summarised in Table 4. The locations of the surveys are presented in Figure 4.

Table 4. Number of koala scat presence/absence detection dog surveys conducted by USC since 2015. These include surveys for Aim 1 and Aim 2 and are distinguished by location.

Location (LGA)	2015	2016	2017	2018	2019	2020	2021
Fraser Coast	150	66	71	22	-	1	-
Gympie	97	268	194	32	25	-	39
Moreton Bay	-	-	26	-	34	-	-
Noosa	54	155	123	65	92	-	1
NSW Northern Tablelands	-	269	-	120	175	-	100
Redland City (incl. Stradbroke Is.)	-	-	-	532	104	68	238
Sunshine Coast	7	20	178	6	3	4	-
Toowoomba	-	-	-	86	-	-	-

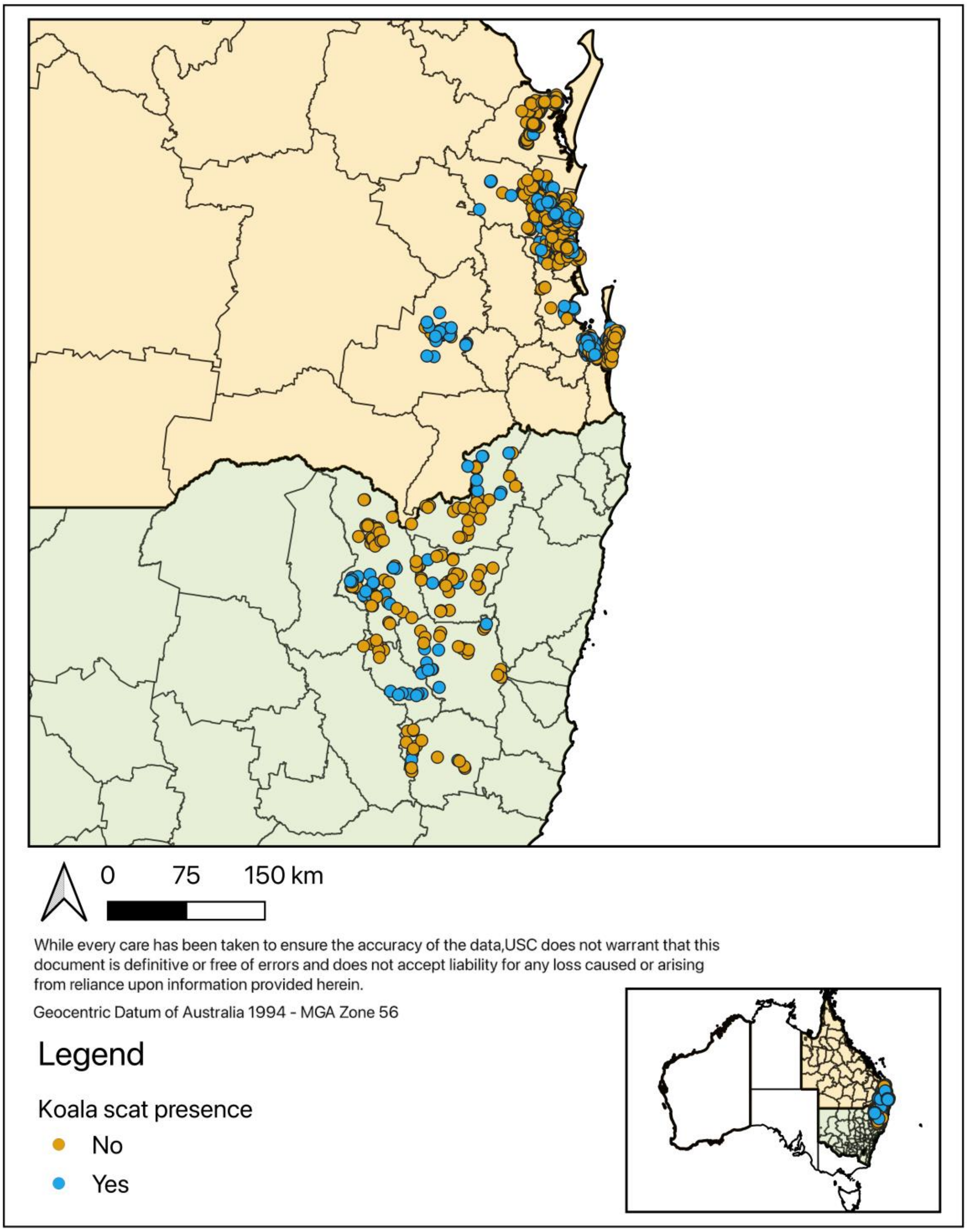

Figure 4. Koala presence/absence surveys conducted by USC koala scat detection dogs used in Research Papers 5 and 6 of Aim 2

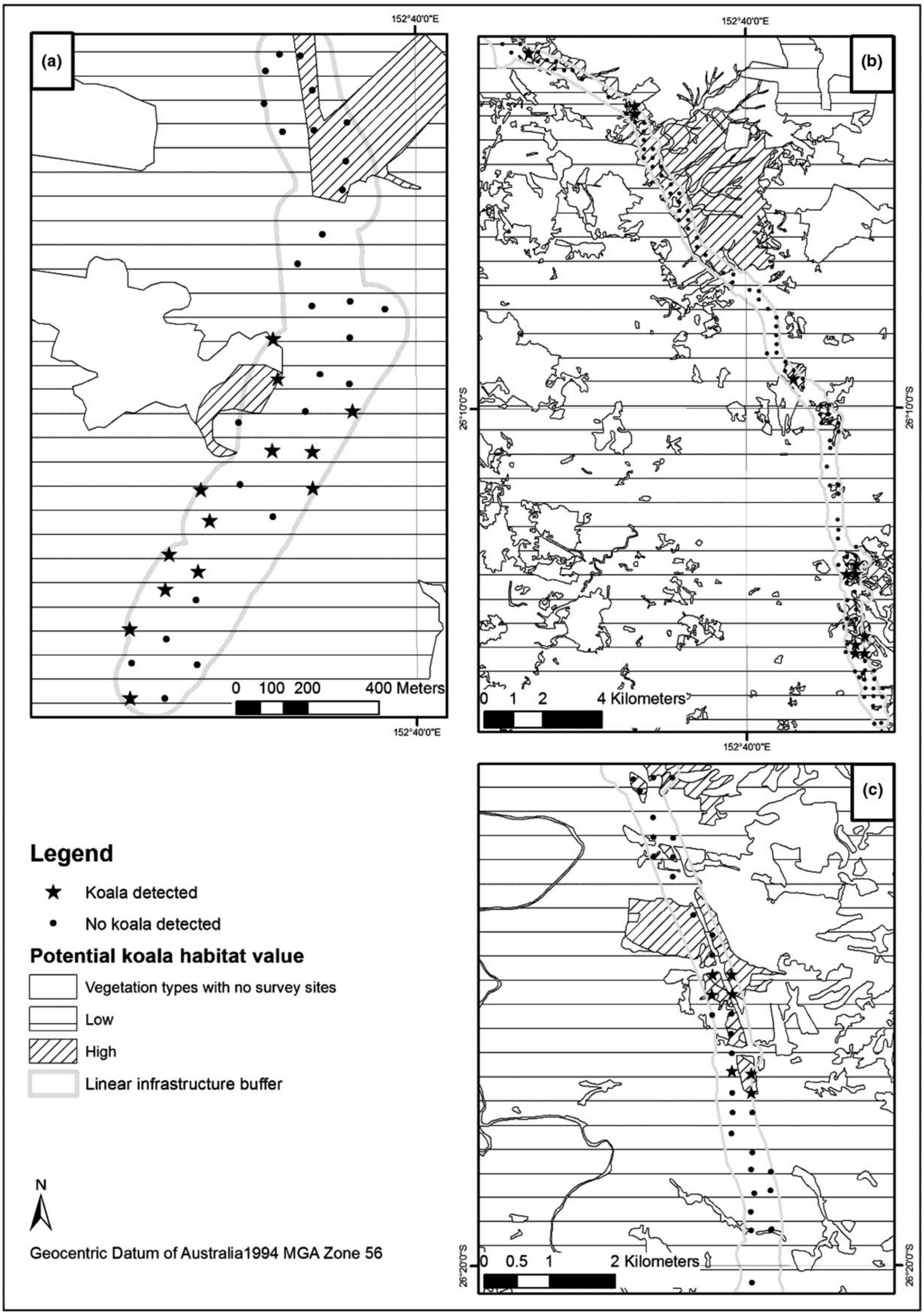

Figure 5. Koala presence and absence at sites surveyed for the purpose of Aim 2, Research Paper 4

3.3 Research Paper 4

Cristescu R H, Scales K L, Schultz AJ, Miller RL, Schoeman DS, Dique D & Frère CH (2019) Environmental impact assessments can misrepresent species distributions: a case study of koalas in Queensland, Australia. *Animal Conservation*. 22 (4), 314-323.

3.3.1 Background

Ongoing human population growth is placing escalated clearing pressures on vegetation (Hansen *et al* 2013). Linear transport infrastructure in particular has far-reaching ecological consequences for vulnerable species such as the koala due to increased road strikes, road avoidance, fragmentation of populations and the formation of barriers to dispersal (Forman & Alexander 1998). In order to combat these risks, legislation requires an Environmental Impact Assessment (EIA) before approval is granted for clearing activities so that mitigation and offsets can be designed accordingly. It is therefore crucial that EIAs are accurately evaluating habitat so that appropriate mitigation strategies are put in place.

Currently, Australian EIA survey guidelines recommend concentrating survey efforts for elusive species such as the koala, by the following steps:

- Stratification of the landscape based on criteria that are known to influence the probability of occupancy by the species
- Ranking the strata and prioritizing sampling in strata with higher probability of occupancy

However, the accuracy of which this process is estimating koala occurrence has never been verified by ground truthing. In particular, the current guidelines to estimate koala occurrence are based on the on the presence of food trees. This method therefore relies on the assumption that koalas can only occur in food trees despite recent literature to the contrary (Ellis *et al* 2002; Ellis *et al* 2011; Reckless *et al* 2017; Dargan *et al* 2019). Therefore, Paper 4 aimed to test the assumption that EIA guidelines are successful in determining koala occurrence.

3.3.2 Methodology

3.3.2.1 Data collection and selection

This study utilised scat detection dogs to assess koala presence/absence across the Noosa Council landscape, as well the Gympie and Fraser Coast areas which were earmarked for three future linear infrastructure developments. The locations of the survey sites at the landscape scale were selected through random point generation within accessible tenures and on private properties. The locations of the survey sites for the three EIA projects were determined by allocating a uniform spatial buffer around the planned infrastructure projects and generating points following a uniform grid pattern (100m or 300m apart) with a random start point. This resulted in a total of 400 scat surveys conducted (Figure 5).

In order to compare the actual koala presence with the estimated occurrence produced through EIA's, the survey sites were ranked into the following categories based on Australia EIA guidelines (Commonwealth of Australia, 2011, 2014):

- Low habitat quality (no Eucalyptus species- including non-remnant forests)
- High-moderate habitat quality (Eucalyptus species present – both with and without preferred food trees)

3.3.2.2 Statistical Analysis

A GLM was used to estimate whether habitat quality and survey design (as currently defined by governmental survey guidelines) explained a significant proportion of observed koala presence.

3.3.3 Key Findings

Research Paper 4 demonstrates that current recommendations for EIA recommend focusing survey efforts on what is perceived as known or high-quality koala habitat may misrepresent the true occurrence of koalas. The results of this research show that across both individual development projects and at a greater landscape scale, koalas were equally as likely to be present in what is perceived as low-quality koala habitat (including non-remnant habitat) to that perceived as high-quality koala habitat. In one infrastructure project, for example, it was even shown that koalas were more than twice as likely to be found in habitat of low rather than that of a high perceived quality.

This research highlights inherent issues with the deployment of survey/sampling designs that rely heavily on prior or presumed knowledge of koala presence. This is because these methodologies risk becoming self-fulfilling prophecies (i.e. a species is only ever found in areas targeted by the surveys, (Merton 1948)) as they uncritically reinforce underlying assumptions. As such, for both the impacted development zone and for potential offsets of a linear transport infrastructure development, habitat of low perceived quality, including non-remnant vegetation, should be surveyed so that mitigation measures appropriately reflect the impact of the development.

3.3.4 Conclusion

This research highlights the importance of using field verification over presumed or mapped high quality koala habitat. Hence, for infrastructure developments and subsequent EPBC Act offsets, koala usage should therefore be confirmed to best maximise management outcomes. To address this a proposed survey design has been developed in Figure 2 of Aim 2 recommendations.

3.4 Research Paper 5

Gardiner R, Terraube, J, Frère, CH and Cristescu, R (2023) Roads and water availability influence the occurrence of koalas (*Phascolarctos cinereus)* in secondary habitat: a multiscale approach. *Biodiversity and Conservation,* 32, 163–180 (2023).

3.4.1 Background

Anthropogenic activities are increasing habitat loss and threatening species persistence, urging the need for effective protection of wildlife and their habitat (Maxwell *et al* 2016; Horváth *et al* 2019). In such landscape that is already modified and fragmented, it is critical to determine what is important for a species to increase their long-term persistence. Due to accelerating anthropogenic activities (e.g. infrastructure development), the available optimal habitat such as remnant forest (e.g. undisturbed habitat with more than 50 percent forest cover) are becoming increasingly scarce. Therefore, the ability to select and rehabilitate less optimal sites such as non-remnant forest (e.g. disturbed habitat with less than 50 percent forest cover) will be an invaluable asset in preserving vulnerable species such as forest specialists.

This study accounted for known ecological factors that influence koala distribution, to determine whether non-remnant habitat (re-growth or secondary habitat) could be used as a means to protect koalas at multiple scales within the landscape. Previous research has found remnant habitat influenced the occurrence, movement and persistence of koalas - driven by the area and density of preferred food trees (*Eucalyptus* species) (McAlpine *et al* 2006). More recent studies have highlighted that koalas do not discriminate between intact and regrowth habitat, and therefore non-remnant habitat may be of potential value for koalas (Cristescu *et al* 2019). This study investigates the importance of remnant habitat for koala conservation and their ecological characeristics to inform potential offset strategies.

3.4.2 Methodology

The following methodology was adopted for this study:

Data collection

This study utilised scat detection dogs to assess koala presence/absence across seven LGAs in Southeast Queensland between 2015 and 2020 (Sunshine Coast, Noosa, Gympie, Toowoomba, Moreton Bay, Redlands City Council and Fraser Coast). In total 2230 scat surveys were conducted with 959 surveys used for analysis. Koala presence was detected in 575 surveys with 384 surveys being negative for koala occurrence (Figure 1, Research Paper 5, Appendix 1).

To account for koala movement across varying range, the study utilised three buffer sizes: i) 250m, ii) 500m and iii) 1500 m radii. These were placed either around the first scat found of the survey if a site was positive, if a site was negative variables were extracted from the survey's starting point.

At the three buffer scales the following variables were extracted and calculated using ArcGIS (10.8):

- **Non-remnant:** Proportion of non- remnant eucalyptus forest calculated in buffers
- **Forest:** Total eucalyptus forest extent calculated within buffers
- **Water:** Total surface area of fresh water calculated within buffer
- **Road:** Euclidean distance to the nearest major primary road from survey or positive indication
- **Rain:** Average yearly rainfall one year prior to surveying
- **Health:** Mean mass fraction of total phosphorous in soil by weight (percent) within 0-5cm
- **Search:** Survey effort conducted by detection dogs (seconds)
- **Year:** Year survey was conducted
- **Eastings/Northings:** Coordinates of survey start point or first positive indication
- **Council:** Local government area surveys with detection dogs were conducted

Statistical analysis

Generalized Linear Mixed Models (GLMMs) with a binomial distribution were used to evaluate the relative importance of environmental variables at the three buffer scales on the occurrence of koalas. A total of seven models were designed for each buffer size and included all covariates. The interactions chosen were to test the hypothesis that covariates associated to features that represent anthropogenic threats (distance to roads) and habitat productivity (water extent and rainfall) could interact with the proportion of non-remnant forest (enhancing or buffering its effect) to influence koala occurrence. These models were then ranked by best fit using the Akaike Information Criterion.

3.4.3 Key findings

This study found that koalas used both remnant and non-remnant habitat, while the probability of occurrence in remnant was slightly higher. The probability of koalas occurring in non-remnant habitat improved when considering the landscape context. In general koala occurrence was higher in sites with higher soil phosphorous, suggesting soil characteristics promoting vegetation health are important. It was also found that occurrence was higher the closer to the coast (where the majority of Queensland's koalas are found) and in areas with lower rainfall, however these results were likely driven by higher-than-average rainfall events in areas with lower presence. Notably, more than 40 - 60 percent of non-remnant forest sites surveyed had koala scats present. Notably the distance to roads negatively influenced occurrence at the smaller buffer size, while water availability increased probability of occurrence at the larger landscape scale. For each of the buffer zones the key findings were as follows:

250m radii buffer

At the 250m radii buffer, the probability of koala occurrence in non-remnant vegetation was predicted to increase by 25 percent when habitat is more than 18kms away from major roads.

500m radii buffer size

At the 500m radii buffer, the probability of koala occurrence in non-remnant eucalypt forests was predicted to be 50 percent but is expected to increase when there is more than 0.5km^2 of water in the surrounding landscape (which is approximately 15 percent of the total area of the buffer).

1500m radii buffer size

At the 1500m radii buffer, koala occurrence in non-remnant eucalypt forests is predicted to be more than 50 percent but is expected to rapidly decline when water area decreases.

3.4.4 Conclusion

Offsets can only benefit koalas if they are directly protecting habitat that is used by koalas. This study underlined the importance of both remnant and non-remnant forests for koala conservation and identified some characteristics that increased koala occurrence in non-remnant forests. It showed that if non-remnant forests were selected as potential offsets, these should prioritise areas at least 18 kms away from major roads and with the presence of water at the larger landscape scale. In addition, this study indicated that the amount of phosphorous in the soil increases the probability of occurrence due to its positive effect on eucalyptus growth.

3.5 Research Paper 6

Terraube, J., Gardiner, R., Hohwieler, K., Frère, CH. and Cristescu, R. (2021). Forest protection has a minor effect on koala occurrence in Eastern Australia. Biodiversity and Conservation, 292, 4 (2023).

3.5.1 Background

Protected Areas (PAs) are crucial policy instruments for conserving biodiversity. When implemented correctly, PAs can have positive effects on the abundance and distribution of threatened species as well as mitigate and promote adaptation to climate change (Gaüzère *et al* 2016; Cazalis *et al* 2020; Lehikoinen *et al* 2021). However, recent research has also shown that the ecological effectiveness of PAs can vary substantially depending on a variety of factors like local governance, national development indices or external pressures (Barnes *et al* 2016; Amano *et al* 2018; Geldmann *et al* 2019; Veldhuis *et al* 2019). Importantly, increased attention should focus not only on quantity but also on the quality of these Pas (Visconti *et al* 2019).

According to the IUCN, protected areas fall under varying categories based on the level of protection and extraction permitted within the designated areas (worldwide adapted assessment of protection). It is generally assumed that categories with stricter protection will be more efficient at protecting species than less strict categories (add refs from the paper here). Despite their global use, the effect of protected areas on koala populations remains poorly understood, yet evaluating the effectiveness of this conservation action is crucial to improve current outcomes in terms of koala conservation. This study aims to examine the impact PAs have had on koala occurrence across Queensland and New South Wales (NSW) at several spatial scales. It also included variables known to influence koala occurrence across their range such as elevation, rainfall, road distance, forest quality, and soil phosphorous levels. These variables were included in all models to take into account potential confounding factors and better isolate the effect of forest protection status in koala occurrence.

3.5.2 Methodology

Data collection

This study utilised scat detection dogs to assess koala presence/absence across seven LGAs in Southeast Queensland between 2015 and 2021(Sunshine Coast, Noosa shire, Gympie, Toowoomba, Moreton Bay, Redlands City Council and Fraser Coast). In total 2230 scat surveys were conducted with 1463 surveys used for analysis. Koala presence was detected in 861 surveys with 602 surveys being negative for koala occurrence.

In order to account for habitat use across multiple behavioural contexts (e.g. foraging and dispersal), the landscape was divided into two different buffer scales: i) 1km and ii) 3km radii. At the two buffer scales the following variables were extracted using ArcGIS (10.8):

- Forest extent (taken from Forests of Australia 2018 dataset created by the *Australia Bureau of Agriculture and Resource Economics and Sciences*).
- Forest type (the amount of Eucalyptus forest classified as non-remnant using Queensland Spatial).
- Extent of protected forest (calculated using the World Database of Protected Areas).
- Water area (calculated from the *National Surface Hydrology* spatial layer).
- Soil quality (calculated by extracted mean mass fraction of total phosphorous in soil by weight (percent) within top-soil layers of 0-5cm from the *Soil and Landscape Grid National Soil Attribute*).
- Distance to major road (calculated using the *Near* tool in the spatial layer of *Global Roads Inventory Project dataset*).
- Elevation (taken from Digital Elevation Model based on 30m grid cells – pid.geoscience.gov.au).
- Average rainfall one year prior to scat collection (calculated from the *Bureau of Meteorology*).

Statistical analysis

A total of eight different models were designed, including two estimates of percentage of protected forests, i) for all protected area categories (IUCN categories I-VI) and ii) only for strictly protected areas (IUCN categories I-IV), for each buffer size (1km and 3km), with and without the additional ‘habitat quality’ covariates.

3.5.3 Key findings

This study found a small yet significant effect of PAs on koala occurrence across the study at the 1 and 3 km buffers when considering forests protected by all IUCN management categories. The small effect (0.5 to 1 percent of the variance in koala occurrence) may be because Australian PAs have historically been created on unproductive lands at higher elevation and with low human population size (Watson *et al* 2009), far from the high-quality forests in lowlands that seem optimal habitat for koalas. It must also be acknowledged that the small PA effect on koala occurrence may be due to the sampling regime which was not designed specifically to investigate the effect of PAs. Conducting surveys in similar and adjacent habitats located inside and outside PAs of different IUCN categories would allow refining the results.

3.5.4 Conclusion

This study highlights the needs for the creation of new protected areas targeted to protecting koalas, such as, for instance, higher quality habitat (like remnant forests). Notably, most habitat that is suitable for koalas is situated where humans live, therefore engaging private landholders and providing an incentive to maintain and protect their habitat will play a vital role for koalas in the future. It also acknowledges the importance of legally securing offset sites for the long-term protection of koala habitat.

3.6 Recommendations arising from Aim 2

During the design of an offset proposal it is important to identify and understand the characteristics of the koala population so offsets can be developed appropriately in the EPBC Act Preliminary Documentation Assessment phase. This should include the following:

1. A desk-top or literature analysis of current known koala population distributions to determine areas of higher success for koalas to use offsets
2. A ground-truthing of sites using robust and accurate survey methods
3. A desk-based analysis of the surrounding area to identify potential offsets that meet the below criteria
4. Conducting baseline surveys to establish koala presence (density preferred over presence/absence) in surrounding areas and if present, an assessment of their current state.

To ensure that the koala presence in an infrastructure project is accurately documented and that offsets appropriately select habitat used by koalas a proposed survey design has been developed in Figure 6 below.

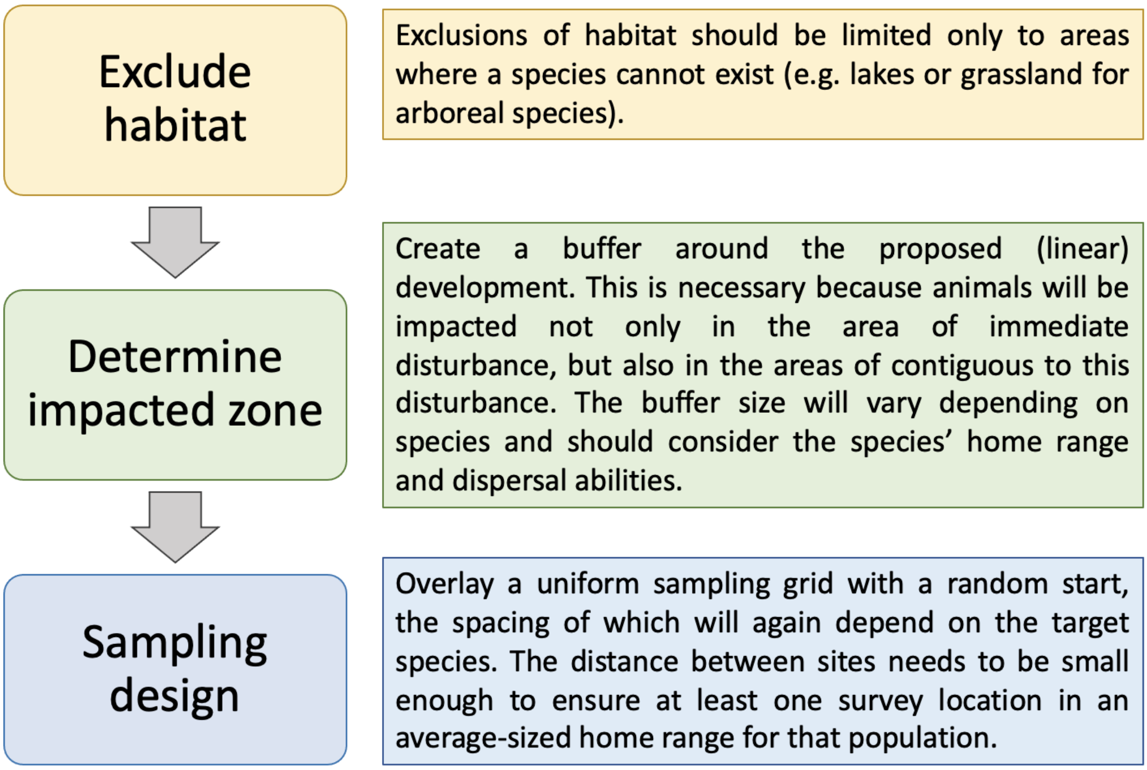

Figure 6. Proposed survey design. *Based on the results of Research Paper 4 – this design aims to prevent the misrepresentation of koala occurrence during future infrastructure development projects.*

The priority should be to combine offsets with rehabilitation close to existing PAs or legally secured sites or remnant habitat. These are considered to be of a higher quality habitat and more resilient to threats such as road mortality and extreme weather events (e.g. droughts and bush fires) and also provide more established habitat for koalas. Where offsets consist of non-remnant habitat, the following should be assessed and targeted:

- Distance to roads. Offsets of non-remnant habitat should be as far away as possible from major roads to reduce mortality and improve connectivity and colonisation.
- Soil quality and potential.
- Water availability. Non-remnant offset should connect or include freshwater bodies such as rivers or lakes in the surrounding landscape to improve habitat quality and act as buffers to climate change and promote habitat resilience.

After an offset has been selected, management should include the following:

- Legally securing the offset site.

- Plan long term maintenance, governance, funding, and ownership of offset site noting that it may be for life of the EPBC Act.
- Design targets for each 5 year (or other) period and associate triggers and management measures if these are not achieved.
- Undertake regular monitoring of the population and assess in relation to the baseline survey first conducted.
- Targeted mitigation measures appropriate for the offset site and population (e.g. koala fencing to reduce road mortality, wild dog management) - priority should be given to replanting and filling connectivity between sites.
- Engagement with private landholders and/or Indigenous native title holders in preserving habitat on their property to protect koalas from further habitat loss.

4 ANALYSIS OF RESULTS AND CONCLUSIONS FROM AIMS 1 AND 2

Whilst the results and recommendations of Aim 1 and Aim 2 have been individually outlined, the combined analysis of research undertaken under both Aims together generates important insights that have salient implications for future planning.

The research conducted under Aim 1 confirmed that maintaining/restoring landscape connectivity is critical to the survival of the koala in anthropogenically altered landscapes. Results from Aim 1 showed that koalas do not exhibit any behavioural mechanisms to avoid breeding with close relatives or Chlamydial diseased individuals. This means that koalas occupying disconnected islands of vegetation, where dispersal capabilities are reduced, will be at significant risk of inbreeding depression, genetic erosion and disease prevalence. Together, these cumulatively increase the risk of local extinction, a trend that has been documented in the last 20 years in Northern NSW and Southeast Queensland where koala population sizes have decreased by 80 percent (McAlpine *et al* 2015).

As such, this research emphasises the importance of the location of design mitigations such as fauna underpasses. Design mitigations should be positioned based on evidence showing the location of likely fauna crossing points to support the required minimum rate of dispersal (individually modelled for each infrastructure project) to maintain genetic connectivity and minimise loss of genetic diversity (see section 2.4).

Aim 1 shows that genetic and disease analysis can usefully inform the location of fauna crossings, however this information can also importantly inform the location of offsets (e.g. can offsets be identified that link isolated impacted populations and restore landscape connectivity to support increase in gene flow?). Taken together, the results of Aims 1 and 2 indicate that if impacts to koalas cannot be avoided or minimised through infrastructure design, and offsets are required, land parcels should be selected to prioritise koala habitat connectivity within the landscape.

So how can this be achieved when remnant vegetation used by koalas is slowly but surely disappearing? Importantly, Aim 2 of this research indicates that while protected areas and remnant vegetation increased occurrence of koalas, non-remnant vegetation is also vitally important to koala persistence (Research Papers 4 and 5). Research Paper 5, for instance, show that koalas do use non-remnant vegetation (e.g. 40 - 60 percent of surveys), and that non-remnant sites provide important habitat which can be targeted for protection via offsets to improve koala conservation.

This means that parcels containing non-remnant vegetation should not necessarily be discounted in the selection of potential offset parcels (as long as efforts are invested to assure habitat quality over the long term), especially if these sites have confirmed koala presence (e.g. scats), provide koala habitat connectivity in the landscape, and meet the criteria outlined in Research Paper 5 under Aim 2. This may expand the potential options available to government when considering offset sites for koala conservation.

Given this, the research here also underpins the importance of a strategic approach to the planning of offsets, to ensure that they either protect or restore habitat connectivity across the landscape. This could be best achieved through a coordinated approach between all levels of government (Commonwealth, state and local). When investigating and identifying potential offsets, potential locations should be prioritised in relation to their relationship to the greater landscape (e.g. whether they abut, or provide new landscape connectivity between, existing offsets or other protected areas e.g. state or national parks). This is because, in most cases, any single offset (even of excellent habitat quality) can have only limited impact on koala conservation if it is isolated and does not support landscape connectivity (and therefore koala dispersal). Such a coordinated approach by all levels of government will help mitigate the cumulative impact of anthropogenic development on the species.

Finally, whether sites with remnant or non-remnant vegetation are identified as potential offset sites, koala use of these sites should first be confirmed through ecological surveys rather than relying on vegetation quality and/or type as an indicator of koala occurrence. For non-remnant habitat offsets (see ecological characteristics which increase probability of koala occurrence in Aim 2 Recommendation), efforts should be placed on improving the quality of the habitat for koalas by, for instance, planting koala food trees. Either way, once offsets are confirmed these should be legally secured to protect them from future development.

In this regard Table 5 outlines the key criteria (in order of priority) for the selection of offset parcels for koalas.

Table 5 - Offset Selection Criteria

Criteria
Does the land parcel provide connectivity?
Are any of the adjoining land parcels protected?
Does the offset land parcel(s) contain remnant or non-remnant vegetation?
Does the adjoining land parcel(s) contain remnant or non-remnant vegetation?
Is the offset land parcel 18kms or more from a major road?
Is the offset land parcel near water?

Together, these questions should be used as guiding criteria for the consideration of any future offsets.

5 LIST OF APPENDICES

- **Appendix 1**: List of links to each of the 6 Research Papers.
- **Appendix 2**: List of links to each of the additional 6 Research Papers.
- **Appendix 3**: Population genetic analysis of Gympie scat samples – Additional Outcome.

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