Minjerribah (North Stradbroke Island) Koala Monitoring

June 2019 Progress Report

Prepared for:

Redland City Council

Prepared by:

University of the Sunshine Coast, Detection Dogs for Conservation

Dr Romane Cristescu, Dr Riana Gardiner, Dr Bethan Littleford-Colquhoun and Dr Céline Frère

Note that this is a preliminary report only. Please do not distribute, and please do not use or quote figures included in this report.

Aims and objectives Project overview

This project is designed to feed into Redland City Council's (RCC) aim of gaining a greater understanding of the status of this island koala population. Previous research suggests that Minjerribah's koalas are facing a different situation to that on the adjacent mainland, with potentially less obvious threats, and therefore the conservation actions required to protect this population into the future are less straightforward and require further investigation.

Redland City Council's Minjerribah koala monitoring long-term aims include:

- Primary

    1. Greater certainty on population numbers/density over time;
    2. Determine strain(s) of *Chlamydia* present on the island;
    3. Enhancement of current genetic information (genetic isolation/barriers); and
    4. Identification of attributes that exacerbate/minimise threats to koalas.

- Secondary

    5. Greater understanding of population dynamics;
    6. Quantify disease incidence;
    7. Describe survival, mortality and recruitment;
    8. Community engagement; and
    9. Impact of fire on the koala population.

These long-term monitoring goals are ambitious within the stated budget and time-frame and will necessitate prioritisation. In this project, we focus on deliverables that are:

1/ assessed as critical in the short term to deliver management that will protect this unique population (identification of *Chlamydia* strains),

2/ able to maximise benefits, because they build on previous projects (enhancement of current genetic information, quantify disease incidence),

3/ best value for money, based on the DDC areas of expertise and ability to provide in-kind contributions (population numbers/density over time, impact of fire on the koala population).

Therefore the Minjerribah 2019 projects had 3 main deliverables:

- Chlamydia analyses of samples already collected by the DDC
- Genetic analyses of samples already collected by the DDC
- Surveys of the 2018/2019 fire footprint (due September 2019, but delivered already)

Project timeline

	Year 1
Procurement process and contract finalisation with RCC	Late January 2019
Pre-start meeting (Milestone 1)	Late January 2019
Project start	February 2019
Genetic / *Chlamydia* analyses	February 2019
Progress report (Milestone 2)	31 March 2019
Data analyses and report preparation for genetic / *Chlamydia* component	May 2019
Proposal submission for project continuation 19/20 financial year	May 2019
Detection dog surveys (1080 dependant)	June 2019
Draft final report for 2018/19 for genetic / *Chlamydia* component (Milestone 3)	30 June 2019
	Year 2
Project rollover (finance dependant)	1 July 2019
Thermal drone surveys	July 2019 to March 2020
Data analyses and report preparation for detection dog survey component	September 2019
Progress report (Milestone 4)	30 September 2019
Data analyses and report preparation for drone survey component	November 2019 to May 2020
Mark-recapture urban surveys	As per Council surveys (November 2019?)
Genetic / *Chlamydia* analyses	December 2019
Progress report (Milestone 5)	31 December 2019
Proposal submission for project continuation 20/21 financial year	May 2020
Draft final report for 2019/20 (Milestone 6)	30 June 2020
	Year 3
Project rollover (finance dependant)	1 July 2020

Thermal drone surveys	July 2020 to March 2021
Mark-recapture urban surveys	As per Council surveys (November 2020)
Data analyses and report preparation	September 2020 to June 2021
Draft final report for 2020/21	30 June 2021

Project deliverables – Year 1

Deliverable	**Date**
Progress report: • Provide detailed account of all genetic work including *Chlamydia* undertaken to date with any basic initial descriptive summary statistics available.	31 March 2019
Final report: • *Chlamydia* status of Minjerribah koalas. • Refine current genetic information for the population. • Quantify disease incidence. • Recommendations arising from results of fieldwork and data analyses to date.	30 June 2019

Part 1 - Chlamydia strain(s) of Minjerribah koalas

Aims and objectives

Until now, there has only been *Chlamydia pecorum* confirmed on the island, and strains have never been determined. Genetic sequencing of a selection of already collected samples that are *Chlamydia* positive should enable us to answer the long-standing question of whether the Minjerribah koalas have the same, different, or a subsample of the *C. pecorum* strains present on the mainland. This will inform appropriate management decisions in terms of quarantine and moratorium concerning the return of sick / injured koalas taken from the island and treated on the mainland.

Methods

C. pecorum-species specific assay screening

All samples have be screened for chlamydia using species–specific qPCR assays (validated for sensitivity and specificity) targeting a 209 bp region of the *C. pecorum* CpecG_0573 gene (Jelocnik et al. 2017) and performed in the Biorad qPCR instruments.

Molecular characterisation of the infecting strains

To evaluate genetic diversity of the infecting *C. pecorum* strains, *C. pecorum*-positive samples will be initially genotyped targeting a 359 bp region of the *C. pecorum* ompA gene (between variable regions three and four) as previously described (Marsh et al. 2011).

Further molecular characterisation will be performed with the standardised *C. pecorum*-specific Multi Locus Sequence Typing targeting seven house-keeping genes, a molecular barcoding tool demonstrated to be representative of the diversity observed across the whole *C. pecorum* genome (Jelocnik et al. 2013).

As chlamydial plasmid was associated with pathogenicity in koala strains, the infecting strains were also screened for plasmid carriage (Phillips et al. 2018).

Progress so far

We are still in the process of analysing results from scats collected in 2018.

In addition, in conjunction with the animal clinic on Minjerribah (Dr Jade Paterson), ocular and urogenital swab samples were taken for five kolas that were recently admitted to the clinic.

Whilst none of these koalas displayed any visual signs of ocular or urogenital chlamydia, a urogenital sample from a male koala located in Dunwich tested positive for *C. pecorum* with a chlamydial load of 22 *C. pecorum* copies per microliter (µl) of DNA. This sample has now been sent for ompA genotying in order to determine which *C. pecorum* strain(s) are present. Whilst *C. pecorum* was detected in this sample, we could not detect the presence of the plasmid.

Part 2 - Enhancement of current genetic information and quantify disease incidence

Aims and objectives

During the 2018 DDC surveys, the DDC collected many more scats than were budgeted for, leaving 204 scats from Minjerribah not analysed (compared to 156 already analysed). Therefore, by analysing currently available samples, enhancing genetic and disease knowledge is possible without further field surveys, greatly decreasing the investment required to achieve this aim. The same method as the DDC 2018 report, DArTcap, was used so that the results are directly comparable to the previous dataset and can be analysed together as one. The molecular method is briefly outlined below.

Methods

Fresh koala scats were collected in a sterile tube and kept on ice until they are stored in a -80 C freezer. DNA from the scats was genotyped using next-generation sequencing protocol similar to the one described in Schultz et al. (2018).

Koala and other DNA was extracted from intestinal epithelial cells on sampled scats. DNA isolates for each individual were genotyped in collaboration with Diversity Arrays Technology, Canberra, using proprietary DArTcap, a specific method derived from DArTseq™ technology, but where probes were specifically designed to improve SNP overlap between individuals.

These SNPs are used to assess gender, measure levels of genomic diversity (heterozygosity (H)), inbreeding (Fis) and estimates of population structure (gene flow, Fst), as well as presence of *Chlamydia*.

The genetic analyses can deliver the following information:

1. Koala genetic diversity, inbreeding;
2. Minimum number of individual koalas, sex ratio;
3. Chlamydia prevalence.

FastSTRUCTURE was used to identify the degree of admixture in individuals and infer the population of origin of specific individuals (Raj et al. 2014). In doing so, we were able to identify number of genetic populations present on Minjerribah. FastSTRUCTURE was run with the number of genetic groups set from K = 1 to K = 10 using default parameters. We applied

ΔK (Evanno et al. 2005) method and the (Ln(Pr(X|K)) method (Pritchard et al. 2000) to infer the number of genetic populations.

Population differentiation between the populations identified using FastSTRUCTURE was calculated using $F_{ST.}$ These values were calculated using 999 permutations in GeneAIex (Peakall and Smouse 2006).

Spatial autocorrelation analyses were conducted in GeneAlEx 6.5 (Peakall and Smouse 2006) using a genetic distance matrix, representing the total genetic distance over 660 loci, and a geographic distance matrix. These analyses identify distances at which individuals are more related than expected if there was no fine-scale spatial structure of related individuals. We used sequentially increasing distance classes ranging between 1 to 15 kilometres.

Patterns of genetic diversity were assessed using GeneAlEx 6.5 (Peakall and Smouse 2006) using the following genetic diversity measures:

- Shannon's information index (I)
- Expected heterozygosity (H_E)
- Observed heterozygosity (H_O)

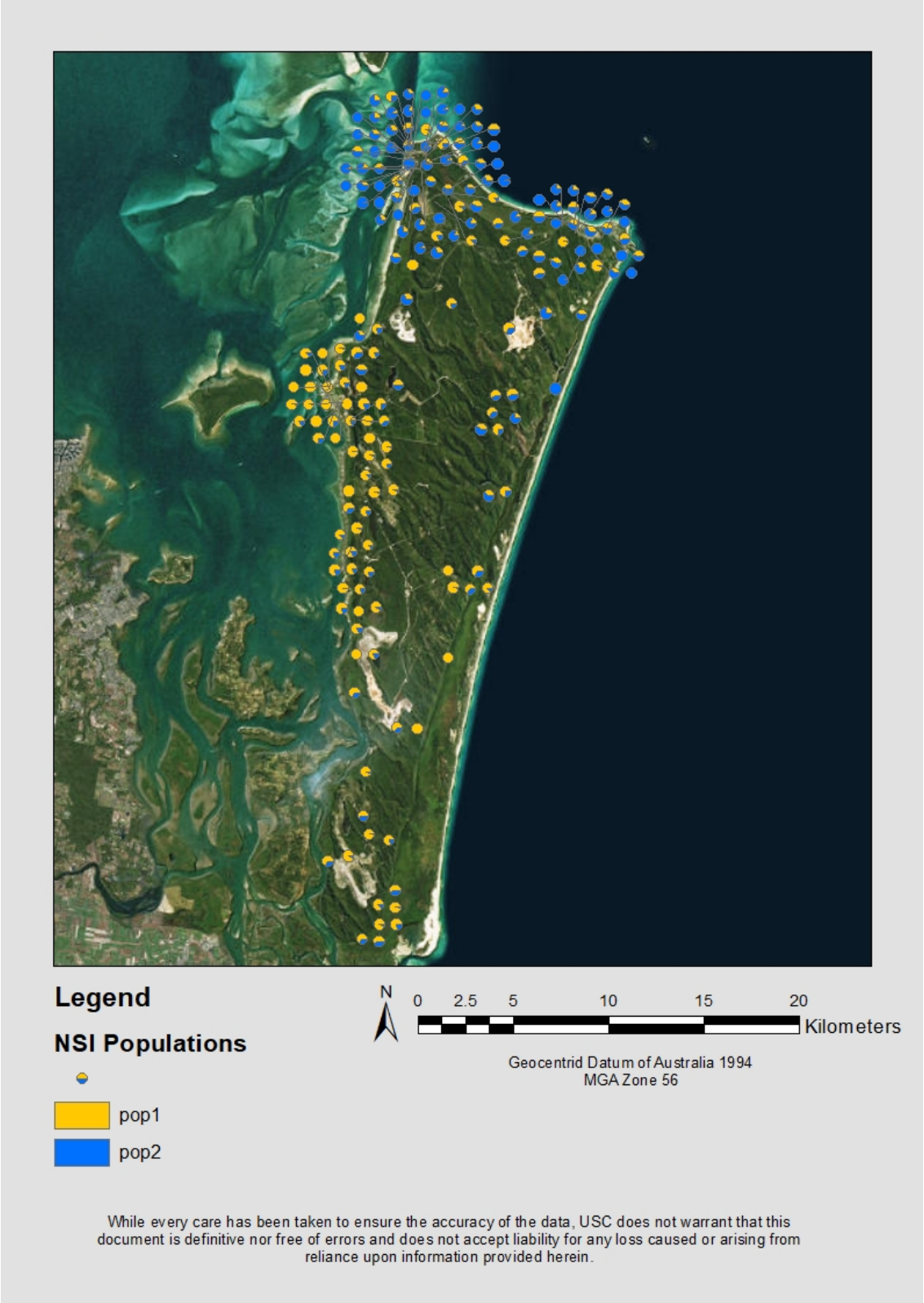
Observed (H_O) and expected (H_E) heterozygosities were calculated at each loci and population to test for possible departure from Hardy-Weinberg equilibrium, using the Markov chain method in GeneAlEx 6.5.

At the population level, the inbreeding coefficient (F_{IS}) was calculated using observed (H_O) and expected (H_E) heterozygosity [i.e., $F_{IS} = (H_E - H_O) / He$].

Progress so far

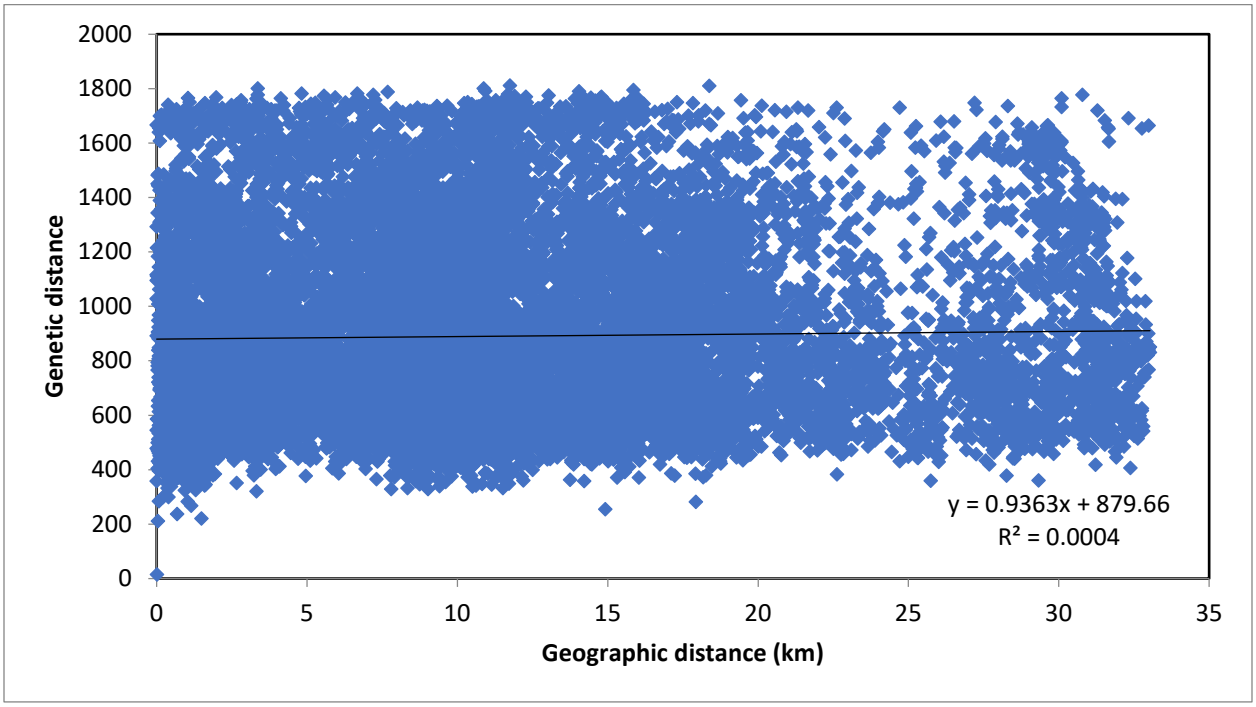
A total of 360 genetic samples were collected on Minjerribah during the DDC 2018 surveys. DNA was extracted of all 360 samples that were collected. After a first quality control, 340 samples were sent to Diversity Array Technology for genotyping. DArT applied a second quality control which caused 15 samples to drop out. We checked the remaining 325 samples for duplicates, and following the removal of these duplicate samples (85 duplicate samples) and downstream SNP quality control, a total of 200 unique individuals remained for use in subsequent genetic analyses.

Using FastSTRUCTURE, a program for estimating spatial population structure based on geography, we found evidence for two ancestral populations across Minjerribah which, based on their geographic locations, are referred to as north Minjerribah (yellow) and south Minjerribah (blue; Part 2 - Figure 1). Whilst two ancestral populations were identified, we found a low but significant genetic differentiation between these two populations (F_{ST} = 0.021, P value = 0.001; F'_{ST} = 0.040). Minjerribah koalas can therefore, be considered one continuous population (i.e. can be considered a single breeding population).



Part 2 - Figure 1: Showing the genetic admixture of each individual on Minjerribah as estimated using FastSTRUCTURE

Additionally, we found no evidence that genetic differentiation increases with increasing geographic distance (genetic isolation by distance) further supporting the fact that Minjerribah is a continuous breeding population (Part 2 - Figure 2).



Part 2 - Figure 2: Scatterplot showing the relationship between genetic and geographic distance for Minjerribah koalas

Minjerribah (North Stradbroke Island) Post Burn Koala Monitoring

Aims and objectives

The DDC was asked to conduct surveys to determine whether koalas had returned and used areas that were burned in January 2019. Specifically, to re-survey areas where records of scats were found during surveys conducted by the DDC in 2018.

Methods

Mapping the extent of the fire

Fire mapping was commissioned and delivered with the GIS information distributed. Part 3 - Figure 1 shows the extent of the fire on North Stradbroke Island extracted using atmospherically corrected Sentinel 2 images acquired on 18th November 2018 and 11th January 2019.

The polygons derived from the raster image was cleaned for wetlands and cloud covers interfering with burn area estimation.

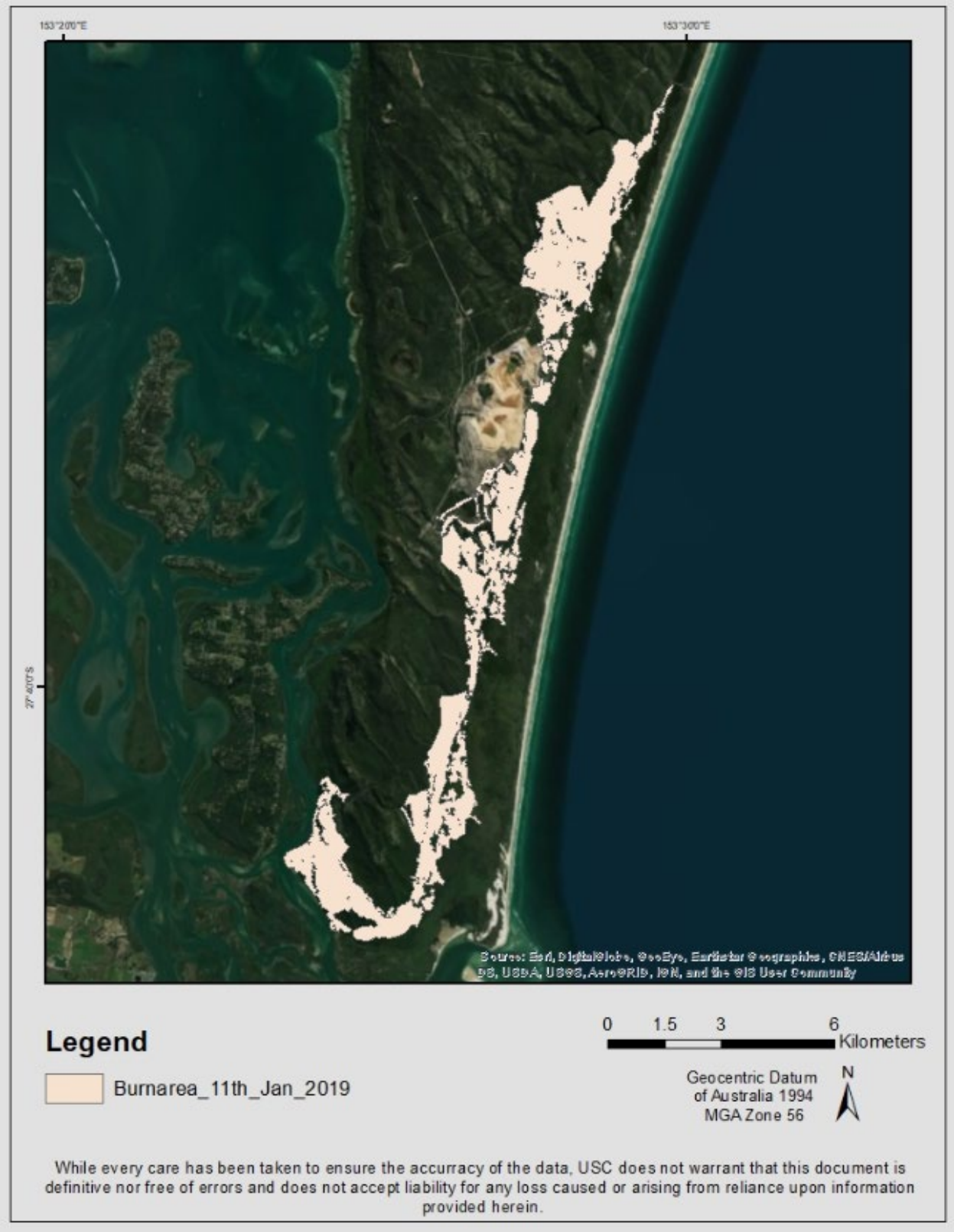
The Sentinel Images were downloaded from European Space Agency's Copernicus Open Access Hub (https://scihub.copernicus.eu/). The final product was derived by Dr Sanjeev Kumar Srivastava using ENVI 5.5 and ArcGIS 10.6 software packages.

Extent

West	153.392023	**East**	153.497420
North	-27.522230	**South**	-27.726941

Scale Range

Maximum (zoomed in)	1:5,000
Minimum (zoomed out)	1:50,000



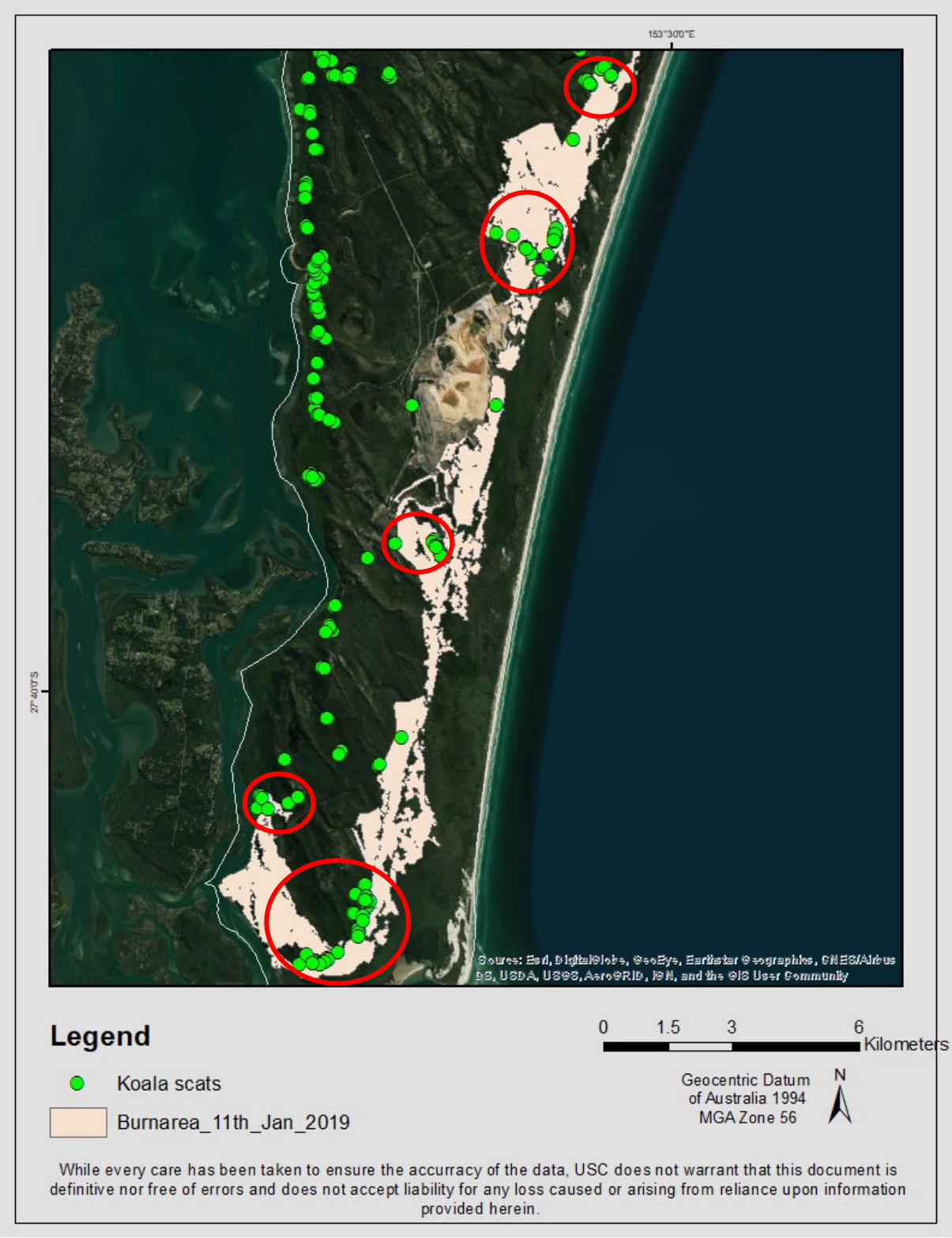
Part 3 - Figure 1: **Mapped fire footprint**

Koala surveys

Survey sites were locations where scats had been recorded during previous surveys in 2018 and overlayed the mapped fire footprint. In 2018, 303 surveys were conducted on North Stradbroke Island of which 360 scats were collected (Part 3 - Figure 2). It was noticeable that there were areas considered hotspots, where a high abundance of scats were found and therefore likely to be highly used by koalas. We used the hotspots that overlapped with the fire footprint as areas to focus our surveys. We attempted to survey as much of the area surrounding a site, if scats were not found we proceeded to the closest survey location.

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).

The dog was then fitted with a GPS collar, motivated with a tennis ball and given the command to search. The GPS tracking of the dog enables us to quantify the survey effort at each site. The dog was rewarded at the end of a survey in which no detections were made, with a planted “reward” koala scat, to ensure a high level of dog enthusiasm during subsequent searches was maintained.



Part 3 - Figure 2: Green circles represent sites where koala scats were found during surveys in 2018, and areas circled were considered hotspot areas used by koalas targeted to re-survey.

For this project, we used casual koala scat survey. The casual surveys are an excellent and fast way to determine whether koalas are present at a specific site. This method is indeed designed to maximise the chance of detecting koala presence in the minimum amount of time. In this project, casual surveys were used to increase coverage.

In the casual surveys, the dog is not constrained by the handler, 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 was chosen where previous surveys were conducted in 2018 and scats were found.

Note that "casual" detection dog surveys were used in the 2018 Minjerribah survey by the DDC, because the goal was to collect genetic samples and not to calculate habitat use. This means that no comparison of habitat utilisation (i.e. proportion of trees used) can be performed. However, we can determine whether areas previously used by koalas are still actively being used.

Typical koala scats (Part 3 - Figure 3) 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.

When scats are found, their age were recorded as well as their GPS coordinates (GDA94). Age of scats (see Part 3 - Table 1) allowed us to classify whether sites were recently used or not.

Part 3 - Figure 3: Typical koala scats

Part 3 - Table 1 Age categories and characteristics of koala scats

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

Summary of findings

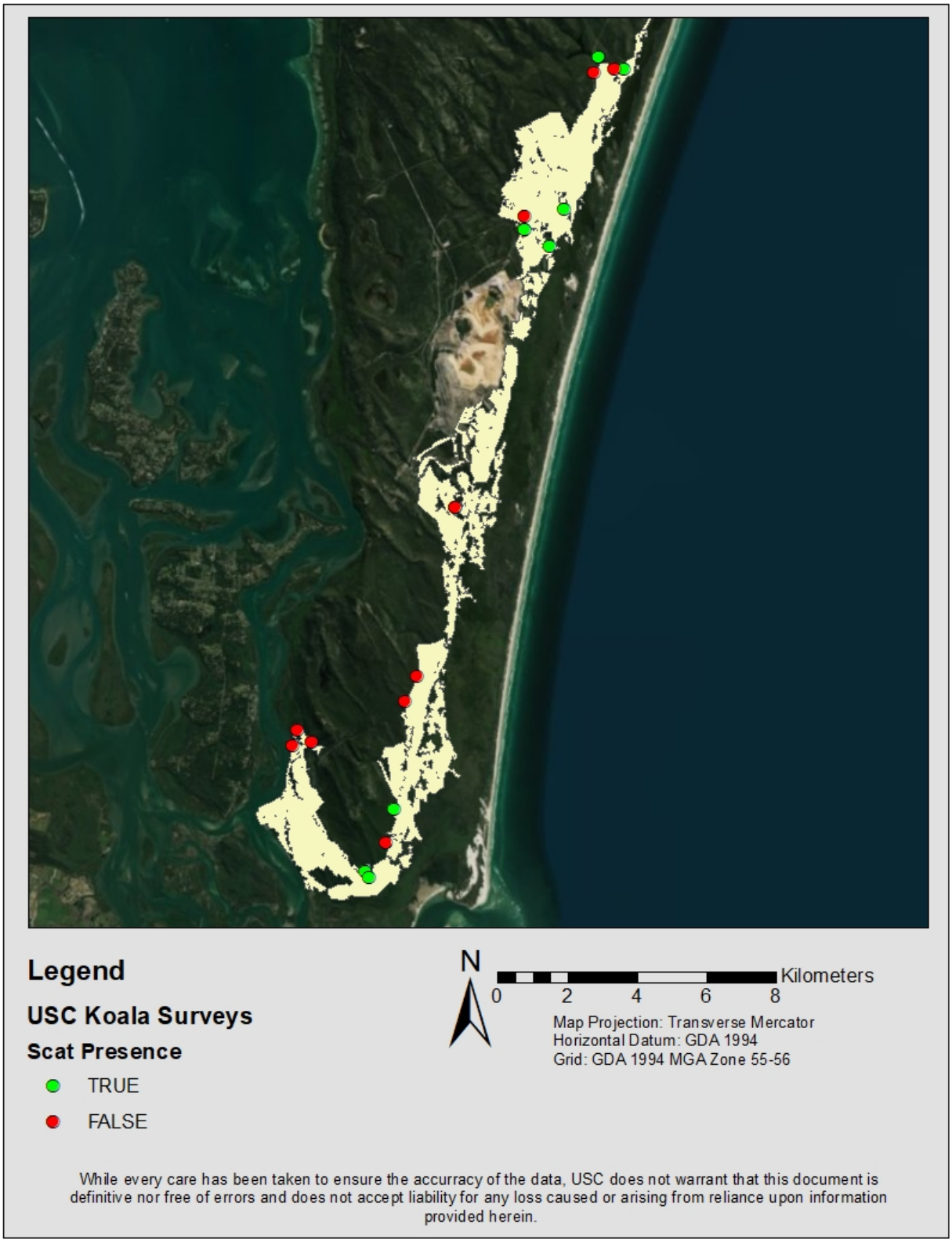
Due to baiting, we completed surveys on 21/05/2019. These were conducted by Dr. Romane Cristescu and Dr. Riana Gardiner with all-age scat detection dogs Maya and Baxter.

We conducted a total of **19 surveys** from which we found koala scats at **9 sites**, and found a lack of presence at **10 sites** (Figure 4). There was a total of 15 indications on koala scats (Part 3 - Figure 5) by detection dogs across surveys. Moreover, we found scats varying in ages between age categories 1-4 (Part 3 - Figure 6; Part 3 - Table 2).

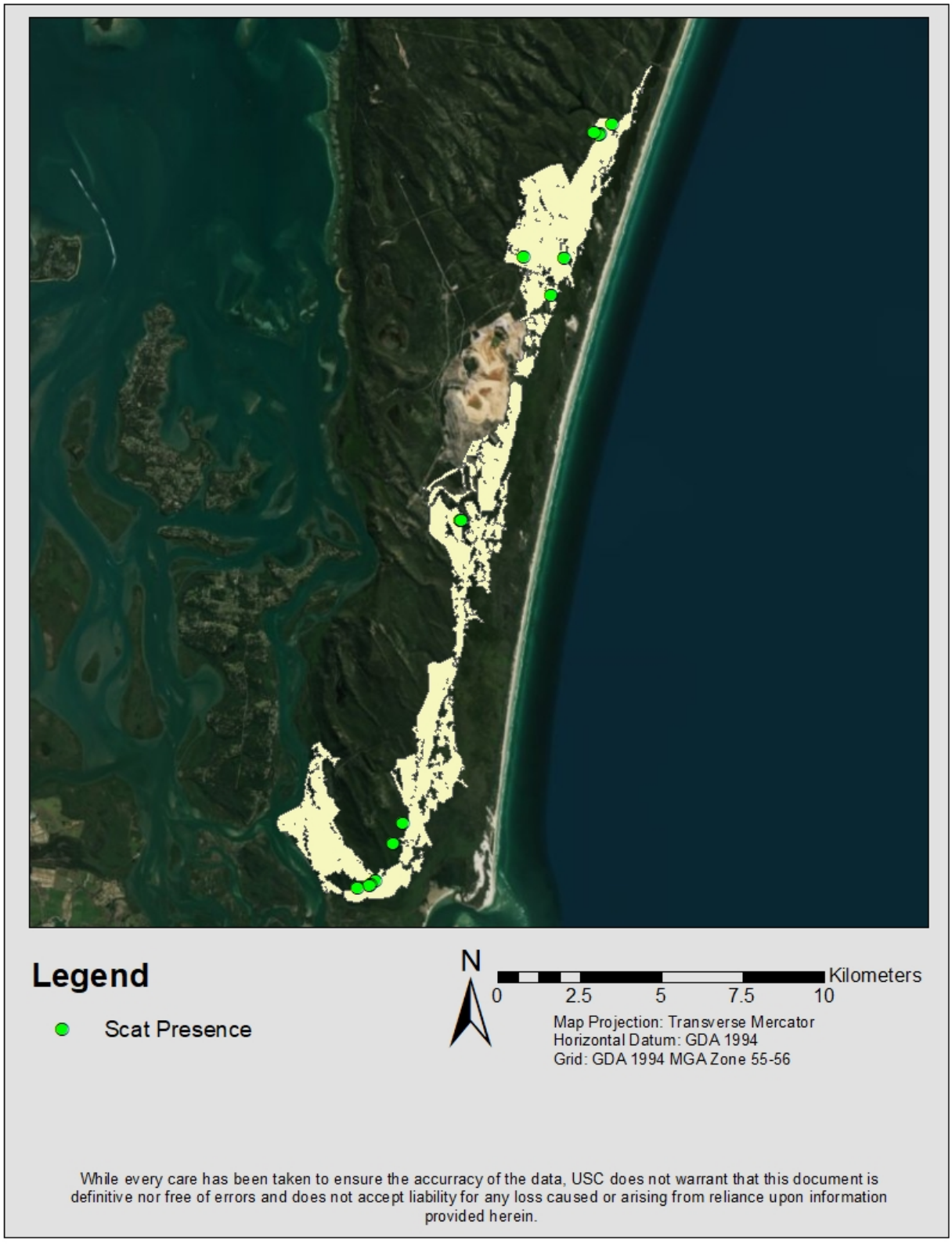
The presence of scats found and the range of age categories, we can assume koalas are successfully using most areas within the fire footprint 4 months after the burn. The only areas we did not find any evidence of koala scats near the 'Fisherman huts'.

Part 3 - Table 2 Categories of scat age found during post-burn surveys

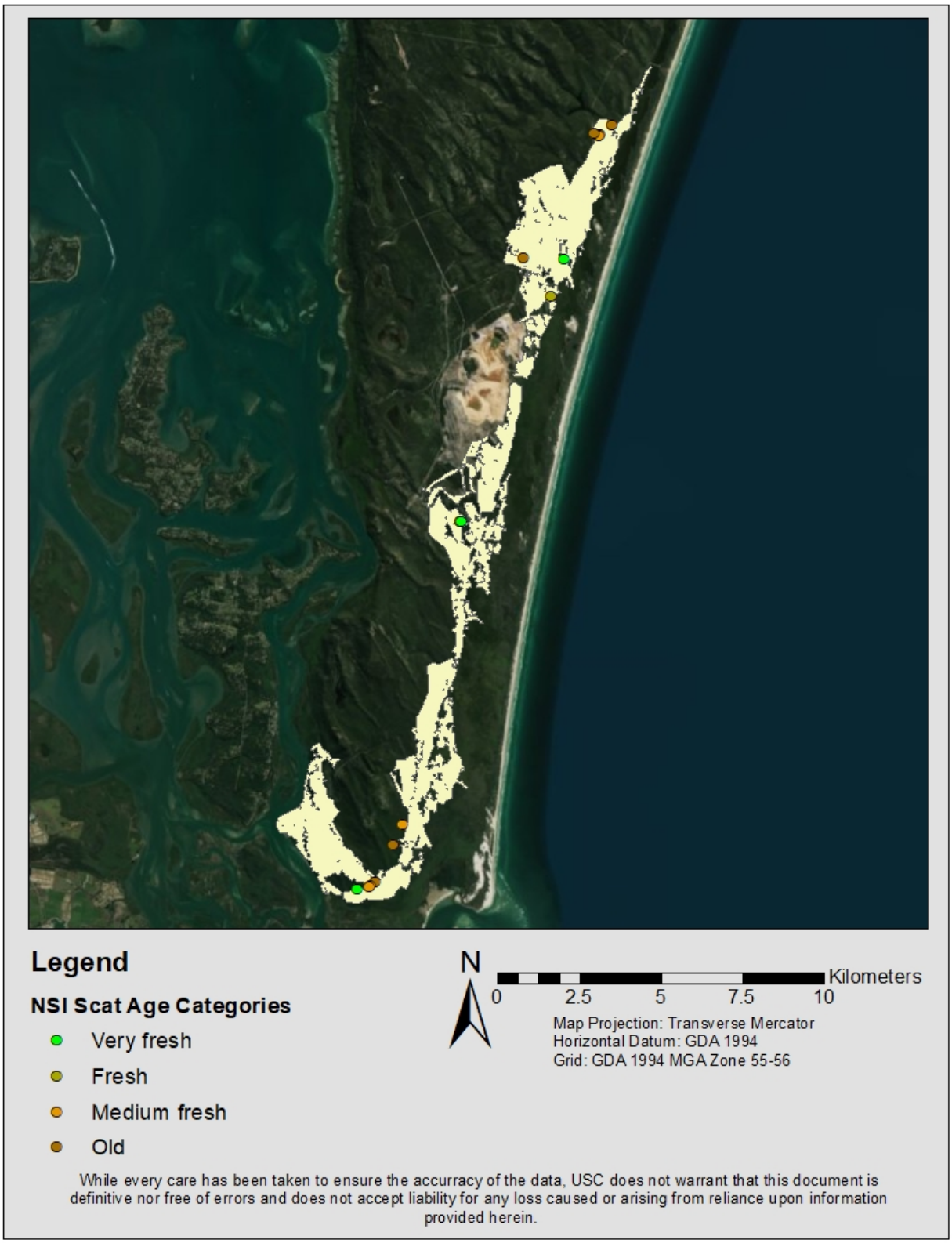
Scat age	Expected Age range	Count
1 Very fresh	1 day old or less	3
2 Fresh	Couple of days old	1
3 Medium fresh	Couple of weeks	4
4 Old	Months old	7



Part 3 - Figure 2: **Koala surveys conducted in the fire footprint. Red circles represent absence of scats at each survey, green circles represent recorded scat presence at each survey.**



Part 3 - Figure 3: **Koala scat presence indicated by detection dogs in the fire footprint.**



Part 3 - Figure 4: Age categories of scats found during surveys in the mapped fire footprint

References

Evanno, G., Regnaut, S., and Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* **14(8)**:2611-2620.

Jelocnik, M., Frentiu, F. D., Timms, P., and Polkinghorne, A. 2013. Multi-locus sequence analysis provides insights into the molecular epidemiology of Chlamydia pecorum infections in Australian sheep, cattle and koalas. *Journal of Clinical Microbiology*:JCM. 00992-00913.

Jelocnik, M., Islam, M. M., Madden, D., Jenkins, C., Branley, J., Carver, S., and Polkinghorne, A. 2017. Development and evaluation of rapid novel isothermal amplification assays for important veterinary pathogens: Chlamydia psittaci and Chlamydia pecorum. *PeerJ* **5**:e3799.

Marsh, J., Kollipara, A., Timms, P., and Polkinghorne, A. 2011. Novel molecular markers of Chlamydia pecorum genetic diversity in the koala (Phascolarctos cinereus). *BMC Microbiology* **11(1)**:77.

Peakall, R., and Smouse, P. E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6(1)**:288-295.

Phillips, S., Robbins, A., Loader, J., Hanger, J., Booth, R., Jelocnik, M., Polkinghorne, A., and Timms, P. 2018. Chlamydia pecorum gastrointestinal tract infection associations with urogenital tract infections in the koala (*Phascolarctos cinereus*). *PLOS ONE* **13(11)**:e0206471. http://doi.org/10.1371/journal.pone.0206471

Pritchard, J. K., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155(2)**:945-959.

Raj, A., Stephens, M., and Pritchard, J. K. 2014. fastSTRUCTURE: Variational Inference of Population Structure in Large SNP Data Sets. *Genetics* **197(2)**:573-589. http://doi.org/10.1534/genetics.114.164350

Schultz, A., J., Cristescu, R., H., Littleford-Colquhoun, B., L., Jaccoud, D., and Frère, C., H. 2018. Fresh is best: accurate SNP genotyping from koala scats. *Ecology and Evolution* **8(6)**:3139-3151. http://doi.org/10.1002/ece3.3765

Triggs, B. 1996. Tracks, scats and other traces: A field guide to Australian mammals. Oxford University Press, South Melbourne.