Minjerribah (North Stradbroke Island) Koala Monitoring

Progress Report

Prepared for:

Redland City Council

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

Introduction

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 timeframe 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 (impact of fire on the koala population and greater certainty on population numbers/density over time).

Therefore the Minjerribah 2019-2020 projects had the following main deliverables:

- Developing methods to estimate population size (drone outside urban areas)

- Chlamydia analyses of samples from Minjerribah koalas
- Genetic analyses of scat samples already collected by the DDC
- Enhance genetic data collection thanks to citizen scientists in urban area,
- Surveys of the 2018/2019 fire footprint

The post fire surveys and updated genetic analyses were reported on in the last report (June 2019), and will not be further reported here.

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. Part of this research project aims 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 could 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. Genetic sequencing of a selection of already collected scat samples that were Chlamydia positive was unsuccessful, and the project is now collecting swabs, a higher quality sample.

Methods

C. pecorum-species specific assay screening

Samples were screened for chlamydia using species–specific qPCR assays. This has been validated for sensitivity and specificity and targets a 209 bp region of the *C. pecorum* CpecG_0573 gene (Jelocnik et al. 2017). The qPCR assays were performed in the Biorad qPCR instruments.

Molecular characterisation of the infecting strains and plasmid

To evaluate genetic diversity of the infecting *C. pecorum* strains, *C. pecorum*-positive samples were 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). As it is suspected that chlamydial plasmid is associated with pathogenicity in koala chlamydial disease, the infecting strains were also screened for plasmid carriage (Phillips et al. 2018). We performed qPCR detection for the *C. pecorum* plasmid targeting a 233 bp fragment of the of the *C. pecorum* plasmid (CDS5 or Pgp3 locus) on all urogenital, ocular and rectal samples. Using specific primers plasF– 5'–AATGGAAGGAGCTGTTGTC– 3' and plasR– 5'–GATGTTGTTTCTGCATTAAGG– 3' and Bio-Rad Sybr-green Itaq master mix, with an initial 95˚C enzyme activation for 5 minutes, then

40 cycles of 95˚C denaturation for 5 seconds, 57˚C primer binding for 30 seconds and 72˚C for 25 seconds with a fluorescence data capture. Finally, a melt profile was generated from 55˚C to 95˚C at 0.5˚C per 2 seconds per step (Phillips et al. 2018).

Results

The results below, in Table 1, include urogenital, ocular and rectal swab samples taken for six koalas (13 swabs) that were recently admitted to the Minjerribah clinic (Dr Jade Patterson). 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 was sent for ompA genotying in order to determine which *C. pecorum* strain(s) was present, but unfortunately the Omp A genotype was not typable. Whilst *C. pecorum* was detected in this sample, we could not detect the presence of the plasmid. An additional 12 koalas have been sampled and are waiting to be processed. Results from swabs collected from Ormiston koalas, on the mainland, are given for comparison. Omp A genotype A was detected in two koalas in Ormiston.

Table 1: Results of the *Chlamydia pecorum* detection, genotyping and the presence of plasmid for 43 swabs collected from 16 koalas (six island koalas and 10 mainland koalas)

Koala Name	Date of Collection	Location	Sample type	Sex	Date of DNA extraction	Beta Actin (control)	C. pecorum copies/ ul of DNA	C. pecorum copies/ ul of sample	Plasmid copies/ ul of DNA	Plasmid copies/ ul of sample	ompA genotype
Dunwich	22/04/2019	Minjerribah	Urogenital	Male	9/06/2019	Detected	22	5500	0	0	Not Typable
Dunwich	22/04/2019	Minjerribah	Ocular	Male	9/06/2019	Detected	0	0	0	0	
Waller C.A	23/02/2018	Minjerribah	Urogenital	Female	9/06/2019	Detected	0	0	0	0	
Waller C.A	23/02/2018	Minjerribah	Unrecorde	Female	9/06/2019	Detected	0	0	0	0	
Flinders	1/03/2018	Minjerribah	Urogenital	Male	9/06/2019	Detected	0	0	0	0	
Flinders	1/03/2018	Minjerribah	Ocular	Male	9/06/2019	Detected	0	0	0	0	
Abdohemia	31/05/2019	Minjerribah	Urogenital	Male	9/06/2019	Detected	0	0	0	0	
Abdohemia	31/05/2019	Minjerribah	Ocular	Male	9/06/2019	Detected	0	0	0	0	
Abdohemia	31/05/2019	Minjerribah	Rectal	Male	9/06/2019	Not Detected	Unassesable	Unassesable	0	0	
Church	26/01/2018	Minjerribah	Urogenital	Male	9/06/2019	Detected	0	0	0	0	
Church	26/01/2018	Minjerribah	Ocular	Male	9/06/2019	Detected	0	0	0	0	
Maveric	15/04/2017	Minjerribah	Ocular	Unrecorded	9/06/2019	Detected	0	0	0	0	
Maveric	15/04/2017	Minjerribah	Rectal	Unrecorded	9/06/2019	Detected	0	0	33	8170	Not Typable
Monty	1/05/2019	Ormiston	Urogenital	Male	9/06/2019	Detected	0	0	0	0	
Monty	1/05/2019	Ormiston	Left Eye	Male	9/06/2019	Detected	0	0	0	0	
Monty	1/05/2019	Ormiston	Right Eye	Male	9/06/2019	Detected	0	0	0	0	
Banjo	24/04/2019	Ormiston	Urogenital	Male	9/06/2019	Detected	0	0	0	0	
Banjo	24/04/2009	Ormiston	Left Eye	Male	9/06/2019	Detected	0	0	0	0	
Banjo	24/04/2019	Ormiston	Right Eye	Male	9/06/2019	Detected	0	0	0	0	
Brian	17/04/2019	Ormiston	Urogenital	Male	9/06/2019	Detected	0	0	0	0	
Brian	17/04/2019	Ormiston	Left Eye	Male	9/06/2019	Detected	0	0	0	0	
Brian	17/04/2019	Ormiston	Right Eye	Male	9/06/2019	Detected	0	0	0	0	
Kimo	17/04/2019	Ormiston	Urogenital	Female	9/06/2019	Detected	0	0	0	0	
Kimo	17/04/2019	Ormiston	Left Eye	Female	9/06/2019	Detected	0	0	0	0	
Kimo	17/04/2019	Ormiston	Right Eye	Female	9/06/2019	Detected	0	0	0	0	
Ted	1/05/2019	Ormiston	Urogenital	Male	9/06/2019	Detected	2	432	52	13000	Not Typable
Ted	1/05/2019	Ormiston	Left Eye	Male	9/06/2019	Detected	0	0	0	0	
Ted	1/05/2019	Ormiston	Right Eye	Male	9/06/2019	Detected	0	0	0	0	
Lucky	17/04/2019	Ormiston	Urogenital	Male	9/06/2019	Detected	1070	267000	7240	1810000	genotype A
Lucky	17/04/2019	Ormiston	Left Eye	Male	9/06/2019	Detected	0	0	0	0	
Lucky	17/04/2019	Ormiston	Right Eye	Male	9/06/2019	Detected	0	0	0	0	
Gumnut	24/04/2019	Ormiston	Urogenital	Female	9/06/2019	Detected	0	0	0	0	
Gumnut	24/04/2019	Ormiston	Left Eye	Female	9/06/2019	Detected	0	0	0	0	
Gumnut	24/04/2019	Ormiston	Right Eye	Female	9/06/2019	Detected	0	0	0	0	
Lulu	24/04/2019	Ormiston	Urogenital	Female	9/06/2019	Detected	0	0	0	0	
Lulu	24/04/2019	Ormiston	Left Eye	Female	9/06/2019	Detected	0	0	0	0	
Lulu	24/04/2019	Ormiston	Right Eye	Female	9/06/2019	Detected	0	0	0	0	
Cuddles	1/05/2019	Ormiston	Urogenital	Female	9/06/2019	Detected	0	0	0	0	
Cuddles	1/05/2019	Ormiston	Left Eye	Female	9/06/2019	Detected	0	0	0	0	
Cuddles	1/05/2019	Ormiston	Right Eye	Female	9/06/2019	Detected	0	0	0	0	
Bruce	1/05/2019	Ormiston	Urogenital	Male	9/06/2019	Detected	206	51600	1380	345000	genotype A
Bruce	1/05/2019	Ormiston	Left Eye	Male	9/06/2019	Detected	0	0	0	0	
Bruce	1/05/2019	Ormiston	Right Eye	Male	9/06/2019	Detected	0	0	0	0	

Part 2. Use of citizen scientists to maximise genetic data collection within urban areas (fresh scats collection)

Aims and Objectives

The DDC team suggested to conduct a fresh scats survey alongside the RCC citizen scientist surveys, to enhance the collection of larger genetic datasets. This dataset can be used to determine koala sex and enhance genetic diversity and inbreeding estimates. We have been considering the use of this dataset for population estimate (mark-recapture), however, this would require the survey to occur two weekends which is logistically challenging.

Methods

First, citizen scientists conducted their visual searches of koalas in each township (Amity Point, Point Lookout and Dunwich), if a koala was spotted the citizen scientists recorded the location and flagged the tree in which the animal was found. These locations were provided to the USC team ahead of their search.

Team members Romane Cristescu, Riana Gardiner and genetics scat dog Billie-Jean then targeted the locations in which koalas were spotted. The team confirmed the presence of koalas and recorded their location and of any others that were spotted. If there was fresh scat below the animals, these were collected in sterile tubes and kept on ice until they were stored in a -80C freezer.

Progress so far

The surveys were completed on the 12th and 13th of October 2019. Within the two days, the USC team spotted a total of 33 koalas (Table 2; Figure 1-Figure 3). In some cases, the koalas had already moved on and could not be found. From the surveys, the team managed to collect a total of 66 fresh koala scat samples (Table 1; Figure 4-Figure 6) from the three townships - double the number of koalas spotted (these included samples from females and their joeys). The samples are currently stored in USC's freezers and will await further funding before they are analysed. As a proof of concept, this exercise worked extremely well and enhanced greatly our ability to collect fresh scats, therefore maximising sample size. We suggest this could be adopted in any area of particular interest to RCC (such as koala safe neighbourhoods).

Table 2: Number of koalas and scats found by the USC team after citizen scientists

Township	No. of koalas	No. of scats collected
Amity Point	13	35
Point Lookout	17	16
Dunwich	3	15
Total	**33**	**66**

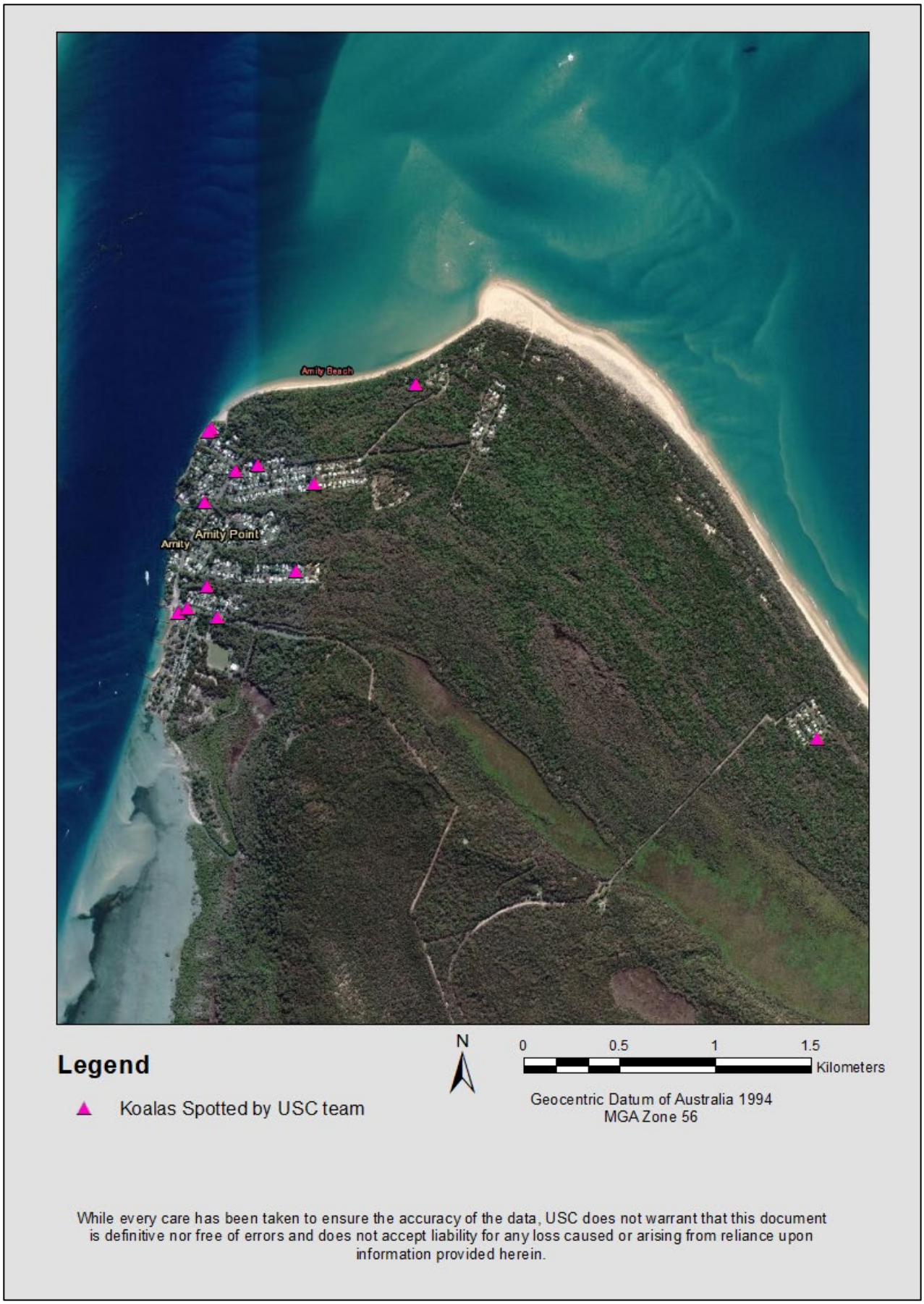

Figure 1: Koalas spotted by USC team in Amity Point

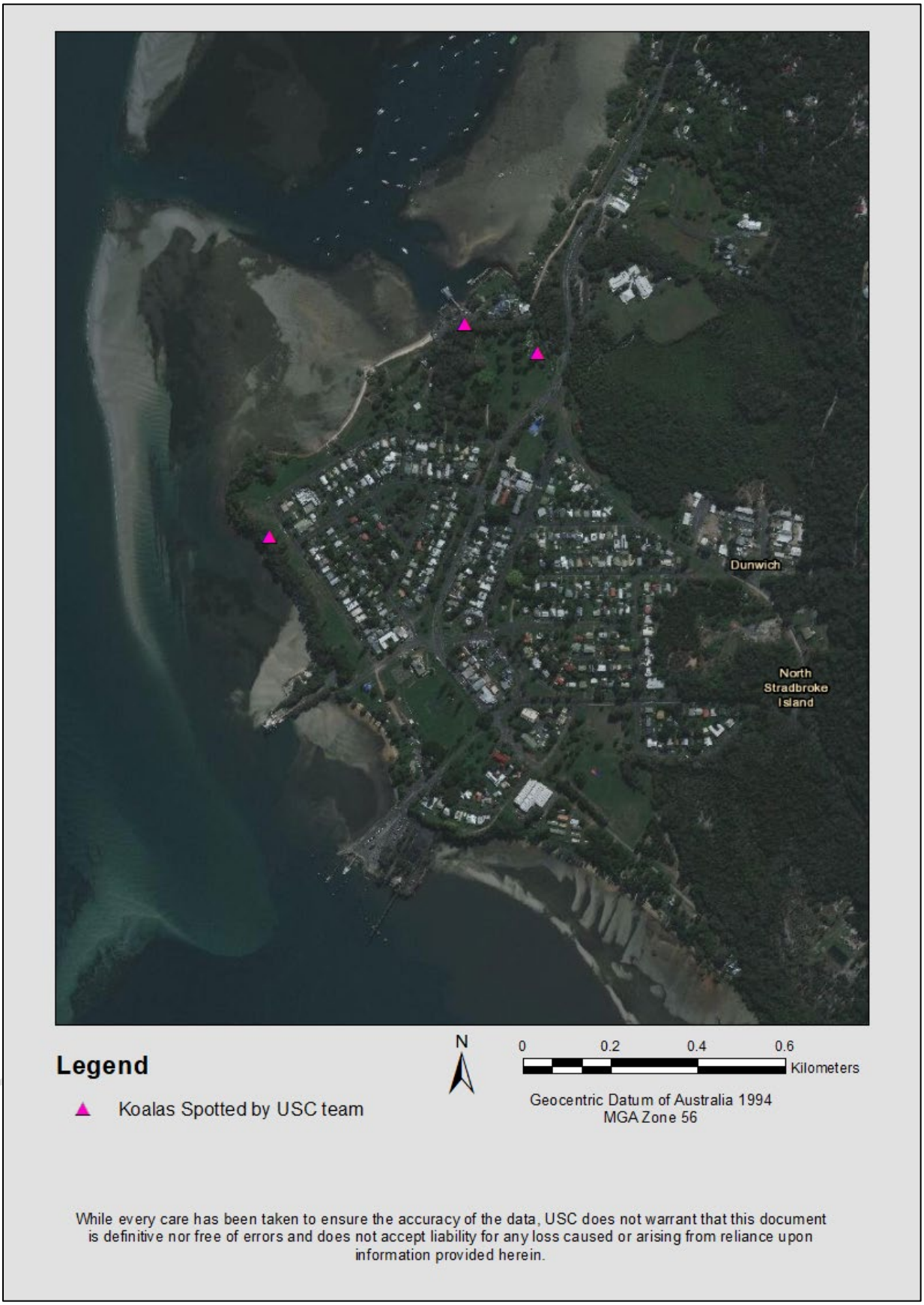

Figure 2: Koalas spotted by USC team in Dunwich

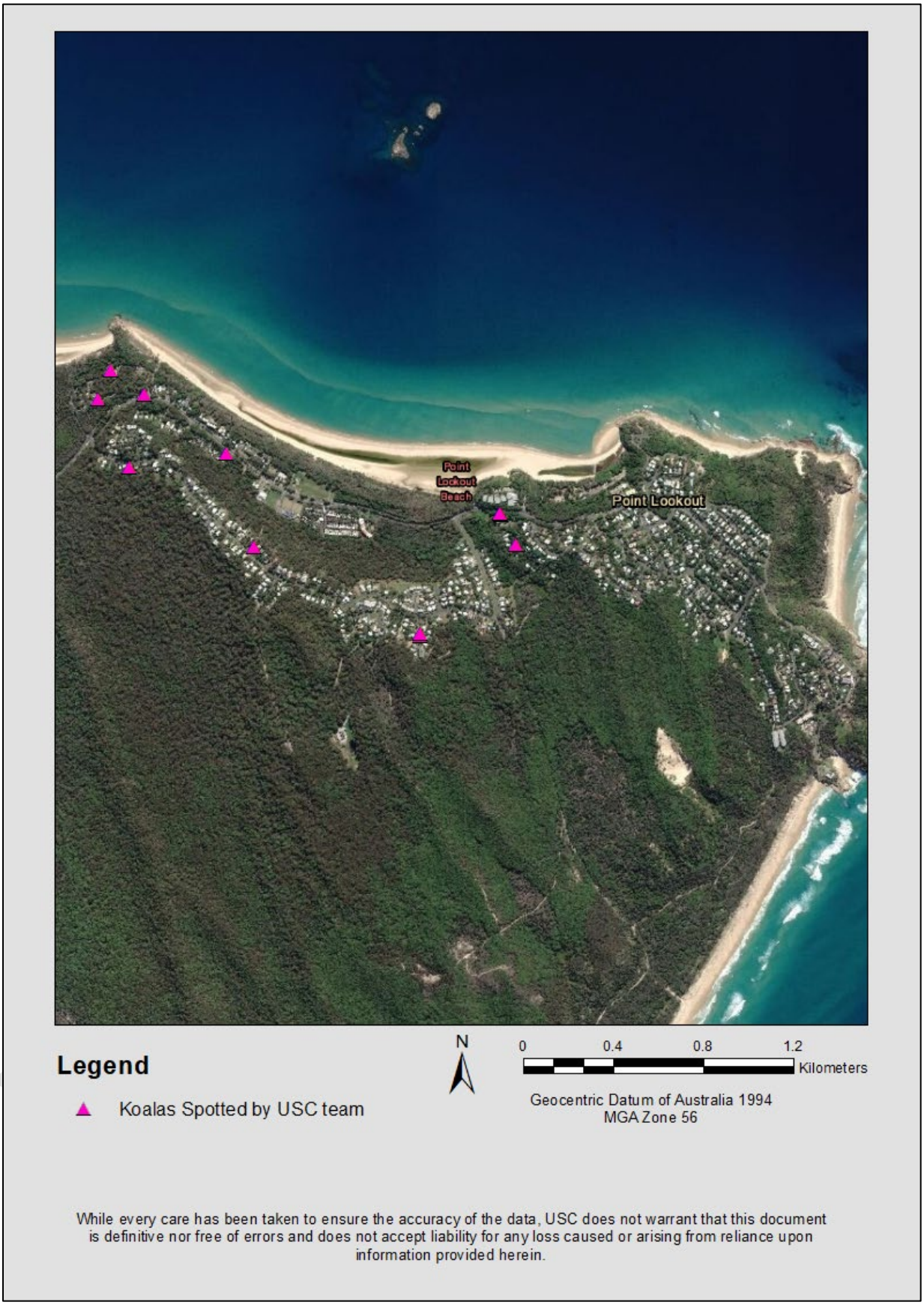

Figure 3: Koalas spotted by USC team in Point Lookout

Figure 4: Scats found by USC team in Amity Point after citizen scientists

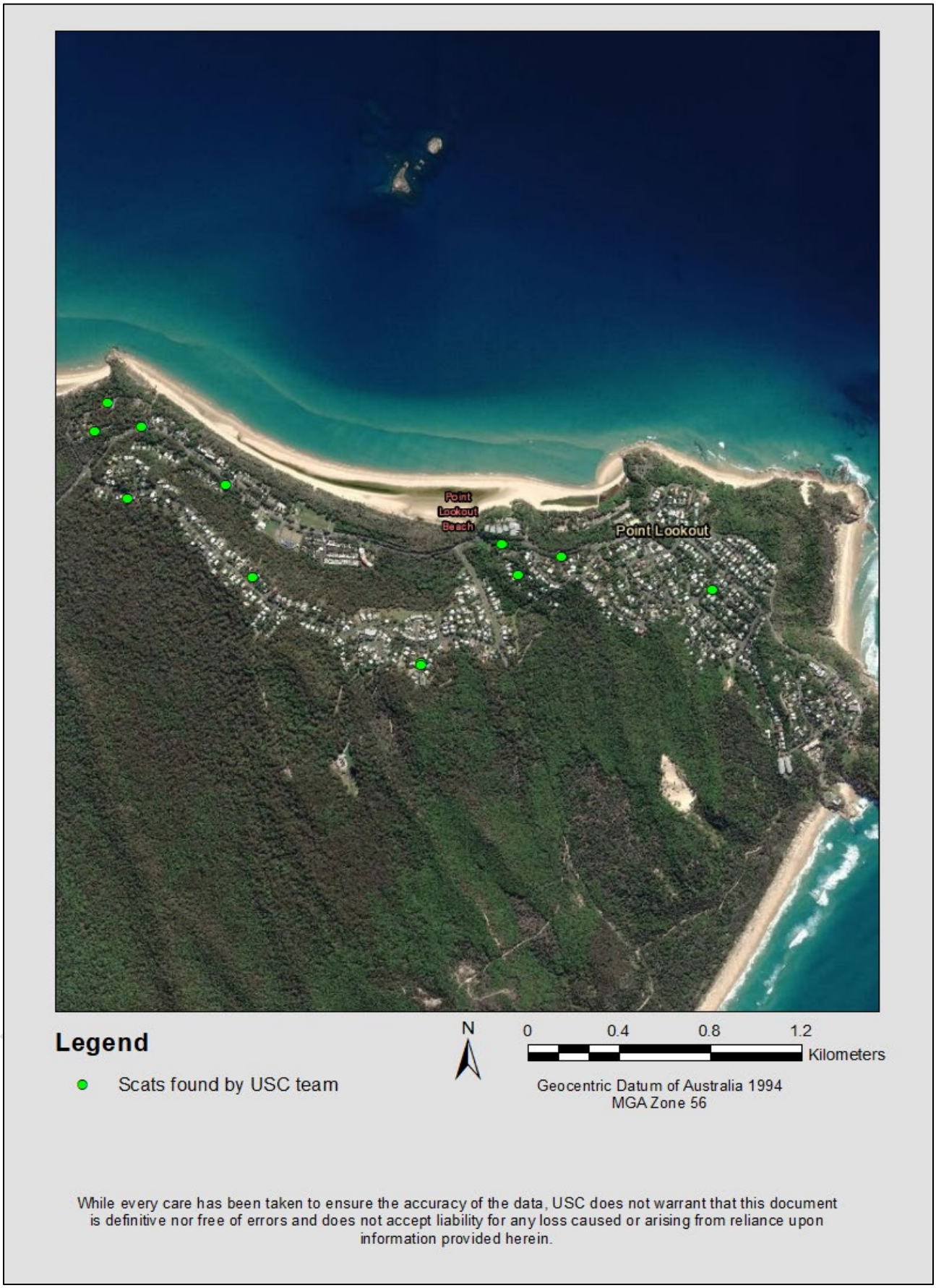

Figure 5: Scats found by USC team in Point Lookout after citizen scientists

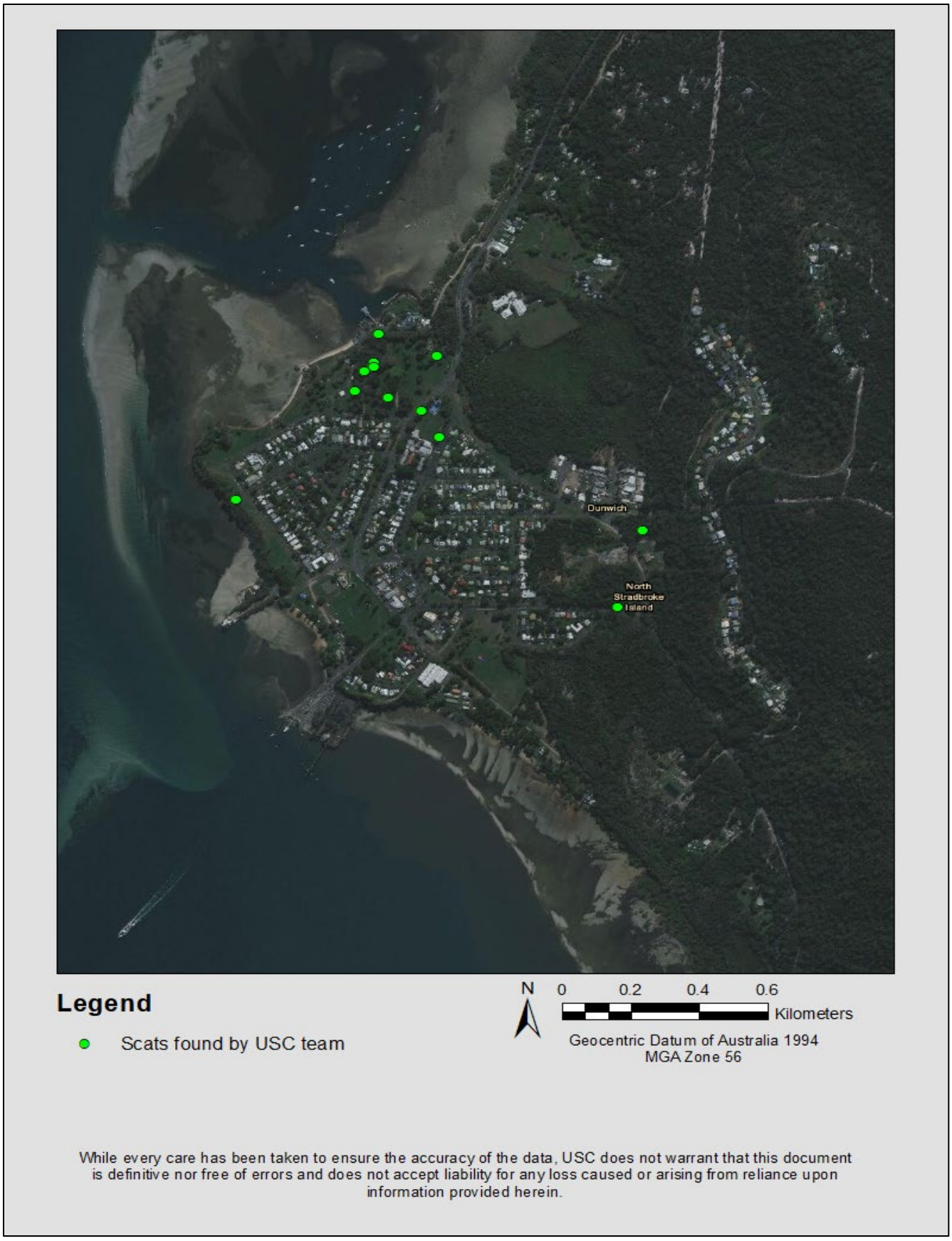

Figure 6: Scats found by USC team in Dunwich after citizen scientists

Part 3. Drone surveys outside urban areas

The DDC team is in the process of obtaining access to site from landholders and expect these surveys to start in March 2020, with the aim is to deliver them by the next reporting date.

References

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