

# ***Cool Country Koala Project 2019 - 2020: Northern Tablelands Koala Habitat and Pilot Genetic Project***

**Prepared for Northern Tablelands Local Land Services**



By the University of the Sunshine Coast, Detection Dogs for Conservation

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*Cool Country Koala Project 2019 – 2020: Koala Habitat and Pilot Genetic*



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## List of Acronyms

Acronym	Meaning
<b>ALA</b>	Atlas of Living Australia
<b>DDC</b>	Detection Dogs for Conservation
<b>IR</b>	Internal Relatedness
<b>NPWS</b>	National Parks and Wildlife Service
<b>NT</b>	Northern Tablelands
<b>NT KRS</b>	Northern Tablelands Koala Recovery Strategy
<b>NT LLS</b>	Northern Tablelands Local Land Services
<b>PCR</b>	Polymerase Chain Reaction
<b>SAT</b>	Spot Assessment Technique
<b>SD</b>	Standard deviation
<b>SNP</b>	Single Nucleotide Polymorphism
<b>USC</b>	in this report: University of the Sunshine Coast's Detection Dogs for Conservation team
<b>TSR</b>	Travelling Stock Route
<b>QLD</b>	Queensland
<b>NSW</b>	New South Wales



## Glossary

This glossary has been compiled from many sources and is given here to facilitate the flow of the report. Each term defined below will be followed by a \* when first used in the text, to alert the reader this term is explained here.

Term	Definition
<b>Activity Level</b>	number of trees used by koalas, as evidenced by the presence of koala scats, divided by the number of trees searched during a systematic survey (as a convention, 30 trees are searched)
<b>Allele</b>	a variant of a gene. The size of an allele can vary in size (e.g. between one nucleotide to hundreds of nucleotides). At the population level, variation in alleles are used to estimate patterns of genetic diversity.
<b>Call rate</b>	describes missing data that stems from the failure to call a SNP because of a mutation or genotyping error. SNPs that failed to be called at a high frequency are usually filtered out of the dataset due to too much missing information.
<b>DNA</b>	deoxyribonucleic acid, a molecule carrying genetic information
<b>F-statistics</b>	<p>is the basic method used to measure the amount of subdivision in populations, and consists of three measures, <b>FIS</b>, <b>FST</b>, and the less commonly used <b>FIT</b>. These measures relate to the amounts of heterozygosity at various levels of a population structure: individual (I), subpopulation (S) and total (T).</p> <p><b>FST</b> estimates the amount of structuring of a population into subpopulations and can range from 0 to 1 (where 0 means complete sharing of genetic material and 1 means no sharing). In this report, <math>F'ST</math>, the standardised <b>FST</b> (produced by dividing <b>FST</b> by the maximum value it can obtain, given the observed within-population diversity) was also calculated to enable comparisons of our results to other studies.</p> <p><b>FIS</b>, also called inbreeding coefficient, is the proportion of the variance in the subpopulation contained in an individual and can range from -1 to 1 (the closest to 1, the higher the degree of inbreeding). Note that inbreeding can not only result from non-random matings (matings between cousins for example), but also from small isolated populations, where all individuals are more closely related than large populations.</p>
<b>Gene flow</b>	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).
<b>Genetic diversity</b>	the extent of genetic variation in a population (or species, or across a group of species), for example heterozygosity or allelic diversity.
<b>Genotype</b>	in diploid species (species with two sets of chromosomes - paternal and maternal copies), genotype is often used to refer to the particular pair of alleles that are carried by an individual. A genotype is described as homozygous if it features two identical alleles and as heterozygous if the two alleles differ. The process of determining a genotype is called genotyping.
<b>Heterozygosity</b>	refers to the presence of two different alleles within a diploid individual, here it refers to the presence of two different nucleotides at a specific SNP locus. $H_O$ = observed heterozygosity, the calculated level of heterozygosity from the allele frequencies of the population under study, $H_E$ = expected heterozygosity, the level of heterozygosity that could be expected based on observed allele frequencies if the population was at the Hardy-Weinberg equilibrium.
<b>Internal Relatedness</b>	internal relatedness (IR) is a measure of inbreeding at the individual level (as opposed to population level, such as $F_{IS}$ ). It is calculated from heterozygosity data and does not require a pedigree (pedigrees are difficult to obtain in wild populations). Internal relatedness is currently the most widespread used index for inbreeding and its main strength is that allele frequencies are incorporated into the measure.
<b>Inbreeding</b>	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.
<b>Locus (plural of loci)</b>	refers to a specific position in the genetic material (such as in a chromosome), for example where a SNP is detected.
<b>Minor Allele Frequency (maf)</b>	is the frequency at which the second most common <b>allele</b> * occurs in a given population.
<b>PCR</b>	polymerase chain reaction, a technique in molecular genetics that permits the analysis of any short sequence of DNA even in samples containing only minute quantities of DNA, such as scats.
<b>Sex ratio</b>	the relationship between the number of males to the number of females. Typically, the sex ratio in natural populations is expected to be 1:1. Risks of extinction are increased if population sex ratios deviate from 1:1. However, a

	small bias of sex ratio towards females can sometimes be desirable, especially in very small or rapidly declining populations.
<b>SNP</b>	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).
<b>Structure</b>	<p>within a species, genetic structure exists because not all individuals are able to breed with all other individuals of the same species (i.e. due to geographic proximity). This can occur even if a species distribution is continuous due to geographic isolation: simplistically, this reflects that individuals that live closely to each other have a higher chance to breed together than individuals further apart. Population structure, i.e. the genetic differentiation of local populations, is increased by mutation, genetic drift due to finite population size, and natural selection favouring adaptations to local environmental conditions; but is decreased by gene flow (the movement of gametes, or individuals). Population structure is higher when gene flow between populations is lower, and so population structure is increased by habitat fragmentation and isolation.</p> <p>Gene flow cannot be directly seen, but population structure can be studied through allele frequencies - this underlines a critical point, that structure can only be inferred with a sample size large enough to calculate robust allele frequencies. This means that sample size dictates, in any study, the unit of comparison and the scale at which the genetic structure can be examined – i.e. depending on the intensity of the sampling design, whether appropriate sample size is reached per park, locality, council or region. In this report, we could achieve fine-scale genetic structure comparisons between localities. In previous studies, Redlands mainland was pooled with neighbouring regions under the name “Koala Coast” and this became the unit for comparison with other regions of Australia (Kjeldsen et al. 2018).</p> <p>Genetic structure can be hierarchically described:</p> <ul style="list-style-type: none"> <li>• <b>Large-scale structure</b> (often studied through a Bayesian statistic programs) usually defines “populations”, these are independent breeding units, each population coming from a different lineage, and with no to very low gene flow. The software usually tests for whether distinct populations can be inferred <b>without any <i>a priori</i> geographic information</b> and identifies migrants (individual belonging genetically to one population, but geographically to another one), and admixed individuals, that are offspring of migrants between populations.</li> <li>• <b>Fine-scale structure</b> (often calculated through Fst, see F-statistics) usually describes sub-populations (also called local populations or demes) where gene flow exists but is restricted. Genetic structure here is studied by comparing allele frequencies <b>between artificially constructed populations</b></li> </ul>



(e.g. between Countries, between States, between Councils...) and then testing whether the populations should be considered one or multiple, and how similar the populations are to one another (pairwise  $F_{st}$ ). • Finally, the distribution of related individual in space, an even finer structure that can be referred to as “cryptic”, can be described through autocorrelation measures, where distances between all individuals and their genetic relatedness are compared.



## Executive Summary

### Purpose

The first aim of this study was to further expand the knowledge of koala (*Phascolarctos cinereus*) distribution and habitat utilisation in areas not previously surveyed during the *Northern Tablelands Cool Country Koala Project*'s 2016-2017 and 2018-2019 fieldwork. In particular, the present distribution surveys were conducted in and around the following localities: Walcha, Woolbrook, Brackendale, Yarrowitch, Brockley, Aberfoyle, Guyra, Bundarra, Cameron's Creek, Llangothlin, Glencoe, Pargo Flat, Wonga Hut, Guy Fawkes, Georges Junction, Stannifer, Wandsworth, Kangaroo Camp, Lollera, Elderbury, and Glen Elgin.

Furthermore, after consultation with Northern Tablelands Local Land Services (NT LLS), information about crown-dieback, standing water, and wet ground, was included in the surveys in order to assess any correlations between koala site use and tree health or water availability. This information should assist in making informed management decisions.

The second aim was to deliver a pilot genetic monitoring study to understand patterns of genetic diversity for two known koala populations. The Armidale / Uralla area and the Inverell / Delungra area have been identified as koala strongholds in previous *Northern Tablelands Cool Country Koala Project* field surveys. Information on the genetic wellbeing of these two koala populations will enable NT LLS to better target conservation and management planning to ensure these populations persist into the future.

### Survey Methods

#### Presence / Absence Surveys

Field-based surveys of koala scats for presence / absence mapping were conducted in or near priority areas that were 1) identified in the Northern Tablelands Koala Recovery Strategy, and 2) not surveyed during previous *Cool Country Koala Project* fieldwork surveys. Survey sites were randomly generated using GIS and were constrained to Travelling Stock Routes (TSR) within or in proximity to identified priority areas, at the exclusion of any zone with risk from 1080 baiting. Koala scat surveys were conducted using detection dogs trained to locate koala scats (faecal pellets). Systematic survey sites comprised the 30 trees closest to a randomly generated point, where all 30 trees are searched for the presence of koala scats. Trees were also checked for the presence of koalas. Field surveys for koala scats presence / absence were conducted between the 2<sup>nd</sup> - 14<sup>th</sup> of July 2019.



## Genetic Surveys

Detection dog surveys for fresh koala scats were conducted in two regions previously identified as koala hotspots during the *Cool Country Koala Project* presence / absence surveys in 2016-2017: 1) Armidale / Uralla area, and 2) Inverell / Delungra area. Survey sites were not randomly generated, instead handlers selected survey sites on the basis of accessibility, 1080-baiting-free and properties where koalas have previously been found. Searches for fresh scats were performed using a specifically trained fresh scat detection dog to increase detection efficiency. This dog indicates on scat fresher than two weeks, this period of time is where the **DNA\*** quality is the most conducive of successful DNA extraction and sequencing. The dog was not constrained to a specific number of trees, and was allowed to search freely. Genetic surveys were conducted between the 16<sup>th</sup> - 24<sup>th</sup> of September 2019.

## Limitations

### Presence / Absence Surveys

The sites were surveyed on only one occasion; therefore, the results presented here provide a snapshot of the population during this period and it can be noted that evidence of koalas found within the study areas are likely to change with repeat surveys as well as seasonally.

A negative site might reflect that koalas are not using the area (true negative) or that koalas are using the area but the survey failed to detect any scat (false negative), which could occur, for example, if koalas have not deposited any scats in the 30 trees searched during each survey or if scats have decayed before the survey occurred. DDC decreased the risk of false negatives in this project by conducting a second survey nearby if no koala scats were found at a site during the systematic survey. This second survey, referred to as a casual survey, was less geographically constrained and the dog was allowed to search more freely.

## Genetic Surveys

Compared to high quality samples such as biopsies or swabs, scat DNA is degraded and presents multiple extraction difficulties due to inhibitors present from the koala dietary component of the scat. Here, the DDC utilised a new genotyping method that was customised for koala scats which allows the genotyping of >1000 **loci\***.

More samples were collected than **genotyped\*** due to budget and time constraints. The DDC selected 96 samples to be used for DNA extraction that represented a good spread across the two regions.

Very fresh scats yield the best quality DNA. In the field, each scat was therefore assessed for its age and categorised them from age 1 (very fresh with mucus) to age 5 (very old and



degraded) (see Table 1) and usually only collected scats of age 1 and age 2 for genetic analyses. In this study, aging of the scats in the field was at times difficult due to the pronounced effects of the drought. Very fresh koala scats were much drier than usual and would appear older than they actually were. The DDC therefore also sampled scats that appeared to be of age 3 to avoid this issue.

## Summary of Findings

### Field work

#### *Presence / Absence surveys*

Koala presence was detected at nine of the 127 survey sites. Live koalas were not detected during the surveys. For each systematic survey site, the activity level was calculated which is defined as the number of trees used divided by the number of trees searched. The average activity level per positive site was determined to be 3% (SD = 0).

These results were added to data from previous iterations of the NT LLS *Cool Country Koala Project* (2016 and 2018 surveys) to create a map of the whole region. Additional recommendations to those put forward in 2016 and 2018 were proposed. Recommendations in this report were selected for their potential for rapid and achievable implementation.

#### *Genetic surveys*

A total of 47 casual surveys were conducted, with fresh koala scats found in 44 surveys (94%). Across these survey sites, a total of 115 fresh scat samples were collected. In addition, 24 adult koalas were sighted, of which five were females with joeys, bringing the total number of koalas sighted to 29. Only two of these sightings occurred within the Armidale / Uralla area, whereas the remaining 27 koalas were sighted in the Inverell / Delungra area.

### Population Genetics

All 115 fresh koala scat samples were collected, of which a selection of 96 were sent for sequencing after being screened for quality. Of these, 90 were genotyped successfully. Through their unique DNA fingerprint, 76 distinct individuals were identified. Genetic analysis showed that koalas from the Armidale / Uralla region and Inverell / Delungra present two distinct populations that are weakly but significantly differentiated. Levels of **genetic diversity\*** were comparable to other koala populations in NSW and QLD. Further genetic sampling would be required to investigate the landscape in between the two populations to evaluate whether this differentiation is due to recent fragmentation or historical isolation.



## 1. Introduction

### 1.1 *Cool Country Koala Project 2019-2020: Northern Tablelands Koala Habitat and Pilot Genetic Project*

In 2019, University of the Sunshine Coast's Detection Dogs for Conservation team (DDC) was contracted by the Northern Tablelands Local Land Services (NT LLS) to undertake the *Cool Country Koala Project 2019-2020: Northern Tablelands Koala Habitat and Pilot Genetic Project*, with two key aims.

- Firstly, to conduct field-based surveys of koala presence / absence in additional priority areas of the Northern Tablelands not covered by previous projects; and
- Secondly, to conduct a pilot genetic monitoring study at two areas of high koala activity (Armidale / Uralla and Inverell / Delungra), as identified through previous projects.

Presence/absence surveys were conducted using two detection dogs working concurrently in a two-week period from July 2<sup>nd</sup> to July 14<sup>th</sup> 2019. To establish koala presence/absence, DDC used detection dogs trained to locate koala faecal pellets (scats) in sites in the priority areas, a method which was proven to be accurate and efficient (Cristescu et al. 2015). DDC surveyed sites for koala scats in and around the following localities: Walcha, Woolbrook, Brackendale, Yarrowitch, Brockley, Aberfoyle, Guyra, Bundarra, Cameron's Creek, Llangothlin, Glencoe, Pargo Flat, Wonga Hut, Guy Fawkes, Georges Junction, Stannifer, Wandsworth, Kangaroo Camp, Lollera, Elderbury, and Glen Elgin. Information about crown-dieback, standing water, and wet ground, was also recorded during the surveys in order to assess any correlations between koala site use and tree health or water availability.

Genetic sampling surveys were conducted over 10 days using a fresh-scat trained detection dog, from 16<sup>th</sup> to 25<sup>th</sup> September 2019. DDC used a detection dog trained to identify fresh (< 14 days old) koala scats to use for DNA extraction and genetic analysis (Cristescu et al. 2019). Surveys were focussed in and around the Armidale / Uralla area and the Inverell / Delungra area.

### 1.2 Background

The koala (*Phascolarctos cinereus*) is an iconic Australian marsupial that is broadly distributed across south-eastern Australia, particularly in regions that have experienced high levels of habitat fragmentation due to human residential, commercial and industrial activities (Martin and Handasyde 1999). Despite their iconic status and global appeal, koala conservation has become a growing national concern (McAlpine et al. 2015). The combined koala populations of Queensland (QLD), New South Wales (NSW) and the Australian Capital Territory are listed as Vulnerable under the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999* (Shumway et al. 2015). In NSW, the koala is listed as Vulnerable under





the *Threatened Species Conservation Act 1995*. Koala conservation is emphasised heavily in policy and planning at both Federal and State government levels, as is demonstrated by the development of the *National Koala Conservation Management Strategy 2009-2014* and the *New South Wales Recovery Plan for the Koala 2008*. In addition, NSW has developed a scheme that focuses on threats to koalas: 1) *NSW Koala Research Plan 2019–28, A 10-year plan under the NSW Koala Strategy NSW*, 2) *Securing the Koala in the wild in NSW for 100 years, Saving Our Species Iconic Koala Project 2017–21* and 3) *Koala Research Plan: Expert Elicitation* (Office of Environment and Heritage 2017, Hemming et al. 2018, Office of Environment and Heritage 2019). In 2019-2020, bushfires have been unprecedented in both their extent and intensity (Figure 1). Fieldwork for this report was conducted prior to the fire season, and the areas surveyed were not impacted by the fire. However, the number of koalas, especially in NSW, have changed, and this will heighten the conservation significance of the Northern Tablelands koala population. For example, the fire ground in NSW covered 8.4 million hectares (10% of the state), including (as published by the State Department on February 3rd 2020, prior to the end of the bushfires) 2.7 million hectares in national parks (37% of the NSW park system). There are calls to up-list the koala status to endangered in NSW, with some reporting a loss of a fifth of the state population (Lane et al. 2020).

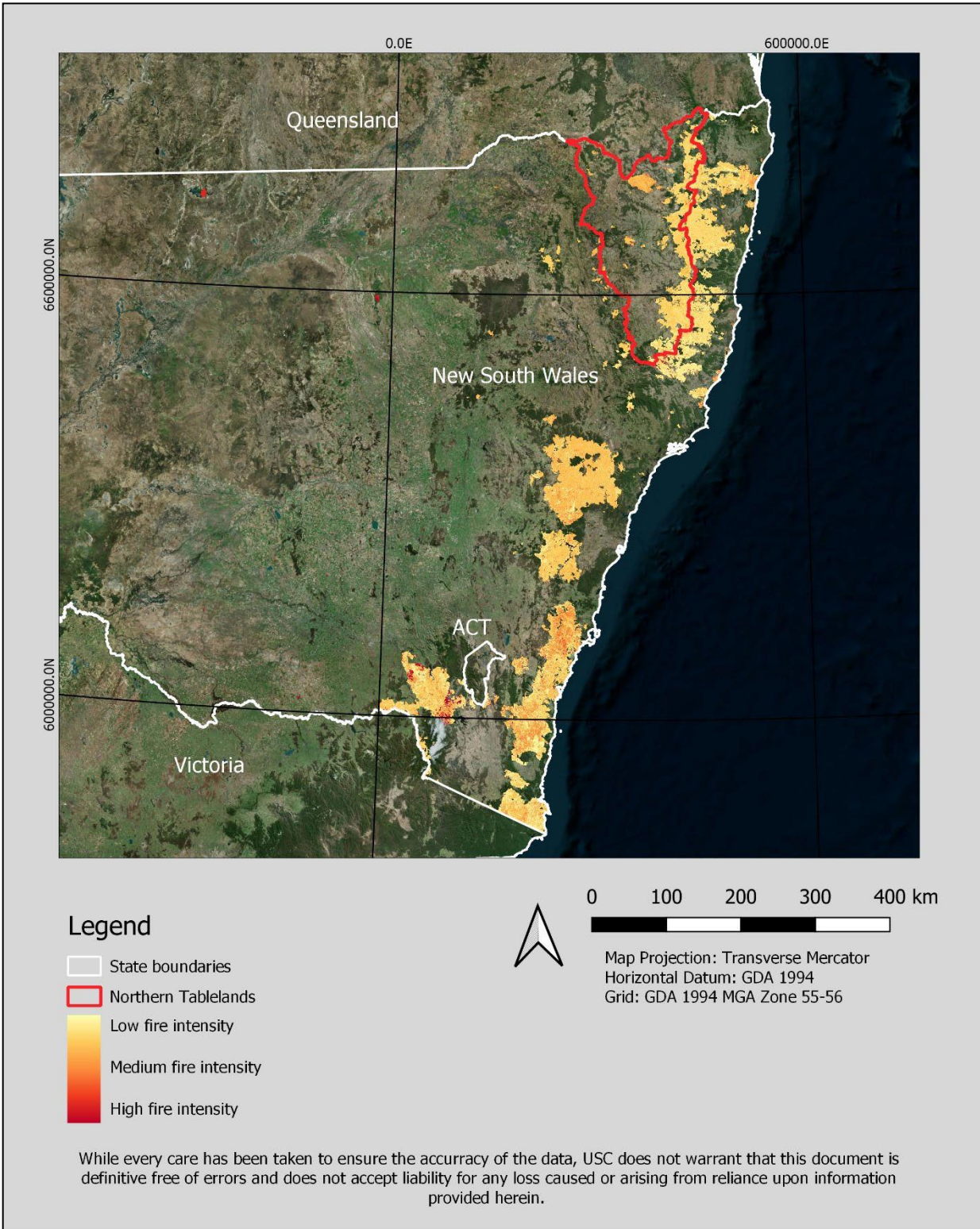


Figure 1: Fire extent during the 2019-2020 season in NSW

### 1.3 Previous Work

The DDC was engaged by the NT LLS to deliver the Northern Tablelands Koala Habitat Project in 2016 (in parallel with Stringybark Ecological), which was supplemented with further fieldwork in 2018. This research delivered a comprehensive analysis of the presence of koalas in the main priority areas as defined in the *Northern Tablelands Koala Recovery Strategy* (NT KRS). In 2016 and 2018, DDC conducted 388 surveys across identified priority areas in the Northern Tablelands. Altogether, the NT LLS *Cool Country Koala Project* surveys found relatively high areas of koala activity in some regions (e.g. Inverell / Delungra and Armidale / Uralla), while finding very low koala activity levels in other areas. Some of these areas showed high historical levels of koala activity (e.g. Ashford), suggesting large declines in these regions.

As a whole, the *Cool Country Koala Project* contributed to the objectives of the NT KRS to gain a baseline knowledge of koala distribution and abundance, promote recovery, avert any ongoing decline, and minimise the risk of extinction of koalas within the Northern Tablelands region in NSW while building community engagement.

## 2. Objectives of the *Cool Country Koala Project 2019 - 2020: Northern Tablelands Koala Habitat and Pilot Genetic Project*

The Northern Tablelands *Cool Country Koala Project 2019 – 2020: Koala Habitat and Pilot Genetic Project* contributes to the objectives of the NT KRS, which are to:

- Consolidate and improve baseline knowledge of koala distribution and abundance, threatening processes and impacts upon koala populations on the Northern Tablelands; and
- Develop a recovery strategy in collaboration with stakeholders that prioritises actions for koala protection and areas for effective investment on the Northern Tablelands.

This study contributes to these objectives by addressing the following aims:

- To address data deficiencies through systematic, field-based surveys – in this study the DDC focussed on areas not covered in previous projects;
- To inform future investment in koala habitat restoration and revegetation; and
- To undertake a pilot genetic monitoring project of two koala populations identified through previous surveys.



This report;

1. describes the outcomes of the 2019 field-based surveys in Travelling Stock Routes (TSRs) across the Northern Tablelands,
2. provides the results from the pilot genetic monitoring study undertaken in Armidale / Uralla and Inverell / Delungra.

### 3. Methodology

#### 3.1 Priority Survey Areas / Survey Site Locations

In 2016, priority areas for surveys were determined by a panel of experts gathered by NT LLS, the determination of these areas is defined in the NT KRS (layer provided by NT LLS, Figure 2). Many of the priority areas have been previously surveyed as part of the *Cool Country Koala Project* surveys between 2016 and 2018. For the scope of this study however, DDC surveyed mostly outside of identified priority areas. This was to expand knowledge of koala presence beyond known populations identified in previous projects. Survey sites were randomly generated by NT LLS in and around these priority areas using ArcGIS. Survey sites were constrained to fall in TSR lands that were further constrained to be at least 2 km away from areas that had been baited with 1080 dog baits within the past 12 months. These parameters were decided in collaboration with NT LLS. Ongoing baiting programmes resulted in necessarily expanding the survey areas beyond those in the original project proposal (*Northern Tablelands Koala Habitat and Pilot Genetic Project 2019 – 2020 Proposal*).



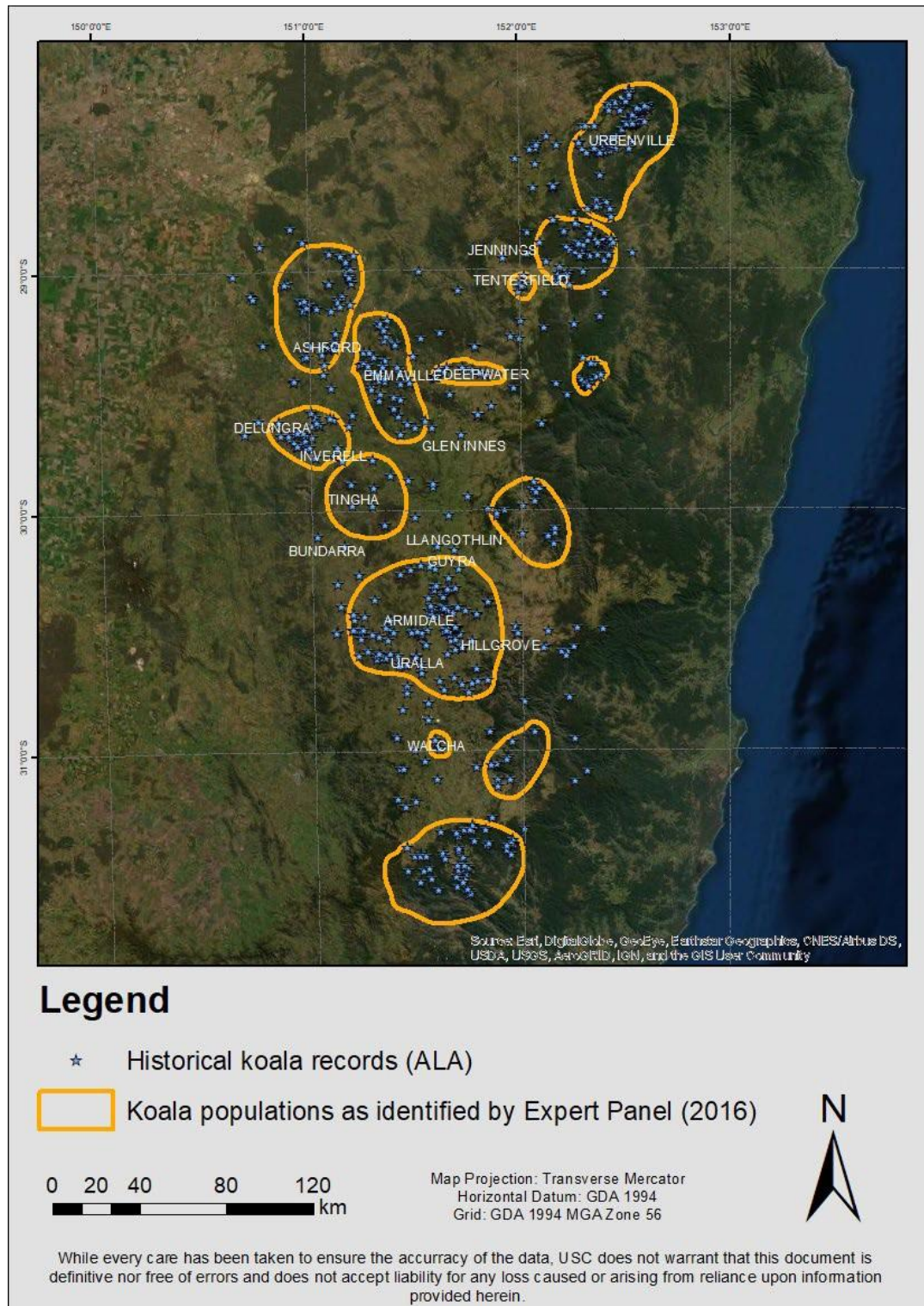


Figure 2: Priority areas of the Northern Tablelands, taken from the Northern Tablelands Koala Recovery Strategy

## 3.2 Field Methods

### 3.2.1 Koala Presence / Absence Surveys

#### 3.2.1.1 Dog Handling and Data Recording

Direct observation of animals is time-consuming, particularly for koalas, which have a cryptic nature, occur in low densities, and have large home ranges (Tyre et al. 2001, Kéry 2002). Locating secondary evidence of animals (fur, scats, feathers, tracks, etc.) is a common technique for establishing species occurrence across large areas because secondary evidence indicates species presence and use of the habitat even when the animal is not on site (Putman 1984, Wilson and Delahay 2001). The best method to confirm koala presence and habitat utilisation is to use detection dogs specifically trained on the odour of koala scats. DDC has proven that the detection dog method surpasses human-only teams in both accuracy and efficiency (Cristescu et al. 2015, Cristescu et al. 2019).

Detection dogs ‘Baxter’ and ‘Maya’, both Border Collie, were used for the koala scat presence / absence surveys (Figure 3). For each survey, the dog was then fitted with a GPS collar, motivated with a tennis ball and given the command to search. Two dog handlers conducted the surveys, Dr Anthony Schultz (AS) and Kirra McDougall (KM), both research assistants with the University of the Sunshine Coast.



Figure 3: Detection dogs Maya (L) resting in between koala scat surveys and Baxter (R) fully equipped for survey work



Upon arrival at the survey sites and prior to the dog deployment, site information was recorded including location name and unique survey identifying number, and site photos captured. Any ecological characteristics that might have influenced the detectability and decay of scats were recorded (e.g. wet areas will increase decay rates; therefore, scats will be detectable for a shorter amount of time (Cristescu et al. 2012a). Evidence of crown dieback (browning leaves in the crown of the tree), nearby standing water, and wet ground was photographed and recorded at each site. Two survey types were used for presence/absence surveys, systematic and casual, as described below.

### *3.2.1.2 Survey Type 1: Systematic koala scat survey*

The survey protocol followed the commonly used koala survey method named Spot Assessment Technique or SAT (Phillips and Callaghan 2011), which was adapted for use with a detection dog as per Cristescu et al. (2015). At each survey site, 30 trees with a diameter at breast height of more than 10 cm were searched for the presence of koala scats using the detection dog. The trees were centred around the random survey point.

Typical koala scats (Figure 4) have the following characteristics (Triggs 1996):

- symmetrical and bullet-shaped (not jelly-bean shaped);
- generally about 1.5 cm long by 0.5 cm wide (adult koala scat size);
- even-sized and especially fine particles;
- absence of insect parts (koalas do not eat insects); and
- very compact.



Figure 4: Koala scats

When scats were found, the number of scats within a one square meter quadrat, their age category (Table 1) and their size (based on scat width, Figure 5) were recorded as well as their GPS coordinates (GDA94). When only one size of scat and age class (see Table 1 below) is present, the tree is considered less used than when scats of different age classes (indicative of



repetitive visits) and sizes (indicative of different individuals) are present. Age of scats allowed DDC to classify sites as recently used or not.

Table 1: Koala scat age categories

Scat age categories	Characteristics
1	Very fresh (covered in mucus, wet)
2	Fresh (shine and smell)
3	Medium fresh (shine or smelly when broken)
4	Old (no shine, no smell)
5	Very old and discoloured



Figure 5: Koala scats of different sizes (widths)

If the systematic koala scat survey was positive (i.e. koala scats were detected at the site), the team proceeded to the next random survey site scheduled. If the systematic koala scat survey was negative, a casual survey was conducted. Tree species was not identified during this project.

### 3.2.1.3 Survey Type 2: Casual koala scat survey

The casual surveys, in contrast to systematic surveys, cannot be compared in space and time, nor do they give insight into utilisation rates of the habitat. The casual surveys are however an excellent and fast way to determine whether koalas are present at a specific site. This method is designed to maximise the chance of detecting koala presence in the minimum amount of time. In this project, casual surveys were used to decrease the risk of false negative after one negative systematic survey.



In the casual surveys, the dog is not constrained by the handler to check 30 trees, and can follow its nose roaming over an area of up to two hectares within an approximate 30-minute timeframe, or until the handler deems the search to have covered the site thoroughly. The search duration is usually less than 30 minutes and can be as short as a couple of minutes if koala scats are detected. The start point of the survey can be determined by the handler, on site, and rely on the handler's assessment of the potential for the area to be used by koalas (this increases the chances of finding koala scats but also increases bias); or can be random.

The same scat details (age and size of scats) recorded for systematic surveys were recorded for casual surveys. Only the specific trees with koala scats underneath were recorded (GPS).

#### *3.2.1.4 Survey Type 3: Incidental koala / koala scat surveys*

Researchers conducting the surveys are always on the lookout for incidental, or opportunistic, spotting of koalas and koala scats. This can happen while on foot or in the car, moving between survey sites; or thanks to information passed on to DDC researchers from members of the public, property owners or passers-by. The public is always considered as a source of knowledge and individuals are questioned on koala presence, past and present, whenever possible. When koala scats were located during incidental surveys, the same information is recorded as for other survey types.

### *3.2.2 Genetic Surveys*

Genetic surveys were conducted using a third detection dog (Billie-Jean), a dog specifically trained to find fresh (age category 1-3) koala scats. Areas of known koala presence were targeted to maximise sampling success. Dog handling followed the same procedures as described for "Presence/Absence surveys" (see section 3.2.1.1 Dog Handling and Data Recording). We used "casual" and "incidental" survey types for genetic surveys (see section 3.2.1.3 and 3.2.1.4 respectively). Fresh scats were collected in a sterile tube without direct skin contact to avoid potential contamination and loss of koala DNA from the scat. Tubes were kept on ice until they were stored in a -20° C freezer. DNA from the scats was genotyped using next-generation sequencing protocol described in Schultz et al. (2018) and detailed below.

## *3.3 DNA Isolation and SNP Genotyping*

### *3.3.1 DNA Isolation*

Note that genetic terms are written **in bold and carry a \*** when used for the first time. These terms and their definition can be found in the glossary.



Koala DNA was isolated from the intestinal epithelial cells present on koala scats. Epithelial cells from the surface of each scat were separated by slicing off the top-most layer of the scat using a scalpel. These surface slices were then used to extract koala DNA using the QIAamp PowerFecal Pro DNA Kit (Qiagen), following the manufacturer's protocol, with the following variations: after adding CD1 buffer, samples were incubated at 65° C for 10 minutes, and then vortexed for seven minutes at maximum speed using Genie 2 Vortex Mixer (Scientific Industries). Final DNA isolates were eluted in 100µl of C6 elution buffer. Each isolate was tested for quality DNA isolation on a 1.5% agarose gel. Isolates that passed this quality control were then stored at -80° C.

### 3.3.2 Single nucleotide polymorphism (SNP) genotyping

Please note that the genotyping method below is given for completeness but is not essential to the understanding of the genetic results. The reader might choose to skip the genotyping section altogether.

Genotyping of **single nucleotide polymorphism (SNP)\*** genetic markers was conducted by Diversity Arrays Technology, Canberra, using proprietary DArTseq™ technology. DArTseq™ represents a combination of DArT complexity reduction methods and next-generation sequencing platforms (Kilian et al. 2012, Courtois et al. 2013, Von Mark et al. 2013, Raman et al. 2014). Specifically, for this study, SNP genotyping was conducted using a DArTcap approach, which is a targeted application of DArTseq™ technology allowing for the sequencing of targeted markers. DArTcap is used in similar applications as DArTseq, but it applies a selective step after complexity reduction to genotype specific markers from DArTseq representations. This selection is achieved with the use of the nucleic acid “capture probes” that bind to restriction fragments in the representations carrying the specific DArTseq markers. Capture probes were designed by Diversity Arrays using DNA extracted from 189 tissue samples of koalas. Capture probes included sex markers.

DNA samples were processed in digestion/ ligation reactions (Kilian et al. 2012), ligating two adaptors corresponding to the combination of restriction enzymes overhangs. For DNA extracted from koala scat, the combination of PstI and SphI restriction enzymes performed better in polymorphism detection efficiency. The PstI-compatible adapter includes the barcode. The reverse adapter contained the SphI-compatible overhang sequence. The PstI-SphI fragments were amplified by adapter-mediated **PCR\*** as follows: initial denaturation of 94°C for 1 min, followed by 30 cycles of denaturation (94°C for 20 s), annealing (58°C for 30 s), and extension (72°C for 45 s), with final extension phase of 72°C for seven min. The PCR primers were designed to add the required sequences for enabling sequencing in a single-read Illumina flowcell. Equimolar amounts of amplification products from each sample were bulked and applied to c-Bot (Illumina) bridge PCR followed by 77 cycles of single-read sequencing on Illumina HiSeq2500 (Illumina).



The resulting sequences generated were processed using proprietary DArT analytical pipelines. The primary pipeline filtered out poor quality sequences, while applying more stringent selection criteria to the barcode region. In this way, assignment of sequences to specific samples was very reliable. Identical sequences were then collapsed into “fastqcol” files for use in secondary pipeline analysis, using DArT pipeline's proprietary SNP and SilicoDArT (presence / absence of restriction fragments in representation) calling algorithms (DArTsoft14). For SNP calling, all tags from all libraries included in the DArTsoft14 analysis are clustered using DArT PL's C++ algorithm at the threshold distance of three, followed by parsing of the clusters into separate SNP loci using a range of technical parameters, especially the balance of read counts for the allelic pairs. Additional selection criteria were added to the algorithm based on analysis of approximately 1,000 controlled cross populations. Testing for Mendelian distribution of alleles in these populations facilitated selection of technical parameters discriminating well true allelic variants from paralogous sequences. In addition, multiple samples were processed from DNA to allelic calls as technical replicates, and scoring consistency was used as the main selection criteria for high quality / low error rate markers. Calling quality was assured by high average read depth per locus. This process is similar to that used in published literature using DArTseq™ SNPs from animal genetic samples (e.g. Donnellan et al. (2015)).

### 3.3.3 Genetic Analysis

The genotyped samples were checked for duplication, which is the case when the same individual koala is sampled two or more times. This can happen when relying on scat samples. These duplicates must be removed, or they would otherwise bias the results of the genetic analysis. Samples were furthermore checked for quality and were removed if standards were not met. We filtered our set of SNPs with the following criteria: loci with reproducibility of <90%, **call rate\*** of <70% and minor allele frequency (**maf\***) of <1% were removed from the dataset, as well as individuals with more than 30% missing data. Filtering was done in R studio using the R package dartR (Gruber et al. 2018).

The **structure\*** of the population was assessed through the program fastSTRUCTURE (Raj et al. 2014) as well as using a Principal Coordinates Analysis (PCoA). Two measures of population differentiation were calculated: **F<sub>ST</sub>\*** which estimates the amount of structuring of a population into subpopulations and **F'<sub>ST</sub>\***, the standardised Fst that enables comparisons of our results to other studies. F<sub>ST</sub> and F'<sub>ST</sub> can range from 0 to 1 (where 0 means complete sharing of genetic material and 1 means no sharing), however, these estimates depend on the natural history of the species and the sampling scale and are best interpreted in comparison with estimates of other studies on the same species. From F<sub>ST</sub>, we can make some inferences about **gene flow\***. Gene flow refers to the 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. The number of migrants **Nm\***

can be estimated through  $N_m = (1 / F_{ST} - 1) / 4$ . Results of this calculation have to be treated carefully since these numbers reflect historical rates of gene flow and may not represent current gene flow.

Genetic diversity, both in the Armidale / Uralla and Inverell / Delungra koala populations, was measured as **expected heterozygosity ( $H_E$ )\***. Population-level **inbreeding\*** was measured using the **inbreeding coefficient ( $F_{IS}$ )\***, calculated as:  $F_{IS} = [\text{expected heterozygosity } (H_E) - \text{observed heterozygosity } (H_o)] / \text{expected heterozygosity } (H_E)$ . This measure,  $F_{IS}$ , is the proportion of the variance in the sampled population contained in an individual and can range from -1 to 1 (the closest to 1, the higher the degree of inbreeding). Note that inbreeding can not only result from mating between closely related individuals, but also from small isolated populations, where all individuals are more closely related to each other than in a large population. We furthermore calculated the average **internal relatedness ( $IR$ )\*** across individuals, a measure that reflects the parental relatedness (Amos et al. 2001).

Genetic diversity analyses were conducted using the statistical software R v3.2.5 and GenAlEx 6.5 (Peakall and Smouse 2012). Internal relatedness was calculated using GENHET (Coulon 2010) in R.

### 3.4 Health and Safety

A Job Safety Analysis was completed. Detection dogs, Baxter, Maya and Billie have been professionally trained so as not to pose a threat to wildlife. The wellbeing of the detection dogs was assessed by Animal Ethics (USC: ANA18128 and ANS1752, valid until December 2020). Baxter, Maya and Billie were regularly treated against ticks and thoroughly checked for bites after each survey. All DDC detection dogs are insured in the event of a snake bite. Surveys were conducted only after it was confirmed that no known wild dog baiting occurred in the areas to be surveyed, additionally, the dogs wore a muzzle. Baxter, Maya and Billie were thoroughly brushed before entering the area so that no weed propagules were introduced. The handler was always in view of the dog and controlled the movements of the dog by voice, which means the risk of the dog escaping and getting lost or injured was remote. Surveys were conducted under valid wildlife and scientific research permits (OEH scientific license number SL101741 under Part 2 of the Biodiversity Conservation Act 2016, valid until January 2020).

### 3.5 Data Analysis

All data collected in the field was entered into the DDC database. The habitat utilisation was described in terms of activity level (Phillips and Callaghan 2011), which was calculated by dividing the number of trees with scats by the total number of trees searched at the site ( $N = 30$ ) for each site where systematic surveys were performed. Possible environmental drivers of koala presence were tested using a linear modelling approach with a binomial error structure.



Koala scat presence at a survey site was the dependent variable, and standing water (including wet ground) at site and crown dieback at site were the fixed independent variables. Linear modelling was conducted in R Statistical Environment v3.4.3.

Historical sightings from the Atlas of Living Australia database were plotted on maps of the Northern Tablelands and compared with the locations of positive scat searches during the study. This enabled us to examine changes in koala distribution on the Northern Tablelands: i.e. we compared our study with historical koala sightings to identify sites where koalas previously occurred and are no longer present, as well as sites where koalas have not been recorded in the past but where scats were found in the study. All results were mapped in ArcGIS v10.2 and QGIS 3.12.0.

### 3.6 Limitations

#### 3.6.1 Presence/Absence data

The sites were surveyed on only one occasion; therefore, the presence / absence results presented here provide a snapshot of the population during this period and it should be noted that evidence of koalas found within the study areas is likely to change seasonally [as koala movements vary with time (Ellis et al. 2009)].

The rate at which scats decay may also vary significantly between sites due to varying ground layer structure, composition, moisture, sunlight, local weather events and invertebrate activity (Rhodes et al. 2011, Cristescu et al. 2012b). Decomposed scats may lose their unique scent mark and the dog may no longer detect it – however this has not yet been proven to occur (Cristescu et al. 2015).

Failure to detect koala scats in an area is not necessarily conclusive of koala absence. Failure to detect koala scats may suggest either of the following:

- Koalas are not present in the area (i.e. true absence) at the time of the survey. Note that true current absence does not infer that the site has not been used in the past, or could not be used in the future, i.e. it could still be potential koala habitat.
- Koalas occur in the area, however scats were not detected (false negative) because:
  - Scats were present at some stage but decayed and disappeared from the environment before the survey was conducted;
  - The dog did not detect the scat; and/or,
  - The dog indicated the presence of a scat, but it was too decayed to be confirmed.

“The presence of absence does not equal the absence of presence” – to infer true absence, multiple surveys through time are generally necessary (MacKenzie and Royle 2005), from this survey, only presence can be confidently ascertained. However, to increase certainty in the





negative results i.e. to decrease false negatives, the DDC performed a casual search at sites where the systematic search was negative.

### 3.6.2 Genetic study

Genotyping was conducted non-invasively from genetic material contained in the surface of koala scats. This allows for large scale, relatively cheap, unbiased sampling of DNA compared to other available methods (catching koalas, anaesthetising them and collecting blood / biopsies, or relying on wildlife hospital samples). However, compared to high quality blood / biopsy samples, DNA present in scat is degraded which yields lower numbers of high-quality SNPs. However, the DDC was able to optimise scat genotyping for koalas by using the DArTcap method.

The severe drought that lasted while genetic samples were collected potentially further decreased the quality of DNA contained on the scat surface. Even fresh scats were often very dry, potentially due to the lack of water available to the koalas but also the low moisture in the air.

It is important to note that comparisons of genetic diversity cannot be made across studies unless the set of genetic markers used are identical and have been cross referenced when produced by different laboratories.

## 4. Results

### 4.1 Field Surveys

Field surveys to assess koala scat presence / absence occurred between the 2<sup>nd</sup> and 14<sup>th</sup> of July 2019. A total of 127 koala scat presence / absence surveys were performed, including 68 systematic, 58 casual and 1 incidental survey. Evidence of likely increased scat decay was found at 10 sites, and included three sites on steep slopes, three sites along a creek line or road drainage line where scats may be washed away, one site where earth moving equipment and burning were present, and one site where livestock could have trampled scats.

Out of the total 127 koala scat presence / absence surveys, nine were positive for koala scats (7%, Figure 6). Sites that were positive for koala presence were determined through systematic surveys for 22%, 66% through casual surveys, and 11% through incidental surveys. No koalas were sighted during or outside of presence / absence surveys.

Field surveys to collect fresh scats for genetic sampling occurred between the 16<sup>th</sup> and 24<sup>th</sup> of September 2019. A total of 47 casual surveys were conducted, with fresh koala scats found in 44 surveys (94%). Across these survey sites, a total of 115 fresh scat samples were collected. In addition, 24 adult koalas were sighted, of which five were females with joeys, bringing the





total number of koalas sighted to 29. Only two of these sightings occurred within the Armidale / Uralla area, whereas the remaining 27 koalas were sighted in the Inverell / Delungra area.

Figure 7 shows an overview of all sites that were surveyed during this genetic pilot study as well as koala sightings.

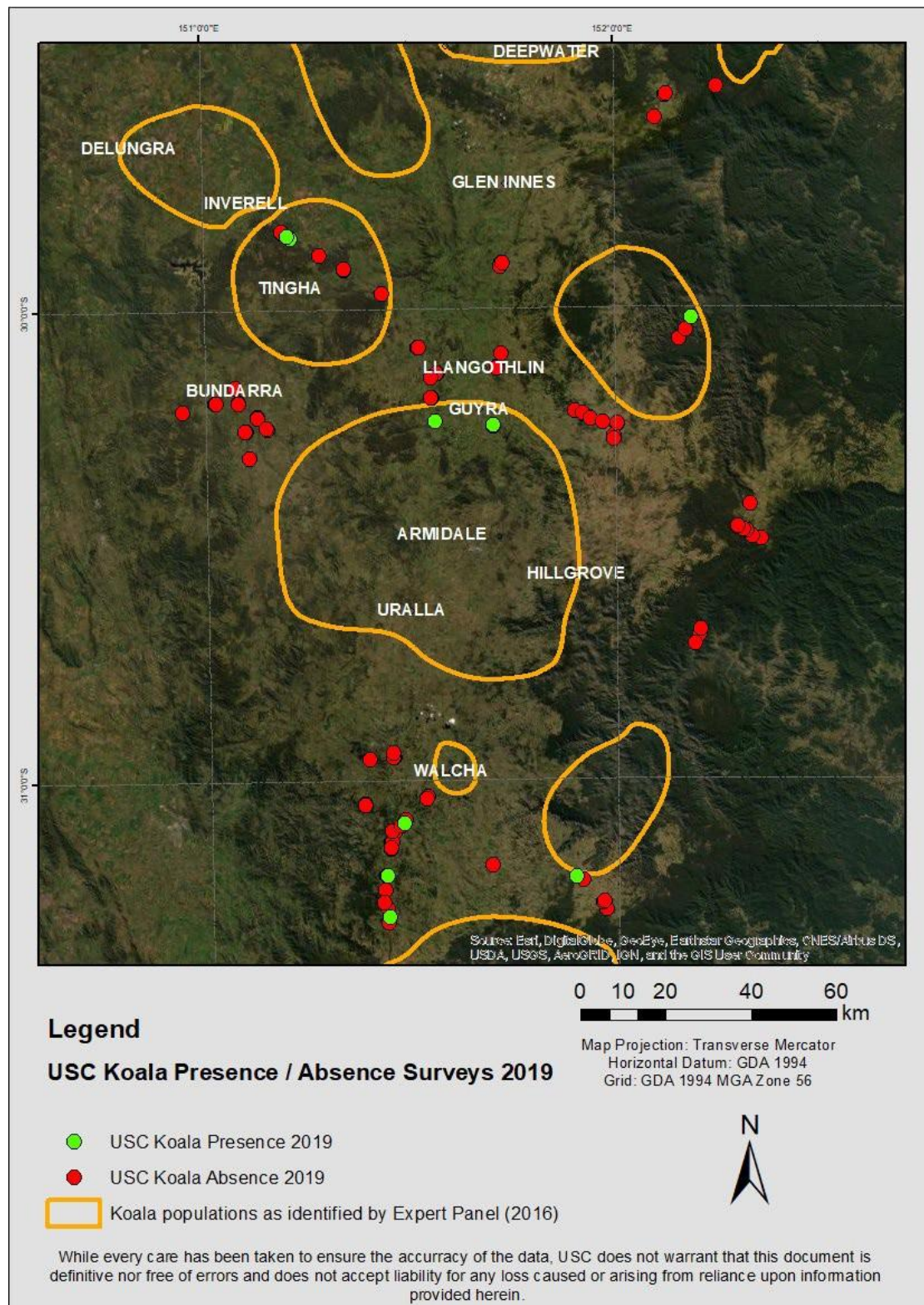


Figure 6: Koala scat presence / absence survey conducted in 2019



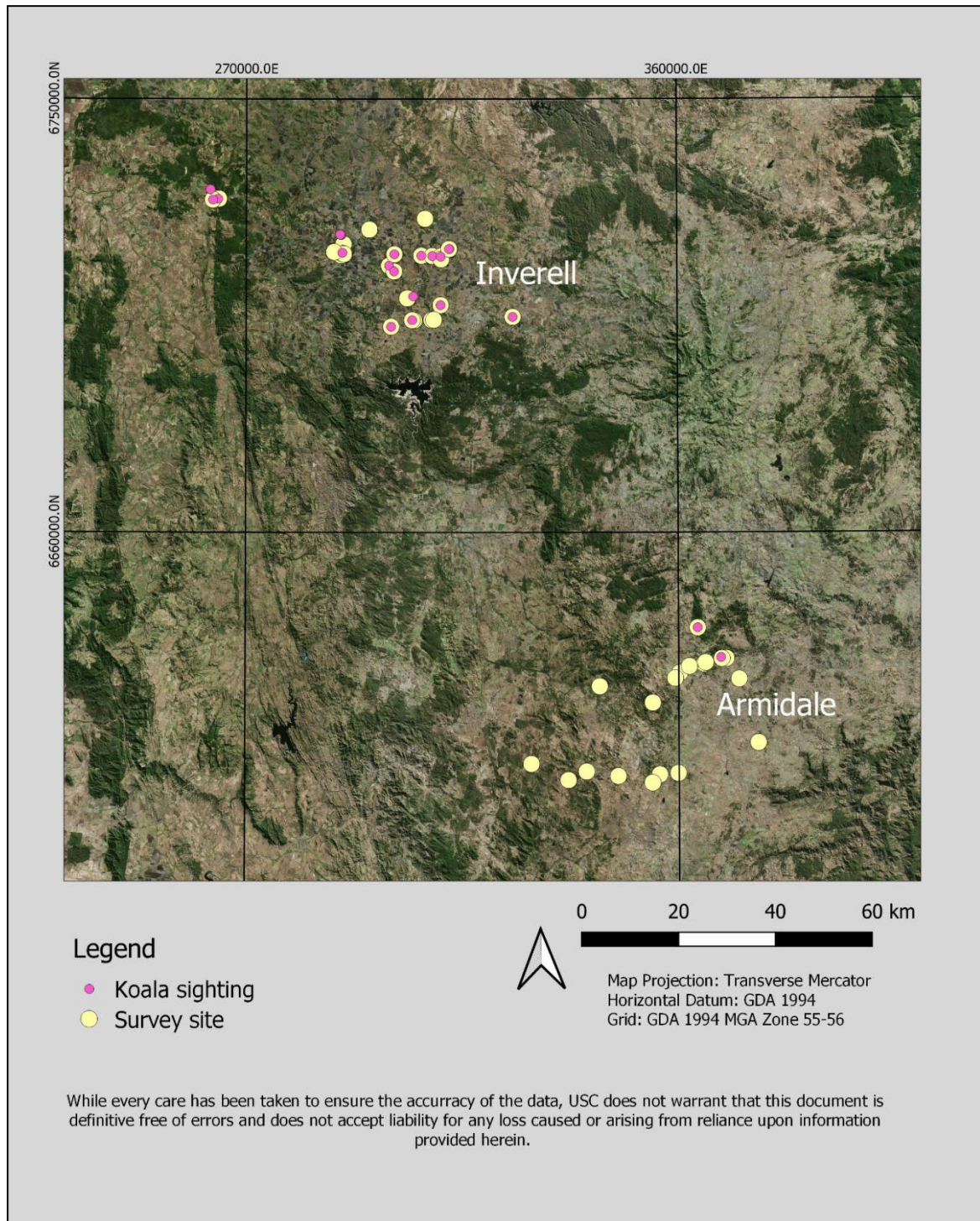


Figure 7: Genetic survey sites and koala sightings during the genetic surveys. A total of 47 sites were surveyed. A total of 29 koalas were sighted of which five were joeys



#### 4.2 Presence / Absence of Koalas and Comparison to Historical Records

The survey sites where DDC found evidence of koala presence typically aligned with, or were near to, sites where koalas have historically (ALA records) been seen (see Figure 8) This is particularly noticeable for the two positive sites near Guyra, the sites south of Walcha, and the sites near Tingha. It is interesting to note that the negative sites east of Hillgrove were close to historical koala territory, as were those near Bundarra, Llangothlin and north-east of Glen Innes. This suggests that koalas are not currently distributed as widely as historical data suggests, and that historical populations may have subsequently been extirpated.



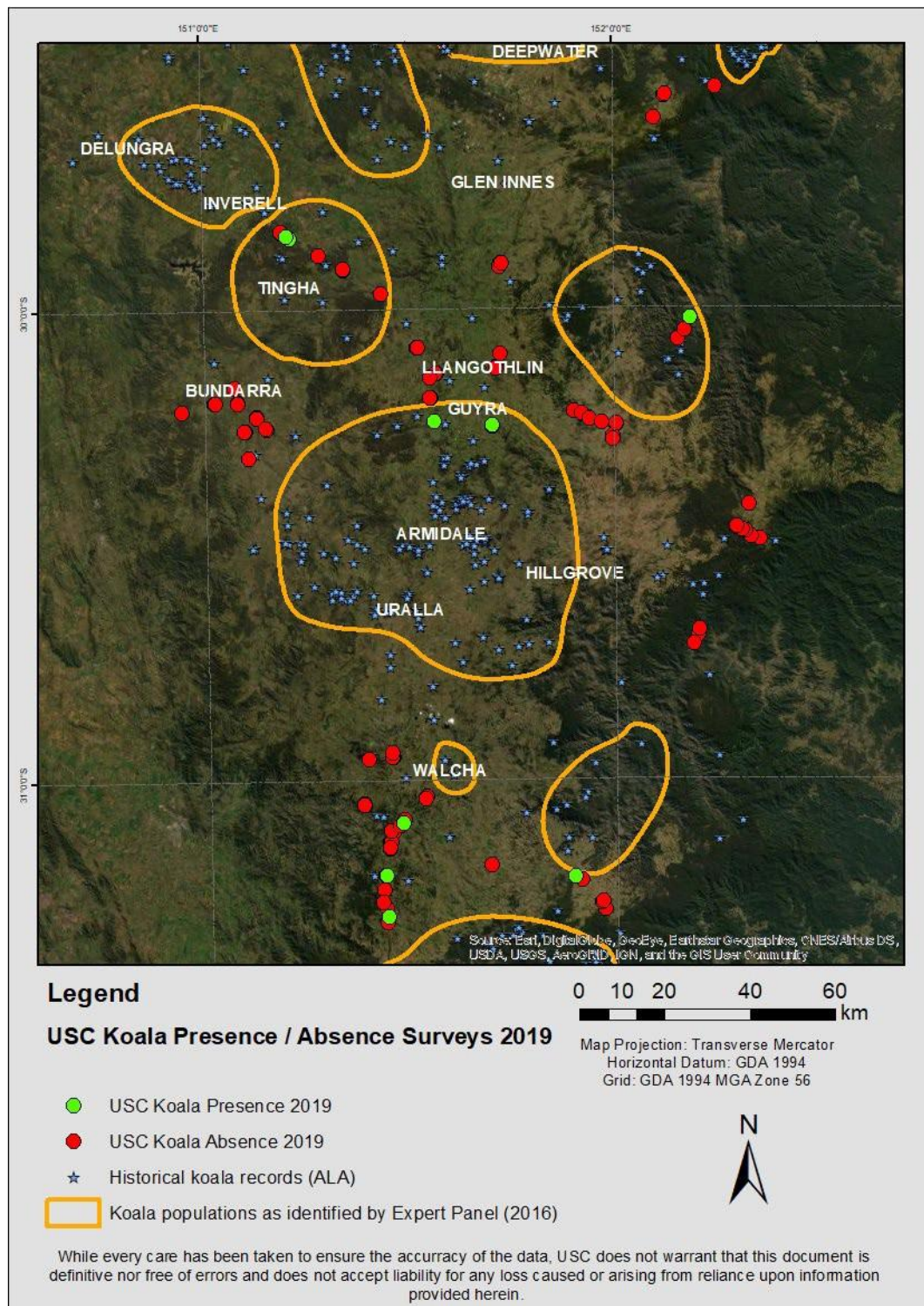


Figure 8: Koala scat presence / absence surveys and historical koala records from the Atlas of Living Australia

### 4.3 Activity Levels

We found very low activity levels across all systematic surveys conducted during the 2019 presence/absence fieldwork. The average activity level at positive systematic survey sites was 3% (SD = 0). Systematic survey sites never had more than one tree with koalas scats present, hence the standard deviation value of 0.

Based on these results, the areas surveyed during this project would be classified as low habitat use (see Table 2, extracted from Phillips and Callaghan (2011)).

Table 2: Activity Levels extracted from Phillips and Callaghan (2011) showing Activity Levels for positive sites in different surveys (East coast includes: Campbelltown, Port Stephens and Noosa; Western plains comprises of Pilliga and Walgett)

Activity category	Low use	Medium (normal) use	High use
Area (density)			
East Coast (low)	-	$\geq 3.33\%$ but $\leq 12.59\%$	$> 12.59\%$
East Coast (med – high)	$< 22.52\%$	$\geq 22.52\%$ but $\leq 32.84\%$	$> 32.84\%$
Western Plains (med – high)	$< 35.84\%$	$\geq 35.84\%$ but $\leq 46.72\%$	$> 46.72\%$

### 4.4 Scat Age, Size and Density

Scats found during the presence / absence surveys ranged from scat age two (within  $\pm 14$  days) to scat age five (oldest age category which could be years; see Table 1 for age categories). Only two of the nine sites with scats (20%) had scats of more than one age present together. This suggests that only 20% of sites where scats were found exhibit ongoing use by koalas over time.

There were no survey sites where koala scats of different sizes were found. This implies that all the scats found within one site could be produced by one individual, and there were no examples of multiple scat sizes found within a site (which might indicate a mother and offspring present together). However, the small sample size (nine positive sites) makes it difficult to draw any reliable conclusions regarding koala size / age structure.

Density of scats was calculated for positive, systematic surveys and ranged from 2 to 3 scats / m<sup>2</sup>, with an average of 2.5 scats / m<sup>2</sup> (SD = 0.7). The average scat density was less than half of previous (2016 and 2018) Northern Tablelands surveys (Table 3).



Table 3: Scat density in this project compared to previous Northern Tablelands surveys in 2016 and 2018.

	NT 2019	NT 2018	NT 2016
<b>Max</b>	3	61	58
<b>Average</b>	2.5	5.1	6.7
<b>SD</b>	0.7	3.6	8.9



#### 4.5 Crown dieback and standing water

Following consultation with NT LLS, the DDC recorded the occurrence of crown dieback (i.e. loss of leaves or browning leaves) of trees within the presence / absence survey sites. The DDC also recorded whether there was standing water within, or in close proximity, to the survey sites. Damp or boggy ground was included as standing water, and was differentiated from sites where the surface soil was merely damp from recent rain.

Regarding tree crown-dieback, the DDC found that 31 sites (24%) showed some evidence of dieback (e.g. brown leaves, leafless branches), with only one of these sites testing positive for koala scats. Standing water was found at 40 sites (31%), with two of these sites providing koala scats. Damp or wet ground was found at 31 sites (24%), with koala scats found at three of these sites.

Of the survey sites where evidence of koalas was found, 89% showed no evidence of crown dieback, while 22% showed evidence of standing water (see Figure 9, Table 4).

The results of the linear model investigating whether crown dieback or standing water may influence koala presence / absence showed that neither standing water (or wet ground) (GLM, estimate = -0.4105, se = 0.7143, p = 0.566) nor crown dieback (GLM, estimate = -1.1413, se = 1.1064, p = 0.302) were statistically significant predictors of koala presence or absence. These results suggest that crown dieback and standing water, as measured in the koala presence / absence surveys, do not predict which sites are used by koalas.

Table 4: Presence of crown dieback and standing water at survey sites which were positive / negative for koala scat

Koala evidence	Crown dieback	No crown dieback	Standing water	No standing water
<b>Positive</b>	1 (11%)	8 (89%)	2 (22%)	7 (78%)
<b>Negative</b>	30 (25 %)	88 (75%)	38 (32%)	80 (68%)

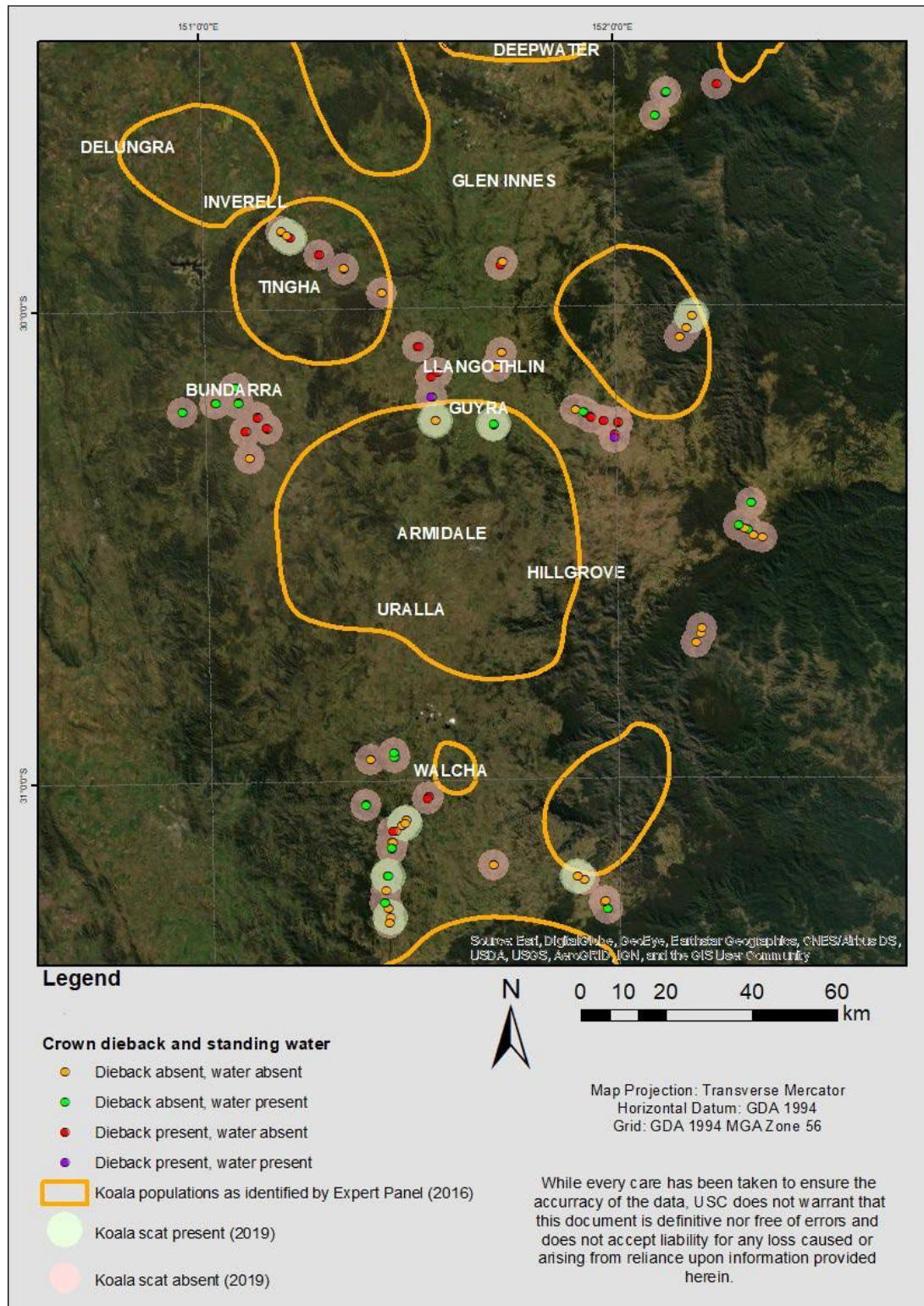


Figure 9: Crown dieback and standing water presence at presence/absence koala scat survey sites



## 4.6 Genetic survey results

### 4.6.1 Overview

Across the 47 survey sites, a total of 115 fresh scat samples were collected of which a selection of 96 were extracted for DNA (funding dependant). Figure 10 shows a map of all genetic samples that were collected. Six of the isolates did not contain enough DNA and were not processed to be genotyped. The remaining 90 samples were successfully genotyped at 1399 SNP loci. After identifying 14 duplicate samples and filtering for SNP quality, a total of 76 individual koalas used in the subsequent analyses. Of these 76 koalas, 40 individuals were from Inverell / Delungra and 36 from the Armidale / Uralla region. Figure 11 shows the identified individual koalas.

Of the 76 identified individual koalas, 30 were identified genetically as females and 46 were males. Figure 12 presents a map that shows the distribution of sexes in each population. This resulted in a **sex ratio\*** of 1:1.53 (female to male ratio) which is biased towards males. It is likely that the small sampling size may have skewed these ratios.

Note that the DDC obtained some preliminary disease status data that is provided in Appendix 1.



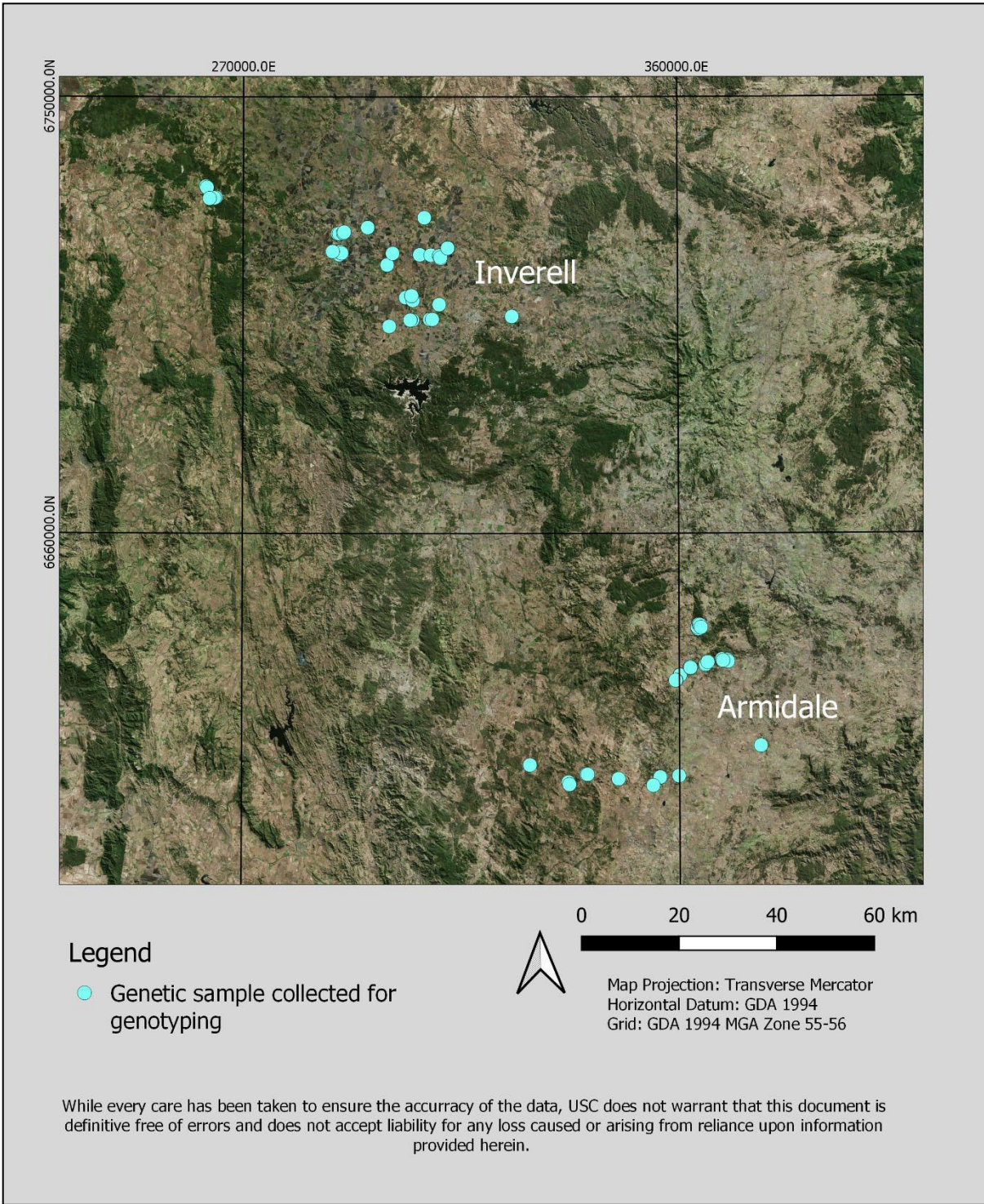


Figure 10: Map of the sampling locations of fresh koala scats



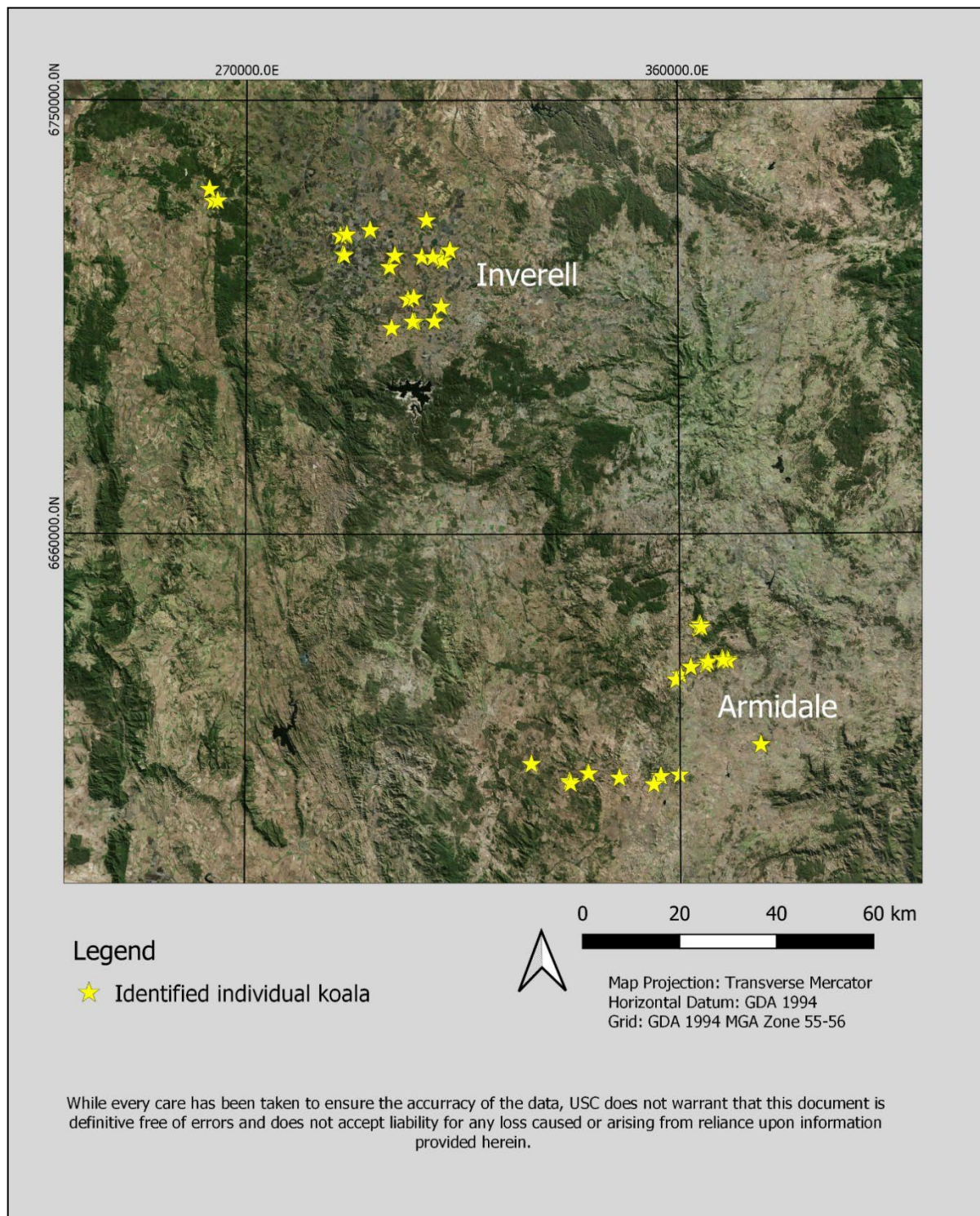


Figure 11: Map of individual koalas (N=76) that were identified through the analysis of genetic samples as part of the genetic pilot study in Northern Tablelands



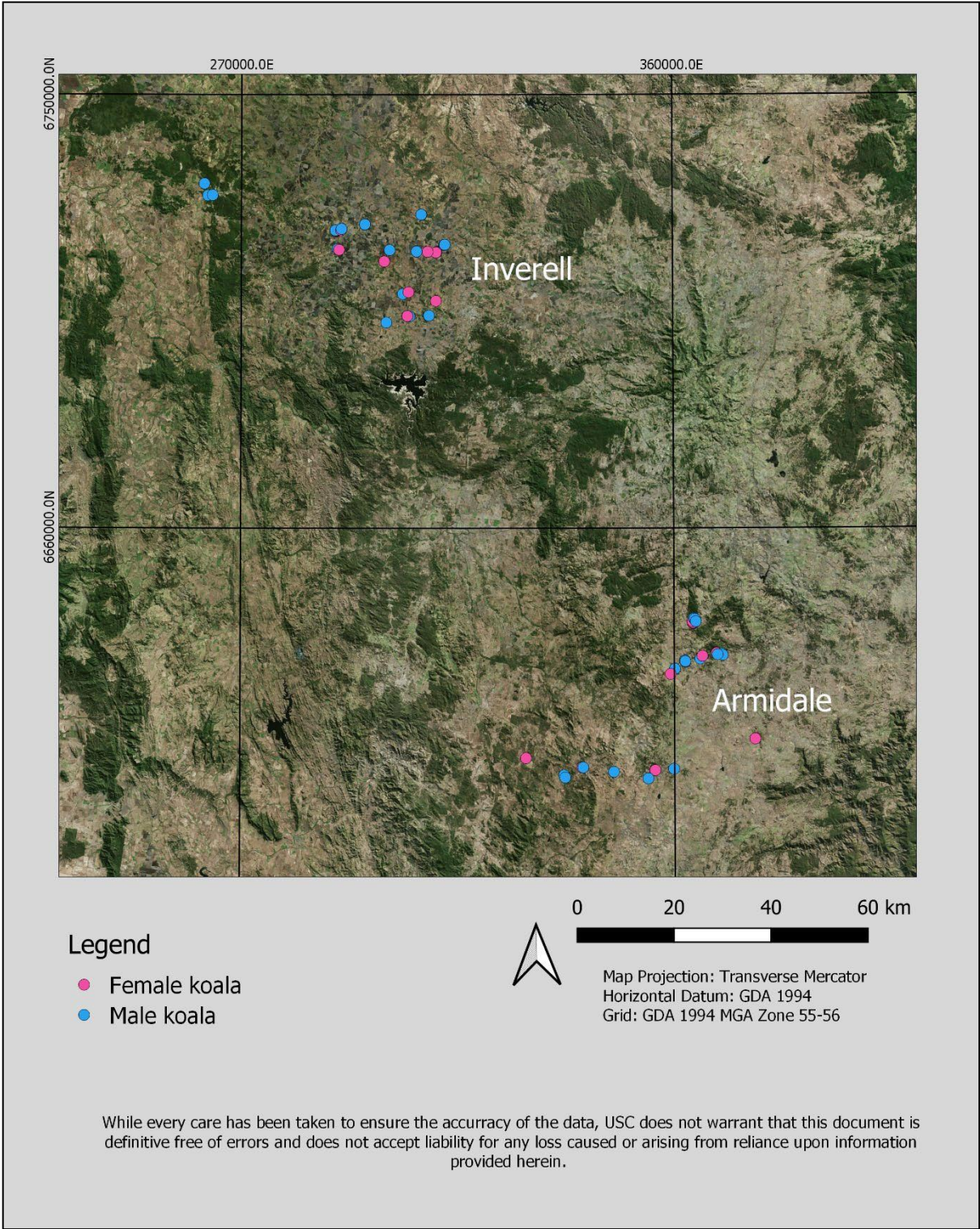


Figure 12: Distribution of male and female koalas in the Northern Tablelands (the sex of individual koalas was determined through genetic analyses)

#### 4.6.2 Population genetics

Individuals of both sampling locations (Armidale / Uralla and Inverell / Delungra regions) were analysed together to assess population structure across the whole landscape. The fastSTRUCTURE analysis as well as the PCoA indicated that there are two populations. Figure 13 shows a graph of the most likely number of populations where the line peaks. Figure 14 shows the results of the PCoA performed across all samples. Individuals are sorted along the axes depending on their genetic similarity. The graph shows two distinct clusters of individuals. One individual in Armidale / Uralla was more similar to the koalas in Inverell / Delungra. Four samples of low-quality genetic information appeared as outliers of the Inverell / Delungra region, which are most likely artefacts of their poor quality. The first and second PCoA axes explained 12% of the differentiation between samples which is informative (any results >10% are considered informative).

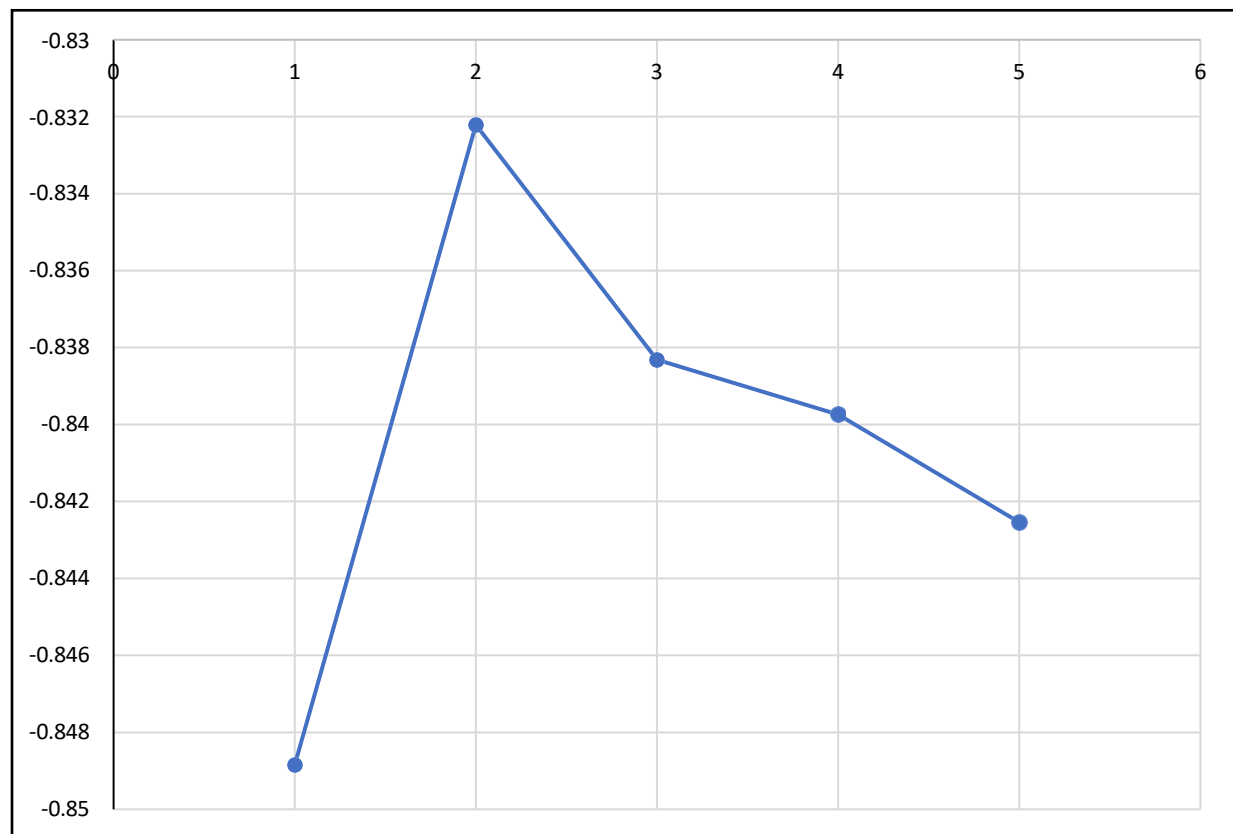


Figure 13: Result of the population structure analysis using the program fastSTRUCTURE. The number of populations is indicated by the peak of the curve.



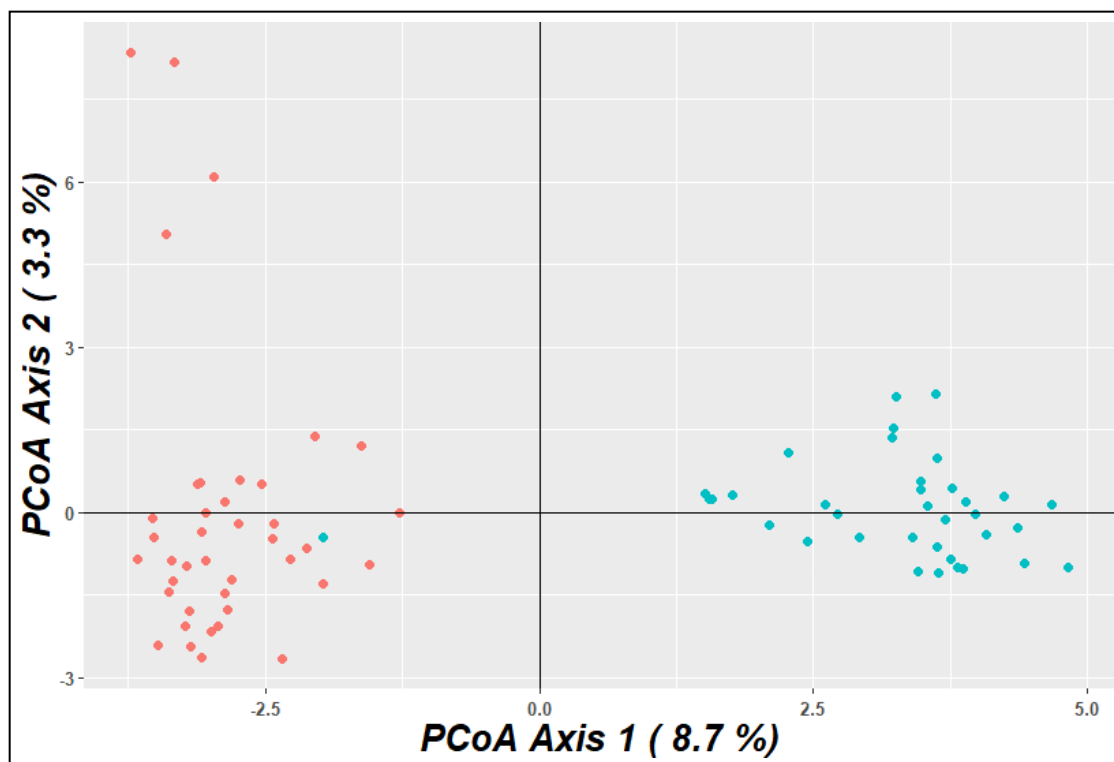


Figure 14: Result of a Principal Coordinates Analysis performed on SNPs of 76 individuals sampled in Armidale / Uralla region (blue dots) and Inverell / Delungra region (red dots) in the statistical environment R using the package dartR. The individuals are sorted along the two axes, depending on their genetic similarity.

#### 4.6.3 Population differentiation and gene flow

The DDC found moderate but significant genetic differentiation between these two potential sub-populations ( $F_{ST} = 0.067$ ,  $P$  value = 0.001;  $F'_{ST} = 0.112$ ). In comparison, the differentiation between koala populations on the mainland of Redland City Council and Noosa Shire was also significant but to a greater extent ( $F_{ST} = 0.193$ ,  $P$  value = 0.001;  $F'_{ST} = 0.305$ ). This is an example of two populations that are geographically discrete (150 km and separated by the Brisbane river).

With an  $F_{ST}$  of 0.067, the number of migrants between populations is estimated to be  $N_m = 3.5$  koalas per generation. In idealised populations, a single migrant is considered sufficient to prevent complete differentiation of two populations. However, in real populations migrants and residents are unlikely to be equally successful in producing offspring which is why more than one migrant per generation is required to prevent differentiation. There is no consensus on an absolute number, but suggestions vary from 1 to 10 migrants. With 3.5 migrants per generation, there is limited exchange of alleles between these two populations.

The DDC tested the two populations for isolation by distance which was weak but significant ( $R_{xy} = 0.02$ , P value = 0.001, Figure 15). This indicates that gene flow between these two populations might be slightly affected by the geographic distance which might be a small factor driving genetic differentiation.

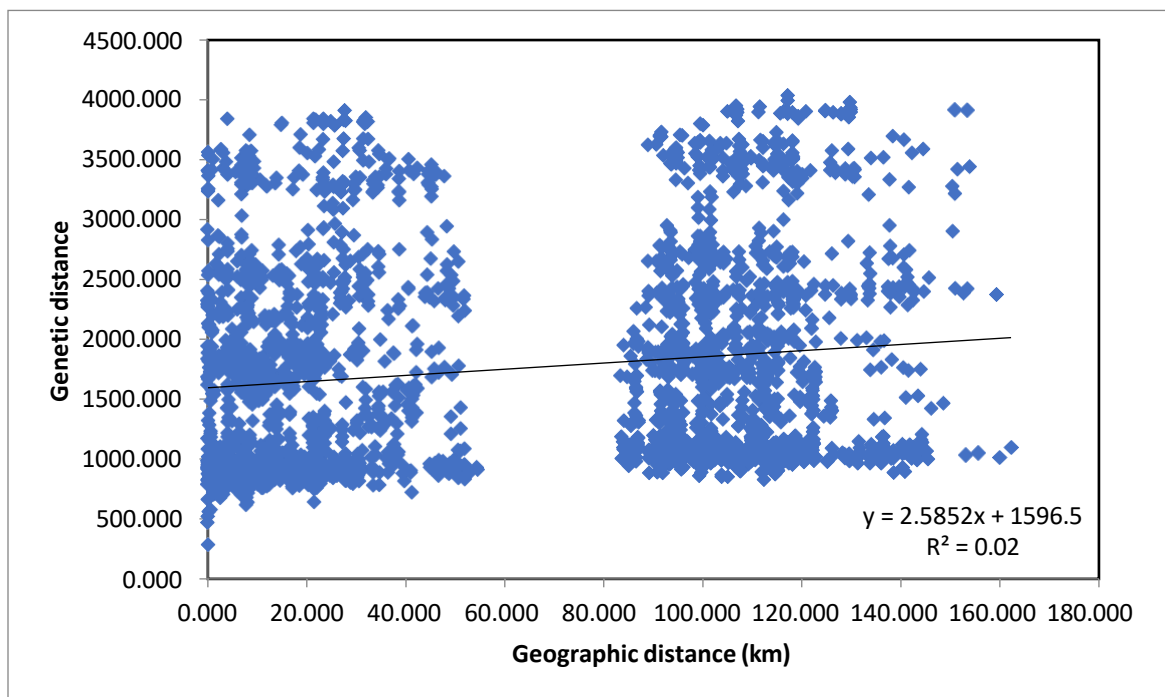


Figure 15: Scatterplot showing the relationship between geographic and genetic distance between koalas in the Armidale / Uralla versus Inverell/Delungra regions. The relationship is significant but weak.

#### 4.6.4 Genetic diversity and inbreeding

Similar to other koala genetic studies (e.g. Kjeldsen et al. (2018), Table 6), the DDC found lower observed heterozygosity ( $H_O = 0.225$ , SD = 0.003) than expected heterozygosity ( $H_E = 0.279$ , SD = 0.003) when looking at both populations together. There was little difference between the two populations (see Table 5).

Moderate levels of inbreeding were found in both populations that compare to results of other genetic studies of koalas (see Table 6, taken from Kjeldsen et al. (2016)).  $F_{IS}$  describes inbreeding at the population level and was 0.2 for the koala population in Armidale / Uralla and 0.18 for Inverell / Delungra.

Table 5: Comparison of genetic diversity and inbreeding measures of koalas in the Armidale / Uralla region and Inverell / Delungra regions

	Armidale / Uralla region	Inverell / Delungra region
<b>H<sub>o</sub> ± SD</b>	0.225 ± 0.004	0.225 ± 0.004
<b>H<sub>e</sub> ± SD</b>	0.282 ± 0.004	0.276 ± 0.005
<b>IR ± SD</b>	0.29 ± 0.37	0.20 ± 0.24
<b>F<sub>is</sub></b>	0.20	0.18

Internal relatedness, which describes inbreeding at the individual level, was comparably higher in Armidale / Uralla koalas (IR = 0.29, SD= 0.37) than koalas from Inverell / Delungra (IR = 0.20, SD = 0.24). These internal relatedness values also compare to those found in Kjeldsen et al. 2016 (Table 6). The difference in internal relatedness in Armidale / Uralla and Inverell / Delungra are shown in Figure 16. These values are highly dependent on sampling size and including more individuals would benefit the robustness of these results.

Table 6: Table taken from Kjeldsen et al (2016) showing genetic diversity of wild koala populations across QLD, NSW and Victoria.

State	Location	n	Ho	He	Fis (P < 0.01)	IR (±SD)	Ne <sub>LD</sub> (95 %CI)
QLD	St Bees Island	19	0.29	0.35	0.23	0.29 (±0.15)	Infinite (∞)
QLD	St Lawrence	19	0.26	0.30	0.20	0.21 (±0.11)	Infinite (∞)
QLD	Koala Coast	24	0.22	0.30	0.32	0.42 (±0.29)	Infinite (921.20–∞)
QLD	Ipswich	23	0.27	0.31	0.19	0.26 (±0.16)	Infinite (∞)
NSW	Port Macquarie	45	0.23	0.28	0.21	0.25 (±0.15)	116.8 (109.8–124.6)
NSW	Campbelltown	9	0.27	0.33	0.27	0.34 (±0.27)	2.7 (2.4–3.2)
VIC	South Gippsland	19	0.24	0.30	0.27	0.31 (±0.34)	Infinite (∞)
VIC	Cape Otway	13	0.24	0.25	0.11	0.20 (±0.11)	46.7 (40.8–54.4)

n = sample size, Ho = observed heterozygosity, He = expected heterozygosity, Fis= inbreeding coefficient, IR = internal relatedness and Ne<sub>LD</sub> = effective population size calculated using linkage disequilibrium.

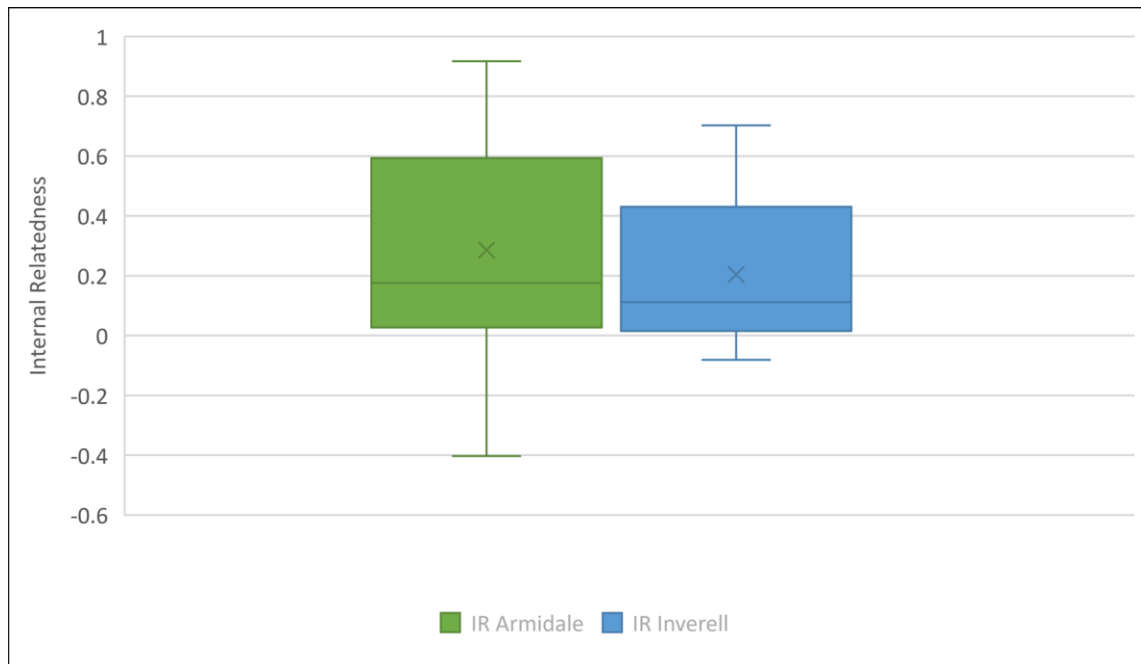


Figure 16: Boxplot showing the internal relatedness (IR) of koalas in Armidale / Uralla vs Inverell / Delungra areas

## 5. Discussion of Results

### 5.1 Cool Country Koala Project 2019 Presence / Absence Surveys

The presence / absence surveys for this project were aimed at extending the knowledge of where koalas occur in the Northern Tablelands. To this end, the DDC mostly surveyed outside of known / expected koala hotspots, with the Tingha area being the exception. DDC conducted 127 koala scat presence / absence surveys, and detected koala presence at **9 of the 127 surveys (7%)**. However, no live koalas were found during the koala scat presence / absence surveys, which were conducted in and around Walcha, Woolbrook, Brackendale, Yarrowitch, Brockley, Aberfoyle, Guyra, Bundarra, Cameron's Creek, Llangothlin, Glencoe, Pargo Flat, Wonga Hut, Guy Fawkes, Georges Junction, Stannifer, Wandsworth, Kangaroo Camp, Lollera, Elderbury, and Glen Elgin.

During systematic surveys, 2040 trees were searched for the presence of koala scats. Of these, 2 trees were found to have scats, therefore the **average tree use was 0.1%**. There were very low activity levels across the positive systematic survey sites, with **average activity level being 3% (SD = 0)**. However, it is important to note that these findings are based on only two systematic surveys where koala scats were found (due to high percentage of negative surveys).



The findings suggest that the surveyed areas have low levels of koala presence, with low levels of activity when presence was detected.

The results of these presence / absence surveys have expanded knowledge of koala distribution in the Northern Tablelands. Due to ongoing dog-baiting activities, most of the sites surveyed fell outside of the priority areas (Figure 2) identified by the NT KRS, although site selection was guided by historical koala sightings recorded in the ALA. In the future, priority areas that were unable to be surveyed should be prioritised but will potentially require dog surveys to occur at a specific time of the year when baiting is not actively occurring.

Of the priority areas that were able to be surveyed, the Tingha area appears to have undergone a population reduction since ALA sightings were recorded. Some evidence of koalas was found in this area, however, the high number of negative surveys suggests that the population is likely to be small. This is particularly interesting to consider given the proximity of the Tingha priority area to the Delungra / Inverell priority area, where the genetic sampling surveys were successful in finding fresh koala scats and live koalas. Delungra / Inverell might provide a source population for recolonisation of Tingha in the future.

All other sites where positive scat detections were made fell either just inside, or just outside priority areas, except for those south west of Walcha. Further investigation focussing on this area would help to determine the size and robustness of the koala population, and whether these koalas should be included in their own priority area.

No evidence was found that standing water or crown dieback could be a significant driver of koala presence in the surveyed areas. Further research is required to uncover what predicts koala presence / absence in the Northern Tablelands.

In comparison to historical records from the ALA (all years till 2016), the 2019 presence / absence surveys highlighted the reduction of koala distribution outside of priority areas. All surveys conducted in 2019 occurred either at or near sites of historical records. Outside of the priority areas, the DDC conducted 106 surveys at locations of historical koala sightings and found no evidence of koala activity. These findings reinforce the accuracy of the identification of priority areas but are also indicative of a potential reduction in the koala populations of the Northern Tablelands in the last 10 years. The DDC also found anecdotal evidence of a koala population reduction through interactions with local residents during fieldwork. Generally, the areas surveyed outside of priority areas seem to show low levels of koala presence, and even the sites where koala scats were found show low levels of koala activity.

By combining the results of the 2019 presence / absence surveys with previous surveys conducted by USC and Stringybark (Table 7), some key koala hotspots in the Northern Tablelands can be identified (Figures 17 & 18). Two of these hotspots (Armidale / Uralla and Inverell / Delungra regions) were selected for genetic sample surveys in this study, and these two areas are likely important strongholds for koalas in the Northern Tablelands. There is also





evidence of a sub-population in the north east of the Northern Tablelands (between Tenterfield and Urbenville), and another south of Walcha (Note that genetic sampling or analyses was not undertaken for these populations). The high numbers of positive survey results within these populations (Figures 17 & 18), reinforced by the numerous negative survey results outside of these areas, suggests a patchy koala distribution in the Northern Tablelands that exemplifies the importance of koala hotspots. Focus should be given to preserving them. This requires ongoing monitoring of the hotspots, as well as assessment of their genetic health and disease statuses.

Table 7: Comparison of koala metrics calculated at sites surveyed during the NT LLS koala scat presence / absence surveys (both USC and Stringybark 2016 and USC 2018 and 2019)

	NT LLS / USC 2019	NT LLS / USC 2018	NT LLS / USC 2016	NT LLS / Stringybark 2016
Percent positive site	7% (9/127) 0.5%	24.80% (30/121) 4.90%	30.30% (81/267) 7.20%	49% (N = 139)
Trees with koala scats	(N = 2048)	(N = 2082)	(N = 5136)	NA
Koala sightings	0 0.1%	0 4.10%	29 6.60%	NA 6.80%
Activity levels	(N = 2040)	(N = 2082)	(N = 4980)	3910
Activity levels at positive sites (i.e., when koalas are present, how intensively do they use a sites)	3%	15.70%	21.00%	NA
Percent of sites with fresh scats	33.3%	5.50%	18.60%	2%
Percent of sites with medium scats	33.3%	14.80%	41.40%	25%
Percent of sites with old scats	33.3%	78.70%	40.00%	75%

NA = Not available in report

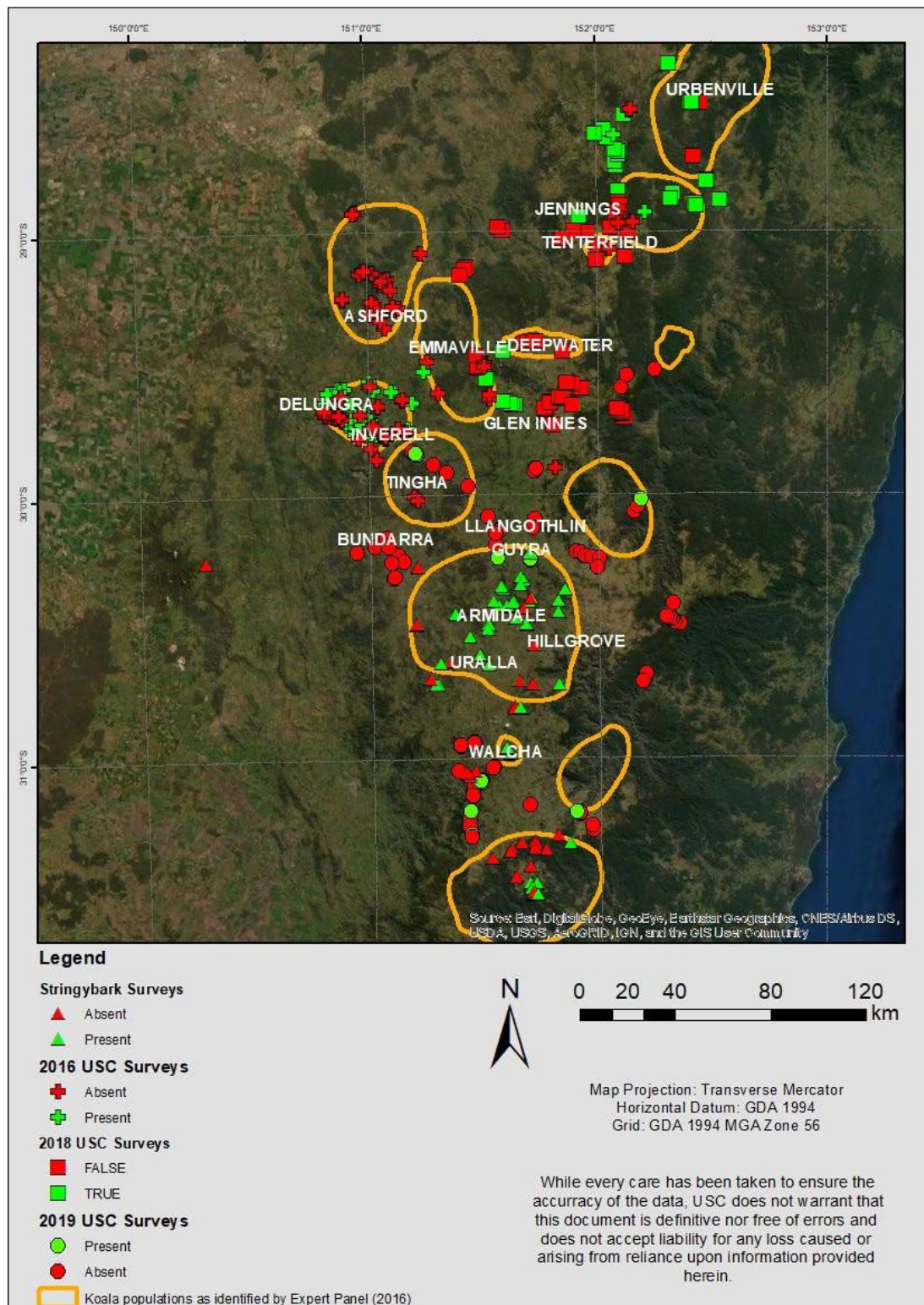


Figure 17: Koala survey sites where koala presence or absence was recorded during 2016, 2018, and 2019 koala surveying projects



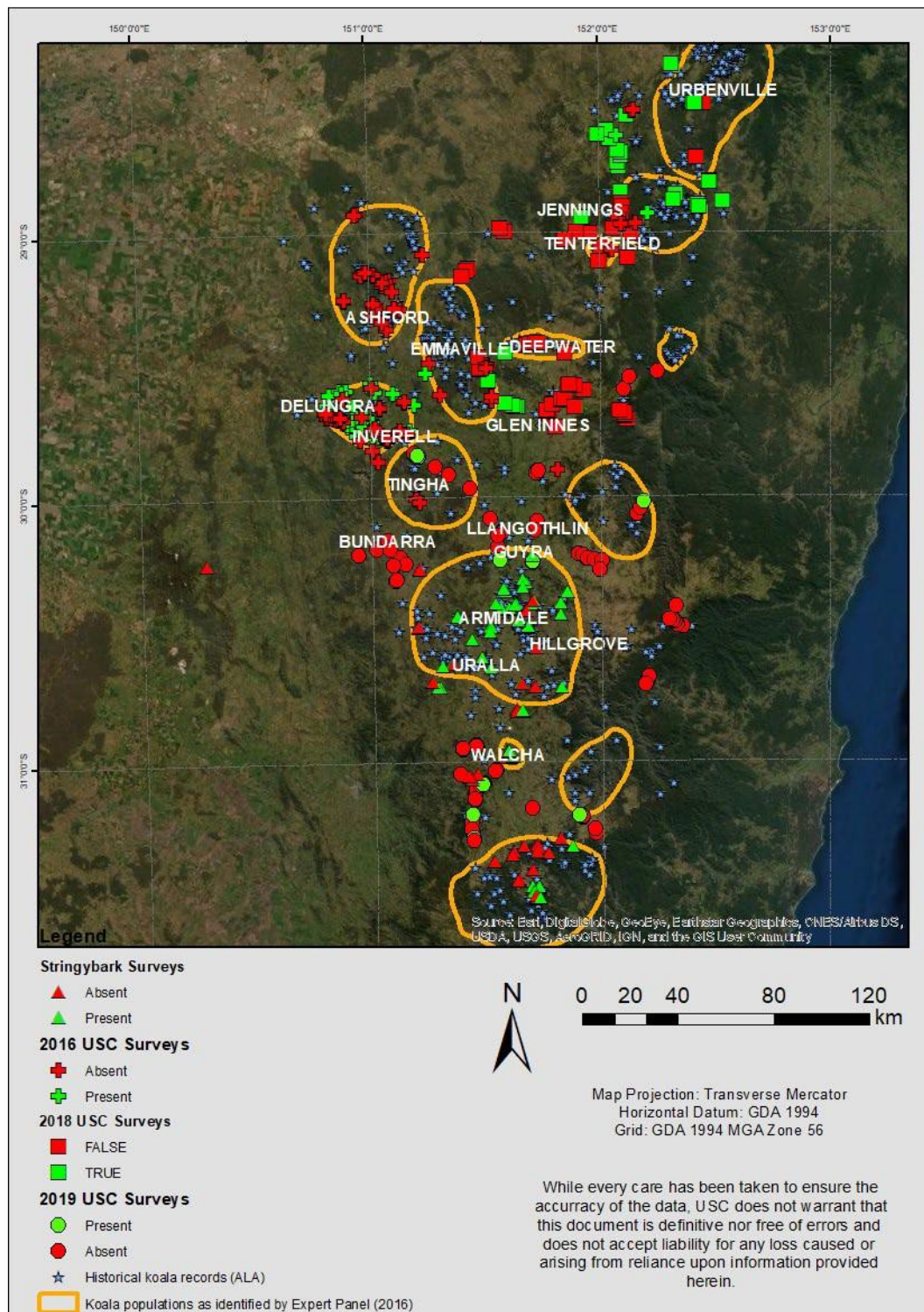


Figure 18: Koala survey sites where koala presence or absence was recorded during 2016, 2018, and 2019 NT LLS / USC / Stringybark koala surveying projects and historical data



## 5.2 Cool Country Koala Project 2019 Genetic Pilot Study

The genetic monitoring pilot study was aimed at investigating whether a full genetic study across the landscape would be feasible and to gain first insights into the population genetics of the koala populations of the Northern Tablelands. The DDC were able to collect 115 samples in areas of known koala occurrence around the regions of Armidale / Uralla and Inverell / Delungra. Of these, 90 samples were successfully genotyped, belonging to 76 unique individuals. Population genetics analyses indicated that koalas from Armidale / Uralla and Inverell / Delungra are clustered into two separate populations. Estimates of population differentiation and gene flow indicate significant but weak differentiation with limited gene flow between the two populations. Heterozygosity and levels of inbreeding found in the two populations are comparable to other koala populations in NSW and QLD.

DDC was able to show that high quality koala scat samples can be obtained in the Northern Tablelands and genotyped successfully. The number of samples that were collected in the time frame of the genetic pilot study is comparable to other studies conducted by the DDC and gives a good estimate for future projects. All results presented in this report are based on a moderate sampling size per population and need to be interpreted with care. Increasing the sampling size would contribute to the robustness of the results.

## 5.3 Management Recommendations

In this report the DDC seek to add to the recommendations included in the 2016 NT LLS and 2018 NT LLS reports provided by USC (and available in Appendix 2). The recommendations from these reports are still valid and should still be considered for implementation. Here, an update is provided taking into consideration the results of both the genetic and field surveys conducted in 2019. This includes an updated distribution and the pilot genetic results, as well as the occurrence of an unprecedented (in extent and intensity) fire season in 2019-2020.

### Recommendation 1 - Prioritise habitat preservation and rehabilitation

The *Cool Country Koala Project* survey results show a reduction in koala presence in comparison to historical records (ALA). More generally, koalas have lost large parts of their habitat, especially due to clearing, including illegal clearing due to agriculture (Ward et al. 2019) and the 2019-2020 extensive bushfires. Official numbers on the impact of the bushfire have not been released, but Government and trusted websites have quoted, for NSW alone: more than 8.4 million hectares have been destroyed including at least 1.3 million hectares of koala habitat burnt, while others quoted as much as 30 per cent of koala habitat, and a fifth of the NSW koala population, have been lost.



This new situation increases the importance of protecting remaining koala habitat as well as continuing efforts in habitat rehabilitation. The *Cool Country Koala Project* confirmed multiple koala hotspots throughout the Northern Tablelands region. Therefore, especially considering the loss of prime koala habitats and populations in the 2019-2020 bushfires across wide ranges of NSW, the importance of the Northern Tablelands region for koalas needs to be re-evaluated.

Large koala habitat rehabilitation programs are currently being developed / delivered, and the Northern Tablelands should be part of these initiatives. For example, WWF's "Towards Two Billion Trees" program, which is very focused on helping private individuals and farmers, plant trees. The NSW Government has announced, in March 2020, \$150,000 in funding to a wide range of organisations for koala habitat rehabilitation, including Lismore City Council, Friends of the Koala, Far South Coast Landcare Association, Border Ranges-Richmond Valley Landcare Network and Bangalow Koalas. The Federal Government has announced \$50m for wildlife impacted by bushfires. It is recommended that NT LLS, in partnership with other local organisations, approach such rehabilitation programs and secure funding for the region.

Note - The reasons behind the decline of some koala populations in Northern Tablelands are unknown, they may be linked to several factors including climate change. This could be investigated, although the current dataset might not be large enough to identify factors of decline.

#### Recommendation 2 – Update the mapping of current population distribution

From the *Cool Country Koala Project* as a whole (2016, 2018 and 2019 surveys), new data has become available for current koala distribution in the Northern Tablelands. Some hotspots have been confirmed, while key areas mapped by experts in 2016 are no longer sustaining populations known historically (e.g. Ashford). Potential new koala populations have been underlined, such as south west of Walcha and north of Tenterfield. Some further surveys may be required to confirm hot spots and populations especially in areas that 1080 baiting prevented surveys in the past.

It is critical, post the 2019-2020 bushfires, that the Northern Tablelands koala populations are updated in State mapping, recognised as significant for the preservation of NSW koalas, and attract funding (State and Federal) that has been in the past focusing on more coastal koala populations.

Predictions are of an increase in bushfire extent and intensity, therefore updated koala maps can inform fire-fighting prioritisation and koala rescue (see "Other Considerations" below).





### Recommendation 3 - Ongoing monitoring of the regional population through scientific surveys

The *Cool Country Koala Project* surveys have underlined that the distribution of koalas in the Northern Tablelands is patchy. Furthermore, some koala populations in areas with historic records seem to have largely declined, such as was the case in Ashford. This underlines the importance of confirming current regions with koala hotspots, such as Armidale / Uralla and Delungra / Inverell regions. This means that koala priority areas, as underlined in the NT KRS in 2016, that have not yet been surveyed, or where surveys covered a small part of the priority areas, need to be surveyed.

The DDC recommend scientific surveys of priority areas that have not been surveyed yet, in particular the priority area south-east Walcha towards Oxley Wild Rivers National Park and north-east Glenn Innes towards Washpool National Park. Furthermore, it is recommended that surveys are conducted within previously surveyed priority areas that have gaps in the landscape, where no surveys have occurred. This would in particular apply for the north-east of Tenterfield, the area around Severn River near Pindaroi, the area near Mt Mitchell around Warra National Park and the area south-west of Nowendoc. Refer to Figure 19 for reference. With one more year of *Cool Country Koala Project* surveys, all priority areas may be able to be checked.

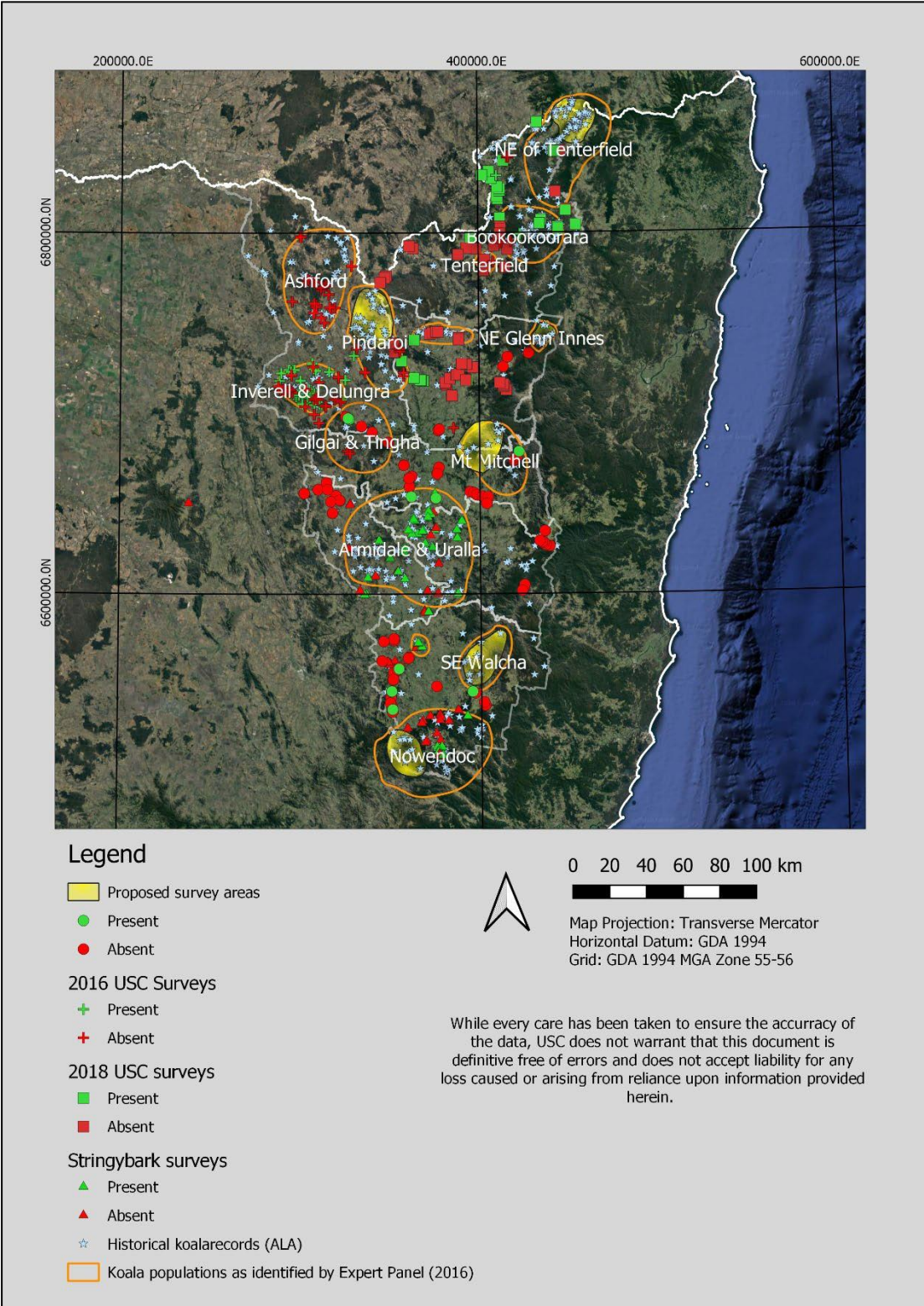


Figure 19: Map of previous koala surveys and proposed survey areas.



#### Recommendation 4 - Ongoing monitoring of the regional population through broad and targeted Citizen Science programs

Longitudinal monitoring of koalas can enhance our understanding of factors affecting the distribution, population trends and specific threats to local koala populations (main threats likely vary between populations). Longitudinal monitoring can be conducted by training members of the public to support koala monitoring. Acknowledging budget constraints, we recommend recruiting the local community to become the guardians of their koalas. Increasing the community awareness of threats to koalas and involving them in active monitoring could improve the detection of emerging hotspots of mortality and disease, which would allow an early intervention in the management of these threats.

In the Northern Tablelands, it is recommended that data collection be promoted through a combination of the following:

- Encourage broadscale reporting to ALA throughout the year: this will continue building the current distribution across the whole of Northern Tablelands

This will be best achieved as a coordinated approach with other Northern Tablelands partners to promote the use of ALA and increase sighting reports. To maintain enthusiasm, this might require continuous communication from NT LLS, with or without external inputs, to increase koala knowledge and the importance of reporting sightings, through flyers and community events.

- Targeted citizen science program in koala hotspots of Armidale / Uralla and Delungra / Inverell regions

Both hotspots occur in areas relatively developed / occupied by humans. This can be both seen as a threat and an opportunity. Events or citizen science programs may include annual phone-in or online surveys encouraging the reporting of sightings at a particular time of the year, such as a “Koala-thon”, a specific weekend each year. These could coincide with national events, using the general heightened enthusiasm for koalas and transforming it into action: *Koala Month* is September, while *Wild Koala Day* is 3rd May each year.

#### Recommendation 5 - Expand genetic sampling of koalas in the Northern Tablelands

The DDC were able to show successful sampling and genotyping of koala scats collected in the Northern Tablelands. Genetic analyses of individuals and populations provide valuable information such as identifying isolated koala populations, risks of inbreeding depression and disease hotspots. Disease mapping of the two hotspots could be conducted from the scats already available from the 2019 genetic surveys.

Increasing the sampling size and the spread of sampling across the Northern Tablelands landscape would contribute to a better understanding of the Northern Tablelands koala



population dynamics and inform the development of conservation strategies, including but not limited to:

- 1) strategic placement of revegetation projects to reinstate genetic connectivity across the landscape,
- 2) identification of disease hotspots to enhance the delivery of disease treatment / vaccination to minimise decline of local populations,
- 3) identification of locations with high inbreeding to inform the possibility of genetically rescuing these (introducing new genes) to reduce the risk of inbreeding depression (the rise of health defect from close relatives mating with each other).

## 5.4 Other considerations

### Plan koala management response to bushfire

The 2019-2020 bushfires have highlighted the difficulty in preventing high intensity (crown) fires, especially considering the prevailing conditions of drought and high temperatures – conditions that are worsening with climate change.

Parts of the Northern Tablelands region have not been impacted by the 2019-2020 bushfires (Figure 20), while some parts have been affected at different fire intensity, therefore a review of fire management and risk to koalas from future bushfires is recommended. Understanding where koalas have survived in the fire footprint, and whether these populations are able to deal with the conditions in post fire habitat could help prepare for future fire events. Planning, and setting aside funding, for an emergency response to bushfires affecting koala populations could help koalas to persist in Northern Tablelands into the future.



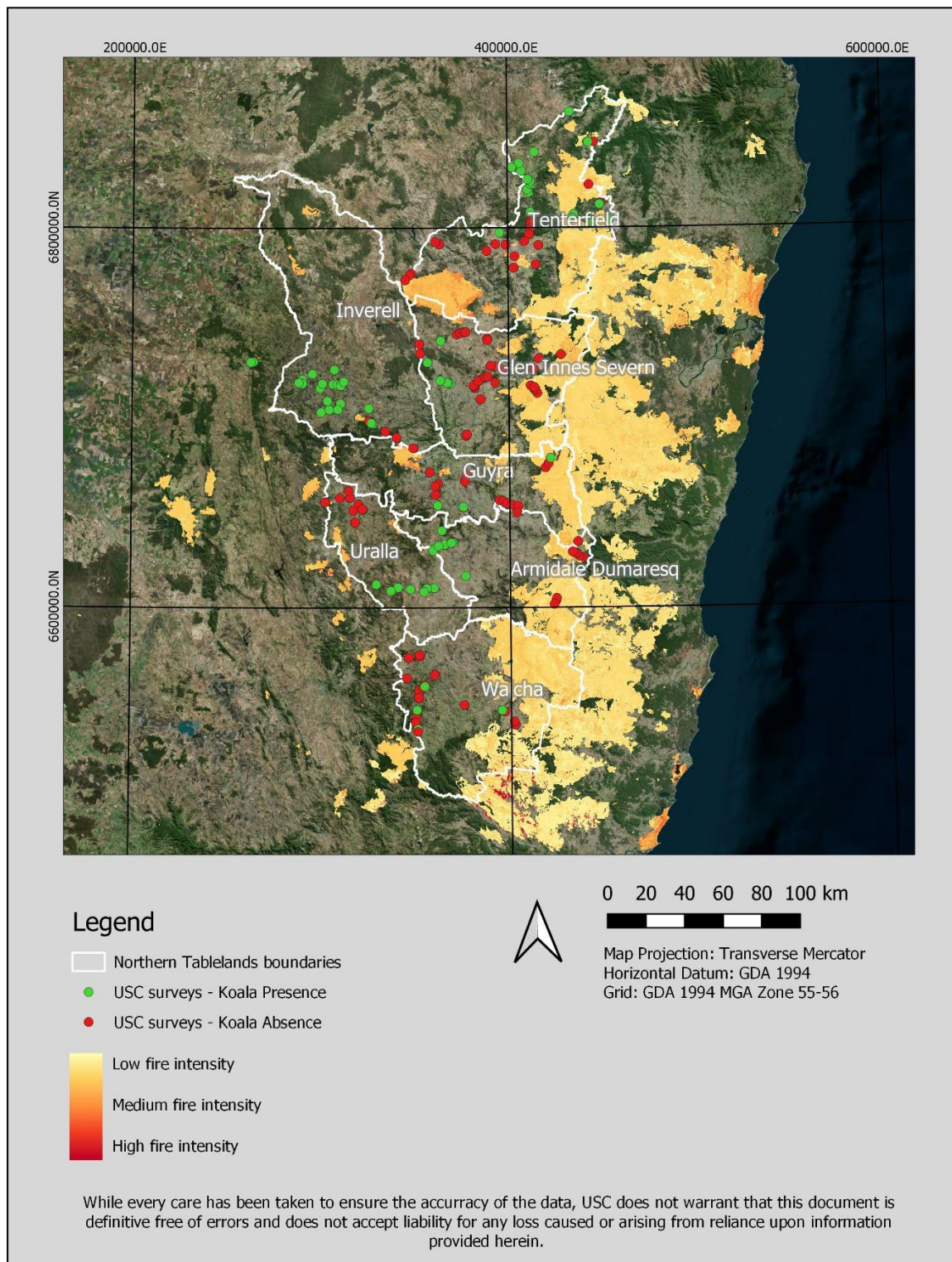


Figure 20: Extent of the 2019 – 2020 fire compared to koala survey sites where koala presence or absence was reported during 2016, 2018, and 2019 NT LLS / USC surveys





For example, during the 2019-2020 bushfires, USC was deployed to locate and rescue koalas through flying drone-mounted thermal camera and deploying detection dogs to:

- locate koalas in burnt areas,
- assess koala health and available habitat,
- rescue koalas when necessary and bring them to veterinarians / wildlife hospitals,
- collected fresh koala scats for health analyses including stress, disease and microbiomes.

A critical part of success in rescue work is to establish a strong network with clear lines of communication, and building trust, before the emergency occurs. In collaboration with welfare and emergency wildlife rescue partners (Ifaw and Vets for Compassion), USC is developing a first responders emergency plan. As part of this plan, further reflections that NT LLS might want to consider, are needed on the following possibilities:

- rescue a percentage of koalas pre fires, and how to 1) work with emergency services to do it safely and 2) determine koala populations to be part of this rescue attempt (for example, if a Northern Tablelands hotspot was predicted to be affected by high intensity fire),
- coordinate with fire fighters to add koalas to the list of assets to be protected during a fire – this has occurred in the past. Of course, human lives and properties were ranked above koalas, however koala populations were also integrated in the fire-fighting planning,
- work closely with state licensing to organise broad-scale permits in advance to access areas as quickly as possible post fires to rescue any survivors,
- what steps to consider in order to prioritise post fire rescue. For example, the following could be undertaken:
  - *prioritise areas of high koala density* to maximise rescue effort benefits in terms of number of koalas, or
  - *prioritise covering a large geographical extent* to protect koalas in several places and allow for recolonisation from multiple populations sources as well as the preservation of more genetic diversity.

#### [Continue to support the koala network on the Northern Tablelands](#)

Because many of the threats to koalas are anthropogenically mediated (i.e., vehicle and dog injury, tree clearing), an educated and empowered community can act to protect koalas. It is recommended that NT LLS continues its efforts to promote threatened species on the Northern Tablelands and to engage landholders to be involved in their protection.

The critical role of wildlife rescuers and koala carers has been publicly recognised during the 2019-2020 bushfires. They are a critical part of the koala network because of their knowledge,



their passion and their constant work rescuing, rehabilitating and releasing koalas. In times of bushfires, their already busy schedule might be stretched beyond capacity and they should receive emergency help.

In preparation, NT LLS should develop ways to:

- Communicate effectively: have contact list ready and updated.
- Recognise and support rescuers / carers effort and investment in the wildlife of Northern Tablelands all year round – for example, several Councils in Queensland now offer “carer grants”, these enable wildlife rescuers / carers to claim petrol and consumables they buy for the wildlife in their care.
- Have emergency funds for disasters emergency relief or be part of a larger emergency network to gain access to these funds rapidly when they are needed.

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## Appendix 1 – Preliminary results for chlamydial disease

Preliminary results extracted from the DArT panel, which includes limited number of markers for the presence of DNA from the pathogen chlamydia, are provided in this section. An important point is that, to date, no study has been able to fully understand the links between chlamydia presence in a koala, chlamydia load and clinical chlamydia disease. The DDC is currently working towards elucidating how DArTCap chlamydia results and clinical signs are linked. These results mainly point out that the chlamydia presence in NT LLS might be worth investigating.

Figure 21 shows the distribution of koalas that were identified to be chlamydia positive. Of the 76 individuals, 24 carried chlamydial DNA (~30%). Of these 24 individuals, only one was found in Inverell / Delungra, all others were in the Armidale / Uralla region. As mentioned before, we are yet to disentangle the meaning chlamydia presence versus disease. Individual koalas may carry the bacteria but show no signs of disease. The DDC is currently working towards elucidating how presence of Chlamydia DNA and clinical signs are linked. The results presented in this report may not reflect the true ratio of sick koalas.

It would be very interesting to peruse the field pictures of koalas in the Armidale region from the *Cool Country Koala Project* in 2016 (Stringybark surveys). During this 2019 survey, The DDC looked for external signs of chlamydia disease but did not conclusively observe them in any koalas.



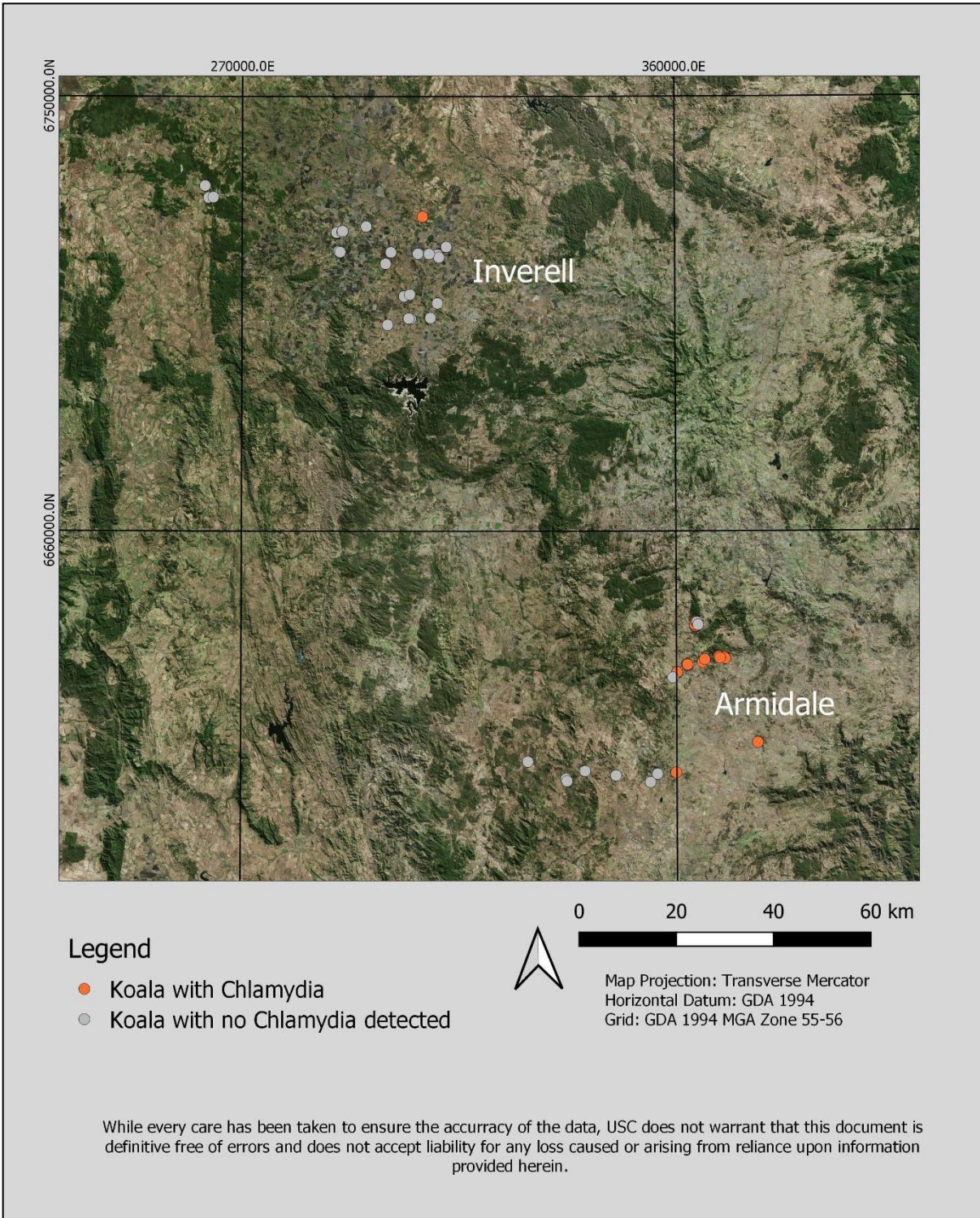


Figure 21: Map showing koalas that were tested positive for chlamydia as a red point versus individuals in grey that were below the threshold and are assumed to not have chlamydia



## Appendix 2 – Past recommendations directly lifted from previous *Cool Country Koala Project* reports

### 1. Management Recommendations from the 2018 “*Cool Country Koalas Project*”

The surveys conducted by USC in 2018 found that koalas are present in the surveyed regions (Tenterfield / Glen Innes), but in low or medium abundance. Historical records indicate a long-term presence in the region and koalas are dispersed widely throughout the landscape, typically in low density with some localised medium to high density populations (see newly recognised population north of Tenterfield).

The longer-term management of NT koala population should make provisions for local and regional changes in the landscape as a result of land-use practices, anthropogenic threatening processes and climate change. Habitat loss was identified as the key threat to the persistence of the KMA4 - Northern Tablelands koala population (NSW 2008). As such, the primary management strategy to conserve this population was the restoration of habitat on public and private lands. Undoubtedly there are a suite of other threatening processes impacting on the Northern Tablelands koalas, resulting in the premature mortality of animals, such as wild and domestic dog trauma, vehicle strikes, trauma from livestock, and disease. However, the general paucity of data on which threats rank higher for each priority area koala population makes targeted management uncertain. As such, the impact for population viability of targeting other potential threats are less certain than the restoration and enhancement of habitat in the region.

The 2016 NT LLS reports (USC / Stringybark) contained many recommendations to NT LLS. In this report, USC seeks to add to the 2016 recommendations, and provide management actions that can be potentially more targeted and therefore easier to implement. However, and before any other actions – NT LLS priority should be to work with Local Councils and State Government so that the approach to koala conservation in the NT is coordinated and strategic.

We provide the following recommendations based on the results of this and past research in the region:

#### **Recommendation 1 - Prioritise habitat rehabilitation**

Typically, better quality habitat for koalas occurs in lower elevation alluvial soils on the plains that have been historically cleared for agriculture and grazing. This is consistent with the majority of the koala records and populations identified by the expert panel as west of the



mountainous regions. There are multiple on-ground actions that can enhance habitat and linkages between populations, and has the dual benefit of enhancing the climate change proofing of the region:

- **Trees on farms program**  
A program to reduce salinity on rural properties in the Gunnedah region (to the south-west) through tree planting was also successful in restoring koala habitat in the area (Lunney et al 2012). Koalas can relatively quickly make use of regrowth vegetation (Cristescu et al. 2013), providing primary habitat and /or linkages between patches of habitat.
- **Landcare grants / Nature Assist / Nature Refuge grants** to encourage, with financial incentives, the restoration and enhancement of koala habitat on private tenure.
- **Local council bushcare groups and other community rehabilitation groups** to coordinate the rehabilitation of strategic linkages between townships and rural areas of koala habitat
- **Community koala tree program** to provide koala food and habitat trees to local residents.
- **Community participation** such as a propagators group could collect local providence seed and propagate preferred koala food and shelter tree species.

## **Recommendation 2 - Conserve habitat in Travelling Stock Routes**

- **Travelling Stock Routes (TSR)** provide important remnant habitat and linkages in the landscape, and may often be the only patches of vegetation in areas of significant farming practices. They should be considered as unofficial reserves and managed as such, in isolation to the usual management practices on rural holdings.

Recommended management actions include:

- weed control of invasive species such as Coolatai Grass and Ox-eye Daisy
- reduce grazing pressure,
- reinstall TSR fencing,
- plant out native species,
- remove rubbish,
- control feral animals such as pigs, goats and deers.





- A review of all TSR survey data available should be conducted, and TSR with high conservation values should be identified. Management plans should be developed to preserve significant species, including koalas, on these high conservation value TSR.
- During this review, NT LLS should also identify well managed TSR to be used as case study for other landholders. This for example could showcase landholders with sound management plans of the TSR that demonstrate grazing and conservation of TSR can both work together.
- If there are information gaps, an additional survey of TSR and how they are treated across NT LLS should be conducted, as our personal observation is that some TSR are appropriated by landholders for private use.

**Examples of suggested management recommendations for specific survey sites (sites are mapped in Appendix 2)**

- Reduce livestock grazing pressure in over-grazed TSRs, e.g. site 2018-11-07-ba10.
- Reinstall TSR fencing where it has been removed/taken down by neighbouring landholders for grazing purposes, e.g. site 2018-11-16-ba2.
- Consider those TSR sites which are of high conservation value to be managed for conservation, e.g. 2018-11-14-ba1.
- Undertake tree plantings on good condition TSR sites with wildlife corridor potential, e.g. 2018-11-07-ba1.
- Manage understory weeds such as Coolatai Grass and Ox-eye Daisy (*Hyparrhenia hirta* and *Leucanthemum vulgare*) which are an emerging threat to good condition TSRs, e.g. 2018-11-16-ba5.
- Manage feral animals, such as goats, deer, pigs, that overgraze TSRs, e.g. 2018-11-14-ba9.
- Discourage the dumping of rubbish in TSRs, e.g. 2018-11-08-ba1.
- Stop the practice of tree clearing in or adjacent to TSRs of high conservation value, e.g. 2018-11-13-ba1.
- Undertake plantings of TSR sites that are susceptible to eucalyptus dieback, e.g. 2018-11-04-ba5.

**Recommendation 3 - Ongoing monitoring of the regional population through targeted and Citizen Science programs**

The longitudinal monitoring of the regional population is needed to understand factors affecting the distribution, abundance and viability of the regional population. Data collection



can be targeted to understand particular aspects of koala biology or ecology, or more general and passive in nature. Data collected through incidental reporting, while somewhat biased, can provide compelling data on the trends and threats to koalas in the region – this is especially relevant in koala hotspots (Delungra / Armidale) that coincide with human populated areas. Citizen scientists are often a cost-efficient way to gain a greater understanding of the threats to koalas that are reported as sick, injured or orphaned and needing treatment and rehabilitation. It is a pro active monitoring that if the community is engaged can provide real-time monitoring of population status as well as monitor the success of management actions.

- Identification of source populations that are healthy and in relative abundance to ascertain the strategic management of these key populations.
- Gather baseline population data on the Delungra and Armidale populations, currently hotspots of signs of koala activity and likely two of the source populations in the region. Further in-depth investigation of the dynamics and ecology of the Delungra and Armidale populations will allow for a more targeted approach to the management of koalas in these areas.
- Monitoring of ‘insurance’ or source populations at a frequency to allow the detection of critical population changes and threats that may negatively impact on the viability of the local and regional koala populations
- Ongoing engagement of the community in koala citizen science programs to get the community actively engaged in koala conservation while providing incidental population data that may otherwise not be reported. These may include annual phone-in or online surveys encouraging the reporting of sightings at a particular time, community-based surveys, etc.
- Improve community awareness of threats to koalas and improve detection of emerging hot spots of mortality and disease in local populations for active management of threats.
- Identify appropriate partners to facilitate the collation of data obtained via citizen science programs for dissemination to regulators and local environmental groups to actively manage emerging threats and support strategies to conserve the koala and habitat in the region. For example, the Armidale Regional Council is actively encouraging their constituents to report koalas: “We urge people who have seen a koala to please register the details on Armidale’s Koala Sightings Register. The sightings are then transferred to Bionet, the NSW Wildlife Atlas record of threatened wildlife that assists ecologists in researching and monitoring threatened wildlife on a national scale.”





This could be extended across NT Councils. Armidale Regional Council register is available at the following link:

<https://epathway.newengland.nsw.gov.au/ePathway/Production/Web/CustomerService/DynamicPages.aspx?CustomerId=42500&PageIndex=0&js=657262340>

#### **Recommendation 4 - Update government mapping to reflect current population distribution**

As data become available, regulatory maps need to be updated to reflect changes in population distribution and emerging important populations. This ensures that strategies to restore koala habitat, for example, are based on the most up to date information about key populations and their distribution in the landscape and where habitat and linkages need to be restored and enhanced.

- Amend maps to acknowledge newly identified high density populations (e.g. NW of Tenterfield).
- Review the expert panel assessment of populations based on current survey data.
- Ensure any survey contractor reports raw data to Bionet as per NSW licencing conditions.

#### **Recommendation 5 - Gain support of local and state government to assist in local on-ground management actions**

- Promote the NT koala populations so that the NT koalas are “put on the map” and become a priority when State Government in particular releases funding. Due to its geographical position, NT koala populations might be less subject to some threats that are high in other KMA (habitat loss and associated anthropogenic threats on the Eastern coast, climate change to the west of NT). This could mean NT populations could potentially act as insurance population and climate refuge. This requires further research but is important as if this is the case, NT is critical for long-term survival of koalas as a species in NSW.
- Seek grants to promote state and local government involvement in on-ground activities that will engage the community and landholders

#### **Recommendation 6 - Scoping an emergency plan document for hotspot populations**



The koala observed in the NT through the 2016 surveys showed no signs of the common koala disease chlamydia. It is less risky and would provide better outcome to monitor population and detect early change of this healthy state. This can be done through citizen scientists (see recommendation 3).

NT LLS might wish to investigate an emergency plan in the event of a chlamydia outbreak. At minimum, if NT LLS is able promote the importance of NT koala population (see recommendation 5), funding in an emergency might be easier to access. The emergency plan should describe cost-effective ways to monitor population hotspots, threshold for management actions as well as which actions should be considered (catching / treating koalas). Research project could also be investigated through partnership with universities and interest groups – for example, whether Drinky Bill water delivery stations can deliver vaccine to wild koala populations in the future.

#### **Recommendation 7 - Spatial analysis as a tool to predict koala current and future landscape use and as a tool to communicate with other stakeholders**

Mapping corridors and climate refuge could be investigated, especially as collaboration with other projects or universities.

#### **Recommendation 8 - Continue support the koala network on the Northern Tablelands**

Many of the threats to koalas are anthropogenically mediated (i.e., vehicle and dog injury, tree clearing) which also means that an educated and empowered community can act to protect koalas. Therefore, NT LLS effort to promote threatened species on the NT and engage landholders to be involved in their protection is critical and should continue. For example, NT LLS should endeavour to pursue these current efforts:

- promote the visibility of the koalas on the NT through social media engagement, including Northern Tablelands Threatened Species Network Facebook page and You Tube channel,
- capitalise on any koala story to develop media releases in order to keep koalas at the front of people minds,
- deliver workshops and public events, with the aim of being practical and breaking all barriers to foster permanent behaviour changes.



A very engaged and committed part of the public are the wildlife rescuers and koala carers. They are a critical, if often extremely busy and already at capacity, part of the network. NT LLS should develop ways to:

- Communicate effectively (for example, carers often cannot go to workshops as they have responsibilities to their animals in care). Long-term rescuers / carers are often very knowledgeable in the local populations, and the value of this on-the-ground experience should not be underestimated.
- Support rescuers / carers effort and investment in the wildlife of NT – for example, several Councils in Queensland now offer “carer grants”, these enable wildlife rescuers / carers to claim petrol and consumables they buy for the wildlife in their care.

## 2. Recommendations from *Cool Country Koala project 2016/2017 Northern Section - Final Report*

### Summary of Threats to the Survival of the Koala

Threats to koala populations have been well-documented throughout their geographic range and include the following (Obendorf 1983, Martin and Handasyde 1999, Dique et al. 2003, Rhodes et al. 2011, Denner and Young 2013, Burton and Tribe 2016, McAlpine et al. 2017):

- habitat loss, fragmentation and degradation (including dieback, grazing and weed incursion);
- vehicle strike;
- disease;
- predation (including wild and domestic dog attack); and
- extreme weather events (bushfires, droughts, heatwaves).

Although the general threats are known, what is lacking and generally difficult to ascertain is the relative importance of these threats across the landscape.

As discussed in Section 4, some areas of the Northern section of the Northern Tablelands appears to experience relatively high levels of koala activity, particularly in the Inverell / Delungra priority area. In comparison, Ashford priority area experienced very low koala activity and, based on historical records and community engagement, it appears that this area has seen a large decline in koala presence, possibly since 2010. None of the threats measured in this project were able to explain this difference.

This study has found evidence that predators such as domestic and wild dogs occur within the priority areas on the Northern Tablelands, although the threat of dog predation is difficult to quantify in koala populations. This is because the density of dogs does not necessarily represent the level of threat. In a research project in Moreton Bay Railway Link, where 500 koalas were radiotracked for up to five years, it is thought that one individual was responsible for most of the mortalities attributed to dog attacks (124 koalas killed, Jon Hanger, personal communication).

Tree dieback and grazing activity were light at the majority of survey sites. It has to be noted that dieback can be spatially limited, and therefore easily missed in the field (Andrew Davidson, personal communication).

Most sites had at least one species of weed, or introduced species, present. However, the impact of these infestations on koalas is unlikely to be significant because the infestations were rarely





considered high enough to prevent regrowth. Personal observations from the USC team also seems to indicate that presence of weed does not always prevent koalas using sites (unpublished data).

Although only a small number of individual koalas were observed during the study, none presented external signs of poor health or disease (i.e. *Chlamydiosis*).

Our models of koala presence / absence as a function of threats (grazing frequency, grazing intensity, presence of weeds, dieback and wild dogs), showed that no threat was significantly associated with the absence of koala (all P-values > 0.5).

As such, the general recommendations (see “Recommended Management Actions”) of this study are more based on the perceived conservation status and threats to koalas on the Northern Tablelands, which has been inferred from known koala threats, rather than on threatening processes observed / quantified during this project.

The recommendations were developed in conjunction with other similar documents produced for koala conservation in New South Wales, including:

- New South Wales Government 2016. Report of the Independent Review into the Decline of Koala Populations in Key Areas of NSW. NSW Chief Scientist and Engineer. December 2016.
- North West Ecological Services 2016. Gunnedah Koala Conservation Plan for the Landcare and Community Groups, prepared for North West Local Land Services.
- Hawes, W., Hunter, J, Lechner, A. & Ede, A. 2016. Northern Tablelands Koala Recovery Strategy 2015-2025. The Envirofactor Pty Ltd, Inverell.

However, below we give some specific recommendations for the two priority areas surveyed through this project, Inverell / Delungra and Ashford, in addition to the general ones.

### **Recommended Actions for the Priority Areas of the Northern Tablelands Cool Country Koala Project (Northern Section)**

#### **Inverell / Delungra Priority Area**

During the *Cool Country Koala Project* (Northern Section), we clearly found the Inverell / Delungra priority area to be a koala hot spot. As a consequence, on-ground actions should be targeted and prioritised in this area where the local population of koalas can be supported and increased immediately.

Recommended actions in the Inverell/Delungra priority area are:

#### ***Rehabilitation:***

Trees on Farms program and other collaborative initiatives (with Landcare groups for example) should be implemented so that the total amount of available habitat is increased. The Inverell /



Delungra priority area is highly fragmented with a low proportion of remnant (i.e. native) PCT remaining (72.5% is cultivated land). Despite this, the area already supports a healthy, breeding koala population. By providing more habitat, we expect that the carrying capacity of the landscape would increase and so would the koala population size.

In addition, the local koala population would benefit from the protection of the remnant habitat.

### *Engagement:*

The community should receive a high level of engagement, such as environmental talks from NT LLS, to increase awareness of the significance of their koala population and promote specific behaviours that strengthen koala conservation efforts.

Particular consideration should be given to the possibility of recruiting a “Koala Champion” in this area to create and support a local Koala Action Group. This group could be in charge of encouraging the community to report koala sightings to the Atlas of Living Australia (ALA), maintaining a Facebook page where the community could upload pictures, organising tree planting days and generally raising the profile of koalas in the area.

### *Road signs:*

Travellers should be notified that they are entering a special koala hot spot, so they can decrease their speed and increase their level of vigilance. Signs can be as simple as koala zone signs (preferably ones with a koala on the ground, not in a tree, and with a direct action message, see examples Figure A1) or can be an interactive sign with the speed of the driver or with the number of koalas hit in the region for the year (toll).



**Figure A1: Example of simple koala signs that show a koala on the ground and have a suggestive message**

### *Disease*



Signs of disease, especially *Chlamydiosis*, should be monitored. This can be achieved by:

- being regularly in touch with the wildlife carers in the area, to inquire whether sick animals have been admitted from this area,
- if a Facebook page is created where members of the public upload their sightings, monitor the pictures for clinical signs of the disease,
- scat monitoring (collection of fresh scats by members of the public and analyses for the presence of *Chlamydia*).

Monitoring of *Chlamydiosis* would permit an early intervention by the NT LLS and other stakeholders to treat / control the disease in case of an outbreak.

#### Heat wave

If a heat wave occurs in the area, NT LLS should encourage members of the public to provide water bowls for koalas. Koalas have now been proven to drink water when freely available (Sydney University, unpublished data), and this might be increasing their survival when they are suffering heat stress (North West Ecological Services 2016).

#### Ashford Priority Area

The Ashford priority area, albeit clearly identified as a potentially significant population based on past records, provided very few signs of koala presence during the 2016 survey. However, no threats recorded during the same survey could explain the population crash which, based on interviews with members of the public during the survey, occurred a few years prior. Anecdotal evidence suggests a heat wave could have been involved (Andrew Davidson, personal communication).

As a consequence, the Ashford area would benefit from a follow up survey based on a questionnaire, for example through a letter drop (Note: this could also be part of USC final community talks). The questionnaire needs to be developed with input from researchers / statisticians with knowledge of community surveys (for example, Dan Lunney, Office of Environment and Heritage NSW, could be approached), so that answers provide quantifiable and robust data.

Areas of interest for the questionnaire:

1. Have you seen a koala in Ashford?
2. Can you circle on the map (provided) where your sighting(s) occurred?
3. How long ago did you see your last koala in Ashford?
4. Is there a moment in time where you saw sick or dead koalas in Ashford?



5. Was there any other change in the environment at the time you noticed sick / dead koalas, or when you stopped seeing koalas in Ashford?

The Ashford priority area, because of the very low koala presence, is not an area where rehabilitation and further work promoting “Trees on Farms” program is considered a priority. In addition, the Ashford priority area presents low fragmentation (90.3% is remnant vegetation).

If the reason(s) for the population crash can be elucidated, directly managing the threats, not habitat rehabilitation, would be the highest priority.

Additionally, the Ashford priority area might present an interesting subject for a student project (Honours for example), where the temperature and precipitation records for the area, from the Bureau of Meteorology, could be data-mined for anomalies. This could be compared to the Atlas of Living Australia records and the questionnaire, to determine whether any specific meteorological event coincides with the koala population crash.

## **Recommended Management Actions**

### **On Ground Actions**

#### *A. Curb habitat loss, increase habitat extent and connectivity*

- A1. Decrease / prevent habitat clearing in key areas for koala conservation (see 5.3.4 Research)
- A2. Increase the extent of koala habitat by supporting tree planting campaigns and promoting natural regrowth (and associated benefits for the land in general)
- A3. Increase the extent of protected koala habitat through: land acquisition, legislative protection and voluntary protection
- A4. Engage with neighbouring Councils / State, environment groups and bushcare and habitat rehabilitation groups on koala corridor projects

#### *B. Identify koala road mortality hotspots*

- B1. Compile available data from all koala Hospitals on road mortality
- B2. Interview wildlife rescue teams (e.g. WIRES, Northern Tablelands Wildlife Carers (NTWC)) and local veterinarians
- B3. Map koala mortality based on B1. and B2.
- B4. Map koala habitat (a first approximation could be by mapping PCT used by koalas from the present surveys, but consider employing an expert to perform a habitat mapping exercise) and underline where koala habitat is divided by roads
- B5. Rank koala mortality risk based on B4., as well as road speed and traffic, which influence koala mortality (Jones et al. 2014). B5. is useful as there might be zones of high risk, i.e. where



koala habitat is divided by high traffic roads, but that was not picked up by B3. if koalas incidents went unreported)

B6. Use B3. and B5. to establish where new koala infrastructure might be needed (see examples Figure A2). Koalas have been proven to use underpasses (Dexter et al. 2016) although not rope bridges (Goldingay and Taylor 2017). Koala fencing is often used to guide koalas to the underpass (Koala Conservation Unit 2012)

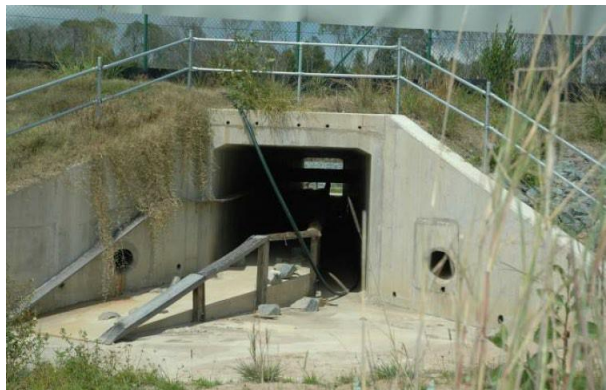


Figure A2: Koala exclusion fencing and fauna underpass with koala furniture. (Pictures from Koala Conservation Unit 2012)

### *C. Audit and prioritise work on koala infrastructure*

C1. Map all current koala infrastructure in the NT (e.g. koala fencing, underpasses, koala signs)

C2. Assess state of all current koala infrastructure in the NT

C3. Prioritise koala infrastructure repairs from C2. and new infrastructure needs from B6.

### *D. Control predation*

D1. Target wild dog control to key areas for koala conservation (see 5.3.4 Research)

### *E. Improve fire Planning*

E1. Review recommendations for control burns in koala habitat

E2. Plan control burns to decrease risks of wild fire in key areas for koala conservation (see 5.3.4 Research)

### *F. Support Wildlife Carers*

F1. Subsidise carers and rescuers (grants)

F2. Organise workshops for carers and rescuers to disseminate knowledge about rescue / care / treatment of koalas

*Cool Country Koala Project 2019 – 2020: Koala Habitat and Pilot Genetic*



F3. Support carers and rescuers to attend koala conferences to stay updated with latest rescue and rehabilitation research findings (grants)

### Legislation and Regulatory Controls

- Develop / support legislation:
  - to protect koala habitat (i.e. Land Clearing laws)
  - to properly offset cleared habitat
  - to decrease carbon emissions, climate change and increase renewable energies (climate change will negatively impact koalas (Adams-Hosking et al. 2011))
  - to control domestic dogs
- Enforce legislation

### Community Engagement

- Foster a community that is: informed, engaged, active on the ground for koala conservation
- Promote responsible dog ownership

### Research

#### *Koala distribution*

- Extend koala presence / absence field surveys to all priority areas and corridors from the NT KRS
- Model koala habitat
- Ground truth the model
- Map genetic connectivity at the landscape level

#### *Koala health*

- Map koala health (diseases) at the landscape level
- Map koala genetics (diversity, inbreeding, effective population size...) at the landscape level

#### *Map key areas for koala conservation*

- Use koala habitat, health and genetics to rank populations and establish key areas for koala conservation

#### *Koala threats*

- Research how climate change will affect NT, map potential climate refuge and investigate connectivity between current koala populations and climate refuges
- Investigate research opportunities to assess the impact of wild dogs, and wild dog baiting, on koala populations



- Investigate research opportunities to assess which weeds (and at what density) impact negatively on koala populations
- Investigate research opportunities to identify and quantify threats across landscape

## **Division of Responsibilities**

### **Local Land Services**

#### *Loss, modification and fragmentation of habitat*

- Organise / promote tree plantings (Trees on Farms program)
- Collaborate with other stakeholders to build a strategic corridor network

#### *Vehicle strike*

- Participate (with Councils) in mortality hotspot mapping

#### *Predation by wild or domestic dogs*

- Continue implementing 1080 baiting

#### *Intense prescribed burns or wildfires that scorch or burn the tree canopy*

- Work with other agencies (especially National Parks) to develop fire regime prescriptions for different plant community types
- Work with other agencies to develop guidelines for implementing control burns in koala habitat

#### *Koala disease*

- Support research into koala health mapping

#### *Heat stress through drought and heatwaves / Human-induced climate change*

- Support legislation on carbon emission, climate change, renewable energies
- Support research on impact of climate change on koala and mitigation measures

#### *Inadequate support for fauna rehabilitation*

- Investigate grant opportunities (with Councils) for wildlife carers / rescuers

#### *Lack of knowledge*

### **Poor understanding of sources of trauma and mortality**

- Interview wildlife carers and veterinarians, and mine wildlife Hospital datasets, to quantify disease in koalas coming into care
- Encourage the reporting of dog attacks on koalas

### **Poor understanding of population distribution and trend**



- Facilitate the development of accurate, scientifically-tested koala habitat mapping for the Northern Tablelands
- Monitor koala trends in key areas for koala conservation

#### **Poor understanding of animal movements and use of habitat**

- Investigate research opportunities to assess animal movements if this information is required for management
- Extend research into koala Activity Levels (see 4.3 Activity Levels) in different habitats (by including more plant community types for example)

#### *Getting the community engaged in koala conservation*

- Distribute educational material to the public about:
  - The importance of koala conservation on private land
  - Rehabilitation programs
  - Koala food and shelter tree species on the Northern Tablelands
  - Reporting koala sightings
  - Reporting sick and injured koalas to wildlife carers
  - Facilitating koala movement across private land and creating “koala-friendly” properties
  - Controlling weeds on private land
  - Options for managing wild dogs on private land
  - Providing watering points for wildlife on private land
  - Opportunities for private conservation initiatives
- Offer expert advice to the public about koala conservation, whenever possible
- Develop and maintain strong relationships between land managers, policymakers, researchers, wildlife carers, veterinarians and other community groups on the Northern Tablelands
- Conduct koala field days to educate and engage the public in koala conservation

#### *In addition:*

- Allocate funding to implement these measures
- Monitor and evaluate the effectiveness of these measures

### **Councils**

#### *Loss, modification and fragmentation of habitat*

- Modify the planning scheme to require a Koala Plan of Management for development in priority areas where koala scats were located. The KPOM needs to ensure a maintained or improved outcome in terms of extent of koala habitat





- Modify the planning scheme to include assessment of potential barriers to koala movement in the development application process.
- Require koala bridges / overpasses / culverts / ladders to mitigate the impact/s of potential barriers
- Implement a policy for replacement / offset of cleared native vegetation for development impacts that do not require a full impact assessment
- Identify Council land that can be regenerated for koalas as a response to developments that do not have space to plant trees
- Protect Council land that has high koala activity from development
- Connect fragmented habitat patches where koala scats were found on Council land (aiming to increase the abundance and diversity of koala food and shelter trees)
- Rehabilitate degraded koala habitat where koala scats were located on Council land (aiming to increase the abundance and diversity of koala food and shelter trees)
- Conduct an audit of existing fencing within each Council area and identify areas where fencing prevents koalas from accessing food trees
- Prepare a tree replacement policy for Council parks and streets that include more koala food and shelter trees
- Manage weeds on Council land to control weeds that threaten regeneration of trees and shrubs, provide a fire hazard or could disable a koala

#### *Vehicle strike*

- Identify koala road and rail mortality hotspots on Council land and target these areas for mitigation measures, such as:
  - Exclusion and guiding fences along roadways and railways
  - Koala bridges, culverts and underpasses
  - Koala ladders
  - Speed limit reductions
  - Koala signage (make it bright or flashing, include local koala death toll)
  - Road markings and traffic calmers
  - Improved street lighting
- Audit the state of existing infrastructure on Council roads

#### *Predation by wild or domestic dogs*

- Educate the Public about the impact of domestic dogs on koalas
- Modify the planning scheme to apply domestic dogs control limitations / land covenants on land where koala scats were found
- Enforce domestic dog control
- Investigate the usefulness of workshops on koala aversion training for domestic dogs
- Conduct wild dog trapping and/or baiting on Council reserves



#### *Intense prescribed burns or wildfires that scorch or burn the tree canopy*

- Manage fire breaks on Council land to prevent the occurrence of uncontrolled fires

#### *Koala disease*

- Support research into koala health mapping

#### *Heat stress through drought and heatwaves / Human-induced climate change*

- Support legislation on carbon emission, climate change, renewable energies
- Support research on impact of climate change on koala and mitigation measures

#### *Inadequate support for fauna rehabilitation*

- Investigate grant opportunities (with State) for wildlife carers / rescuers
- Issue a levy to ratepayers and developers to assist with expenses of koala carers

#### *Lack of knowledge*

##### **Poor understanding of sources of trauma and mortality**

- Share Council database
- Encourage the reporting dog attacks on koalas

##### **Poor understanding of population distribution and trend**

- Facilitate the development of accurate, scientifically-tested koala habitat mapping for the Northern Tablelands

##### **Poor understanding of animal movements and use of habitat**

#### *Getting the community engaged in koala conservation*

- Create incentives for landholders to engage in private conservation (e.g. grants, subsidies)
- Offer expert advice to the public regarding koala conservation, whenever possible

#### *In addition:*

- Allocate funding to implement these measures
- Monitor and evaluate the effectiveness of these measures

### **State Government**

#### *Loss, modification and fragmentation of habitat*

- Strengthen legislative and regulatory controls on vegetation clearing



- Require a Koala Plan of Management for development on State and Crown land in areas known to have/had a resident koala population. The KPOM needs to ensure a maintained or improved outcome in terms of extent of koala habitat
- Include assessment of potential barriers to koala movement in the development application process on State and Crown land and require koala bridges / culverts / overpasses to mitigate the impact of potential barriers
- Formulate a tree replacement / offset policy for small development impacts not requiring a full impact assessment on State and Crown land
- Identify State and Crown land that can be regenerated for koalas as a response to developments that do not have space to plant trees
- Protect State and Crown land that has high koala activity from development
- Connect fragmented habitat patches where koala scats were found on State and Crown land (aiming to increase the abundance and diversity of koala food and shelter trees)
- Rehabilitate degraded koala habitat where koala scats were located on State and Crown land (aiming to increase the abundance and diversity of koala food and shelter trees)
- Prepare a tree replacement policy for State roads that include more koala food and shelter trees
- Manage weeds on State and Crown land, to control weeds that threaten regeneration of trees and shrubs, provide a fire hazard or could disable a koala

#### *Vehicle strike*

- Identify koala road and rail mortality hotspots on State and Crown land and target these areas for mitigation measures, such as:
  - Exclusion and guiding fences along roadways and railways
  - Koala bridges, culverts and underpasses
  - Koala ladders
  - Speed limit reductions and enforcement of speed limits
  - Koala signage (make it gory, bright or flashing, include local koala death toll)
  - Road markings and traffic calmers
  - Improved street lighting
  - Evaluation of the design of road and rail networks

#### *Predation by wild or domestic dogs*

- Conduct wild dog trapping and/or baiting in State forests and reserves

#### *Intense prescribed burns or wildfires that scorch or burn the tree canopy*

- Manage fire breaks on State and Crown land to prevent the occurrence of uncontrolled fires
- Include protection of koalas and their habitat in State Bushfire Risk Management Plans



- Investigate the possibility of detecting / catching koalas ahead of prescribed burns
- Investigate the possibility of detecting / catching and treating injured koalas post prescribed burns

#### *Koala disease*

- Support research into koala health mapping

#### *Heat stress through drought and heatwaves*

- Investigate the possibility of installing water points on State and Crown land

#### *Human-induced climate change*

- Strengthen legislative and regulatory controls on decreasing carbon emissions and promoting renewable energies

#### *Inadequate support for fauna rehabilitation*

- Develop grants for wildlife carers (with Councils)
- Subsidise petrol for wildlife rescuers

#### *Lack of knowledge*

##### **Poor understanding of sources of trauma and mortality**

- Make information about koalas coming into care and their treatment publicly available

##### **Poor understanding of population distribution and trend**

- Invest in accurate, scientifically-based koala habitat mapping and allocate funds to Councils to contribute to the mapping

##### **Poor understanding of animal movements and use of habitat**

- Invest in accurate, scientifically-based koala habitat use mapping
- Investigate the feasibility, and information gained for management, of koala radio-tracking

#### *Getting the community engaged in koala conservation*

- Offer expert advice to the public regarding koala conservation, whenever possible
- Create incentives for Councils to support private conservation (e.g. grants, subsidies)

#### *In addition:*

- Allocate funding to implement these measures
- Monitor and evaluate the effectiveness of these measures





## Private Landholders

### *Loss, modification and fragmentation of habitat*

- Protect existing and potential koala habitat on private land
- Plant koala food / shelter trees to replace trees that are cleared on private land
- Rehabilitate degraded koala habitat where koala scats were located on private land (aiming to increase the abundance and diversity of koala food and shelter trees)
- Encourage natural regrowth (for example, by fencing creeks)
- Engage in private conservation
- Consider barriers to koala movement on private properties and create “koala-friendly” properties
- Examine fencing and identify areas where this prevents koalas from accessing food trees
- Manage weeds on private land to control weeds that threaten regeneration of trees and shrubs, provide a fire hazard or could disable a koala
- Collaborate with neighbouring properties to create strategic koala corridors

### *Vehicle strike*

- Drive responsibly in koala habitat areas and obey all koala speed signs, traffic markings, etc.
- Have wildlife carers phone number stored in mobile phone / vehicle

### *Predation by wild or domestic dogs*

- Restrain domestic dogs
- Support 1080 baiting

### *Koala disease*

- Know koala signs of disease
- Report signs of disease when logging koala sightings to the Living Atlas database
- Contact wildlife carers when diseased koala is identified

### *Heat stress through drought and heatwaves*

- Deploy water bowls for wildlife during drought and heatwaves
- Participate in water stations on farm program

### *Human-induced climate change*

- Support climate change policies

### *Inadequate support for fauna rehabilitation*

- Take injured / diseased koalas to veterinarians or wildlife carers
- Support wildlife carers



### *Lack of knowledge*

#### **Poor understanding of sources of trauma and mortality**

- Report koala attacks and deaths to Council, Wildlife carers or NT LLS
- Report diseased koalas to Council, Wildlife carers or NT LLS

#### **Poor understanding of population distribution and trend**

- Report koala sightings to the Living Atlas database and take photos of koalas sighted

#### **Poor understanding of animal movements and use of habitat**

- Report koala activity in specific tree species (especially feeding)

### *Getting the community engaged in koala conservation*

- Read educational material distributed by NT LLS
- Liaise with other private landholders, policymakers, researchers, wildlife carers, veterinarians, Landcare groups and other community groups
- Participate in koala field days
- Promote koala conservation via social media and through the community