

Woolgoolga to Ballina (W2B)
Pacific Highway Upgrade
Koala Monitoring Program
Year 1 (2017/18)
Annual Report



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1/94 Main Street Alstonville

www.sandpipereco.com.au

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Project team:

Dr D. Rohweder (project management, review)
Dr B. Taylor (project management, field survey, reporting)
Mr M. Jenkins (field survey)
Mr N. Priest (field survey)
Dr S. Fitzgibbon (field survey)
Dr A. Gillett (field survey)
Dr B. Barth (field survey)
Mrs M. Matthes (field survey)
Dr Rob Rankin (power analysis)
Ms Lyndal Hulse (DNA extraction and analysis)

Report prepared for:

Pacific Complete

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ABN: 82 084 096 828

PO Box 401
ALSTONVILLE NSW 2477
P 02 6628 3559 | E brendan@sandpipereco.com.au

Cover Photo: Ecologists viewing a koala on a transect within section 9.

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This report has been prepared in accordance with the scope of services described in the contract or agreement between Sandpiper Ecological Surveys (ABN 82 084 096 828) and RMS. The report relies upon data, surveys and measurement obtained at the times and locations specified herein. The report has been prepared solely for RMS and Sandpiper Ecological Surveys accepts no responsibility for its use by other parties. Sandpiper Ecological Surveys accepts no responsibility or liability for changes in context, meaning, conclusions or omissions caused by cutting, pasting or editing the report.

Executive Summary

Sandpiper Ecological Surveys (SES) was contracted by NSW Roads and Maritime Services (RMS) to implement the Woolgoolga to Ballina (W2B) Pacific Highway upgrade koala monitoring program in accordance with section 8 of the approved Koala Management Plan (KMP) (RMS version 4.4, July 2016), excluding phased resource reduction. The broad aim of the monitoring program is to determine the effectiveness of mitigation measures implemented in Sections 1-11 of the upgrade for koalas. The following report presents results of year one (2017/18) of the monitoring program.

The three main mitigation measures requiring monitoring are koala-proof fencing along the length of the upgrade (Sections 1-11), koala connectivity structures along the length of the highway upgrade (Sections 1-11) and koala food tree plantings (focus mainly in Section 10). Integral to these programs is the need to monitor trends in overall koala population size, particularly the two larger populations along the alignment at Broadwater (Sections 8/9) and Coolgardie-Bagotville (Section 10; hereafter referred to as Bagotville). Both are described as focal populations which could be adversely affected by the highway upgrade (RMS 2016).

Baseline data for the two focal populations were sourced from several population surveys conducted between 2013 and 2015. Bagotville baseline data were also used to inform the preparation of a Population Viability Analysis (PVA) in accordance with the Commonwealth Conditions of Approval (CoA 5 and CoA 7). The PVA for the Bagotville population indicated that this population is projected to decline significantly over the next 50 years unless key threatening processes are controlled. Monitoring of this population is considered critical for determining whether mitigation actions have been effective in slowing population decline. As such, the Bagotville focal population will be assessed against the PVA predictions at years 5, 10 and 15. The Broadwater population, which was not subjected to a PVA, will be assessed against a statistically significant decline at year 15 compared with baseline survey values (KMP).

In addition to population surveys, the year one monitoring program included preparation of a prospective power analysis to determine whether population survey effort had sufficient power to accurately detect population trends; targeted scat collection to enable DNA extraction and analysis; and road mortality surveys along Wardell Road and the Old Pacific Highway at Coolgardie-Wardell. Further, the population survey data were subjected to a Bayesian estimation analysis to derive density estimates for Broadwater and Bagotville populations for both baseline and year one.

Year one population monitoring involved day and night, radial and transect direct count surveys at 100 sites – 50 each in Broadwater and Bagotville. Surveys were completed by teams of three ecologists during spring 2017 and autumn 2018. Bayesian estimation analyses of Broadwater baseline and year one survey data reported a koala density estimate of 0.089 (95%CI: 0.039-0.177) and 0.089 (95%CI: 0.044-0.164) koalas ha⁻¹, respectively. Such data suggest a stable population trend.

For the Bagotville focal area, the Bayesian estimation analysis reported a baseline estimate of 0.092 (95%CI: 0.046-0.165) koalas ha⁻¹ and a year one estimate of 0.085 koalas ha⁻¹ (95%CI: 0.038-0.170). Despite the apparent 7.6% decline between baseline and year one values, no population trend could be discerned from the Bayesian estimation analysis given the high level of model uncertainty. This suggests that more survey data (i.e. subsequent monitoring years) are required to reduce model uncertainty. While acknowledging this, if we apply a 7.6% decline to the PVA baseline estimate of 236 koalas (Kavanagh 2016), it infers a population reduction by 18 koalas. Such a decline is well inside the 17% level (lower bound of the 90% confidence interval) within the first five years as prescribed by the KMP. As such, no corrective actions have been triggered by the KMP. It should also be noted that the focal koala populations may be affected by other impacts outside the control of the project, such as local land development, clearing activities and the 18% below average rainfall

experienced in the study area during the reporting period. Moreover, the Bagotville population was subjected to a wildfire that burnt out 350 ha of forest on the east side of the alignment during September 2017.

No koalas were detected during spring 2017 or winter 2018 road mortality surveys. Records retained by Pacific Complete on W2B Project Wide Koala Observations, which is supplemented by data from Friends of the Koala, reported one koala road mortality during the reporting period. The individual was struck on Old Bagotville Road, approximately 1km west of the alignment during a long-weekend outside of construction hours.

In working towards achieving the key mitigation measure of the PVA to reduce koala mortality by 4-8 individuals per year, RMS have implemented a predator control program, installed six vehicle-activated signs at road mortality hot-spots across the broader section 10 study area and fenced Wardell Road and the existing Pacific Highway. Since installation of fencing, no road strikes have been reported on these two stretches of road compared to 10 in the previous year (FOK, unpublished data).

The prospective power analysis demonstrated that the koala population monitoring program at Broadwater and Bagotville are likely to achieve their target levels of statistical power (>0.7) in order to detect a 30% decline in population over 15 years of monitoring, while maintaining a Type-I error rate of $\alpha \leq 0.3$. The analyses also revealed the challenge of sampling a population at very low densities and drawing conclusions from sparse counts. Subsequent power analyses should better resolve which factors are most important for surveying (such as seasonal or day/night effects) and could strengthen evidence for modifying the survey protocol.

Scat samples were collected from 19 individuals observed during surveys and incidental observations across the Bagotville focal area during year one. From these samples, genotypes across 30 microsatellite loci were generated. Analysis of genetic diversity was performed using the software GENALEX version 6.5 to calculate mean number of alleles and observed and expected heterozygosity. FSTAT was used to calculate inbreeding coefficient.

Genetic diversity analysis revealed that the population exhibits moderate to high diversity (A_{mean} and H_e) when compared to previous studies of koalas in NSW. This was based on comparable microsatellite marker data, despite indications of an increased inbreeding coefficient value. Whereas the data suggest genetic sub-structuring into two genetic clusters within the population, the clusters are not spatially segregated but largely mixed across the focal area. Indeed, evidence from the current study and previous work suggests that gene flow is occurring across the study area.

Overall, the year one monitoring program has completed the considerable task of establishing long-term population and road mortality monitoring sites and completed the first two seasons of surveys. This has been complemented by considerable and largely successful efforts to reduce koala road mortality and predation within section 10. There have also been strong, supportive links developed with landholders during year one, something that is critical to the long term success of the monitoring program. Data analysis protocols have also been developed and reviewed. Going forward, this should enable consistent and robust interpretation of data and well-informed program reviews

Acknowledgements

We wish to thank the landholders who approved access to their properties to conduct the population surveys. Numerous monitoring sites are on private property, so landholder support is critical to achieving a robust monitoring program. We also extend appreciation to the Jali Aboriginal Land Council for their approval and support for conducting surveys on Jali lands.

We received much feedback from members of the Koala Interest Group which both improved and sharpened aspects of the project. In particular, Lorraine Vass, Ros Irwin and Maria Mathers from Friends of the Koala (FOK) deserve special mention for their tireless work in supporting the persistence of koalas in the region.

The final report was improved by comments from Associate Professor Jonathon Rhodes (University of Qld) and Scott Lawrence, Simon Wilson and Julie Ravallion from Roads and Maritime Services.

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1. Introduction

1.1 Background

Sandpiper Ecological Surveys (SES) was contracted by NSW Roads and Maritime Services (RMS) to implement the Woolgoolga to Ballina (W2B) Pacific Highway upgrade koala monitoring program in accordance with section 8 of the approved Koala Management Plan (KMP) (RMS version 4.4, July 2016), excluding phased resource reduction. The broad aim of the monitoring program is to determine the effectiveness of mitigation measures implemented in Sections 1-11 of the upgrade for koalas.

The three main mitigation measures requiring monitoring are koala-proof fencing along the length of the upgrade (Sections 1-11), koala connectivity structures along the length of the highway upgrade (Sections 1-11) and koala food tree plantings (focus mainly in Section 10). Integral to these programs is the need to monitor trends in overall koala population size, particularly the two larger (focal) populations along the alignment at Broadwater (Sections 8/9) and Coolgardie-Bagotville (Section 10; hereafter referred to as Bagotville) (Figure 1). Both are described as focal populations, which could be adversely affected by the highway upgrade (RMS 2016). The two focal areas featured the highest density of koala records along the W2B alignment during environmental assessment population surveys (RMS 2016). Further, the two focal populations reportedly met the criteria for “important populations” according to the *Interim Koala referral advice for proponents* (DSEWPaC 2012).

Baseline data on the focal koala populations have come from a variety of sources. Population surveys of the Broadwater focal area were conducted during 2014 and 2015 (Ecosure 2014, 2015). The Bagotville koala focal population has been the subject of detailed field and laboratory studies (see Phillips and Chang 2013; Phillips *et al.* 2015), which informed the preparation of a Population Viability Analysis (PVA) (Kavanagh 2016). The PVA was conducted in accordance with the Commonwealth Conditions of Approval (CoA 5 and CoA 7) and its outcomes have been used to guide management of koalas within this area.

The PVA for the Bagotville key population indicated that this population is projected to decline significantly over the next 50 years (Kavanagh 2016) unless key threatening processes are controlled. Monitoring of this population is considered important to assist in determining whether mitigation actions have been effective in slowing population decline. As such, the Bagotville focal population will be assessed against the PVA predictions. The Broadwater population, which was not subjected to a PVA, will be assessed against a statistically significant decline at year 15 compared with baseline survey values (KMP).

1.2 Scope of works, program objectives and performance indicators

The monitoring program is designed to provide reliable information with which to inform management of koalas along the highway upgrade. The objectives of the KMP monitoring program for Sections 1-11 of the highway upgrade, as cited in the Ecological Services Brief (RMS 2017), include:

1. Evaluate the success of mitigation measures against the performance measures and corrective actions.
2. Assess the effectiveness of the fauna crossing structures and fauna exclusion fencing to facilitate movement of koalas across the upgraded highway.
3. Determine whether there is a statistically significant decline at year 15 compared with no decline in section 9.

4. Determine whether the corrective actions of the KMP have been triggered by estimated population trends in accordance with predictions of the Population Viability Analysis (PVA).
5. Provide information which supports a program review by RMS at years 5 and 10 in accordance with the KMP.
6. Assess effectiveness of the revegetation program in providing additional habitat for koalas.
7. Support a chronic stress response study being undertaken by RMS and Sydney University (NB. this item is separate to the KMP monitoring program).

Based on the above objectives, the success or otherwise of the monitoring program is determined by program performance against relevant performance indicators (PI). In addition to this, scat sampling will be conducted every three years in section 10 for the purposes of genetic analysis. These analyses aim to provide information on distribution and relatedness of individuals across the study area.

Table 8-4 in the KMP details eight performance indicators and their corresponding thresholds, corrective actions and agency responsible. The performance indicators and their relevance to the current year 1 report are described in Table 1.

As per the scope of works (RMS 2017), the following report documents the methods and results of the year one monitoring period and includes an assessment of statistical power (of population surveys) going forward. Further, it addresses the monitoring objectives and assesses monitoring outcomes against the performance indicators and whether thresholds have been breached and require corrective actions. The year one report is regarded as a brief report and will be used to inform a more comprehensive program review at year five.

Table 1: KMP performance indicators and their relevance to the current report.

Performance indicator	Performance threshold	Relevance to current report
1. Koala 'hot-spot' phased resource reduction:	<ul style="list-style-type: none"> • Separate contract (already completed). 	Not relevant
2. Koala population trends in Sections 10 and 8/9:	<ul style="list-style-type: none"> • Koala population sizes (Section 10) at or above the minimum expected targets of 195-276 at year five, 147-272 at year 10 and 103-261 at year 15. 	Year 1 results.
3. Road mortality	<ul style="list-style-type: none"> • No injury to an individual koala as a result of vehicle strike across all upgraded sections. • Section 10 - No koala road mortality within the fenced areas of the upgrade, on existing Pacific Highway or Wardell Road. 	Year 1 results.
4. Fauna crossing structures	<ul style="list-style-type: none"> • Evidence of at least one completed crossing by koalas at targeted fauna crossing structures. • Evidence of individual koalas using structures and/or breeding on either side of the highway, via scat analysis. • No evidence of high visitation/usage rates by exotic predators. 	Commencing in year 2.
5. Fauna exclusion fence	<ul style="list-style-type: none"> • No breaches in fauna exclusion fence. 	Commencing in year 2.
6. Predator attack near fauna crossing structures	<ul style="list-style-type: none"> • No koala deaths or injuries due to predator attack in the vicinity of fauna crossing structures. 	Commencing in year 2.
7. Habitat revegetation	<ul style="list-style-type: none"> • Years 1-3: annual density of one koala food/shelter tube stock per 20m² across revegetation site. • Year 5: trees within 90% of monitoring plots have an average height >8m. 	Commencing in year 3 (under a separate contract).
8. Koala use of food tree plantations	<ul style="list-style-type: none"> • At least 20% of koala faecal pellet search plots show evidence of occupancy by koalas by year 10 post-establishment. 	Commencing in year 3.

2. Study area

The broader study area includes sections 1-11 of the W2B Pacific Highway upgrade alignment and adjoining habitat (Figure 1). The 155 km-long upgrade stretches from Woolgoolga in the south to Ballina in the north. It is wholly located within the NSW North Coast Bioregion, one of the most diverse in NSW (W2B Planning Alliance 2012). The project boundary is located within a landscape which has been either fragmented or cleared for agriculture and rural development although substantial areas of forest habitat persist across the broader study area (W2B Planning Alliance 2012). The two focal koala populations of Broadwater and Bagotville are embedded within this landscape (Figure 1).

The Broadwater focal population area extends 3-5 km either side of an 11 km portion of the Pacific Highway Upgrade from Lang Hill (northern part of Section 8) north to the Richmond River (including all of Section 9) (Figure 1). The Richmond River forms a major movement barrier to the west and north. The Bagotville koala focal population centers around Section 10 and 11. It extends 13.5 km north of the Richmond River and includes the localities of Bagotville and Coolgardie west of Wardell (Figure 1).

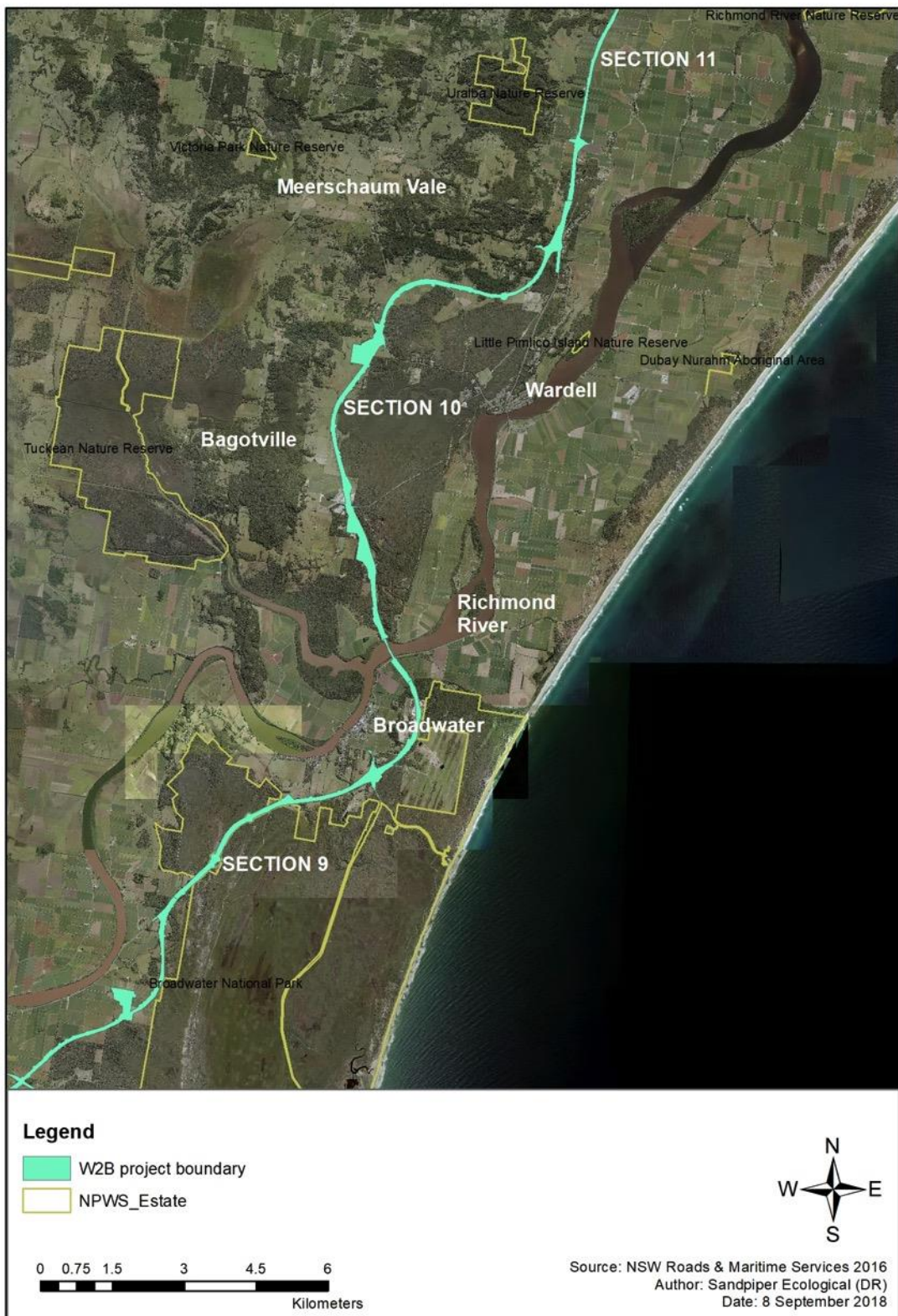


Figure 1: Sections 1-11 of the W2B Pacific Highway Upgrade alignment.

3. Methods

3.1 Population surveys

3.1.1 Site selection

Population surveys were conducted during spring 2017 and autumn 2018 at Broadwater and Bagotville focal areas. Surveys in the Broadwater focal area were conducted at sites used for the 2015 Broadwater Koala Population Survey (BKPS) (Ecosure 2015). These direct count, diurnal surveys provided baseline data for the KMP (2016). The BKPS comprised 54 survey sites, each featuring a 250 m x 40 m transect, and covering all potentially relevant vegetation types. Coastal heathlands and rainforest vegetation communities were generally excluded where suitable koala habitat was absent. For the spring 2017 surveys, landholder access restrictions required abandoning two transects and shifting a further four transects up to 100m each. Fifty-two transects were subsequently surveyed during the spring 2017 session (Figure 2).

For autumn 2018 surveys, a further four sites in the Broadwater area were abandoned due to absence of suitable koala habitat and two new sites were selected. New site selection was based on the same method as that used during baseline survey site selection. That is, a 350m x 350m grid corresponding to existing sites was imposed over the Broadwater focal area. Possible new sites were considered where grid points corresponded with potential koala habitat and were located on public land. Grid points located on private land were not considered due to ongoing uncertainty over access and possibility of clearing. A total of 50 sites were surveyed during the autumn 2018 survey session which is consistent with the number of sites used in the power analysis that informed the KMP (Rhodes and Preece 2016). Refer to section 3.3 for more details on power analysis.

Population surveys within the Bagotville focal area utilised sites assessed during the 2015 Koala Population Survey – W2B Section 10 (Phillips *et al.* 2015). These direct count, diurnal surveys were conducted at 46 sites (250 m x 40 m transects) the majority of which were surveyed in 2013 using a different sampling protocol (i.e. radial surveys of 42 sites; see Phillips and Chang 2013). For spring 2017 surveys, three sites were abandoned due to landholder access restrictions and a total of 43 sites were surveyed (Figure 3). For autumn 2018 surveys, seven new sites were selected based on the method for selecting new sites described above including selecting replacement sites that were in proximity to abandoned sites. In total, 50 sites were sampled in autumn 2018 which is consistent with the number of sites used in the power analysis that informed the KMP (Rhodes and Preece 2016).

3.1.2 Survey timing

Spring 2017 surveys in the Broadwater and Bagotville focal areas were conducted between 27 November and 14 December 2017. Diurnal surveys were generally completed between 1400 hours and 1830 hours. Nocturnal surveys were completed on the same day as diurnal surveys for all sample sites between approximately 2000 hours and 2400 hours. Weather conditions were mostly fine or overcast during surveys and several surveys were completed during light showers. Surveys were abandoned during periods of persistent or heavy rainfall. Temperatures ranged from 18°C to 26°C during the survey period and winds were variable.

Autumn 2018 population surveys were conducted between 9 April and 17 May 2018. Diurnal surveys were generally completed between 1300 hours and 1630 hours. Nocturnal surveys were completed on the same day as diurnal surveys for all sample sites between approximately 1800 hours and 2130 hours. Weather conditions were mostly fine or overcast during the monitoring surveys with several surveys completed during light showers. Temperatures ranged from 16°C to 27°C during the survey period and winds were mostly calm to moderate.

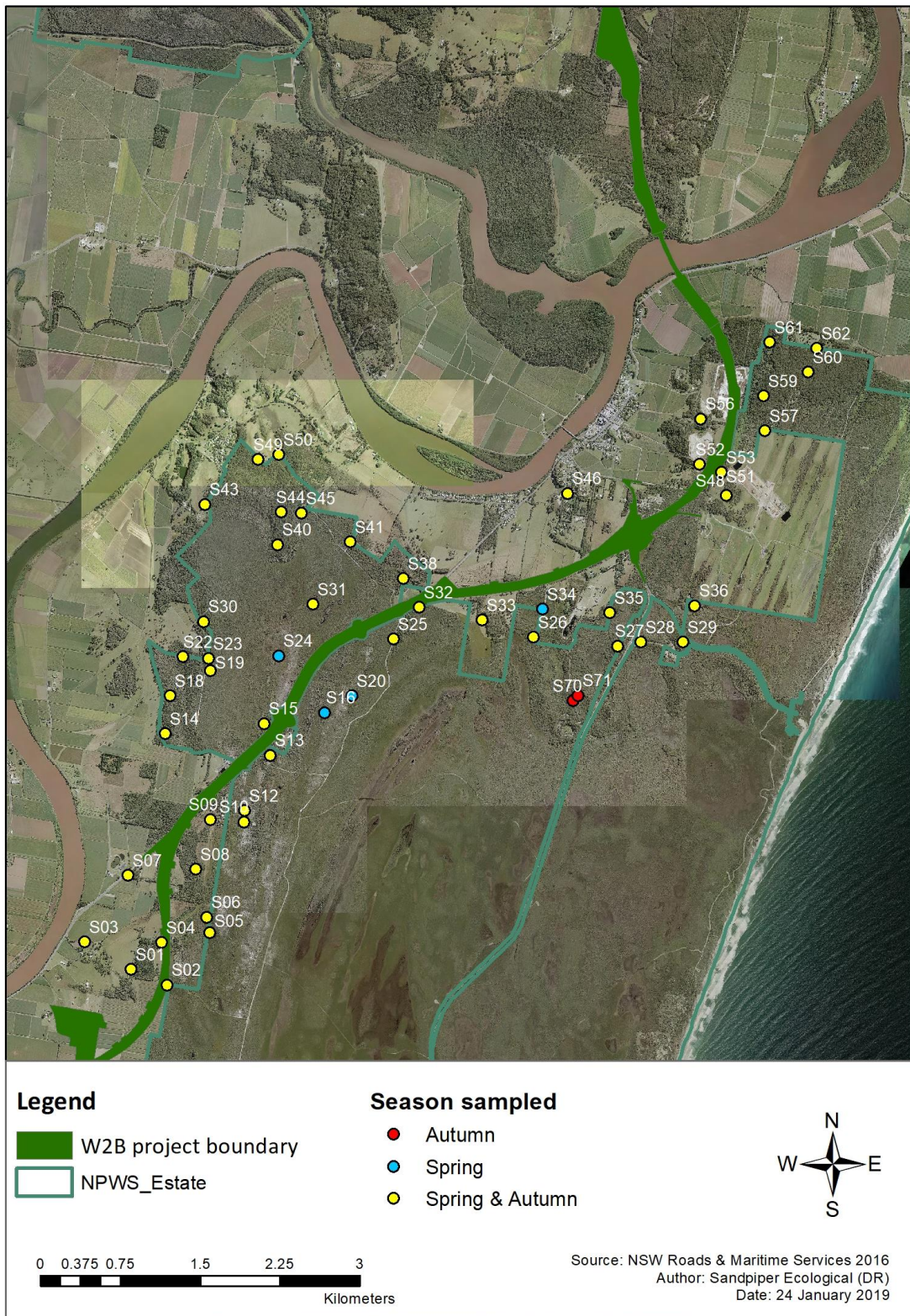


Figure 2: Broadwater (section 8/9) sample sites.

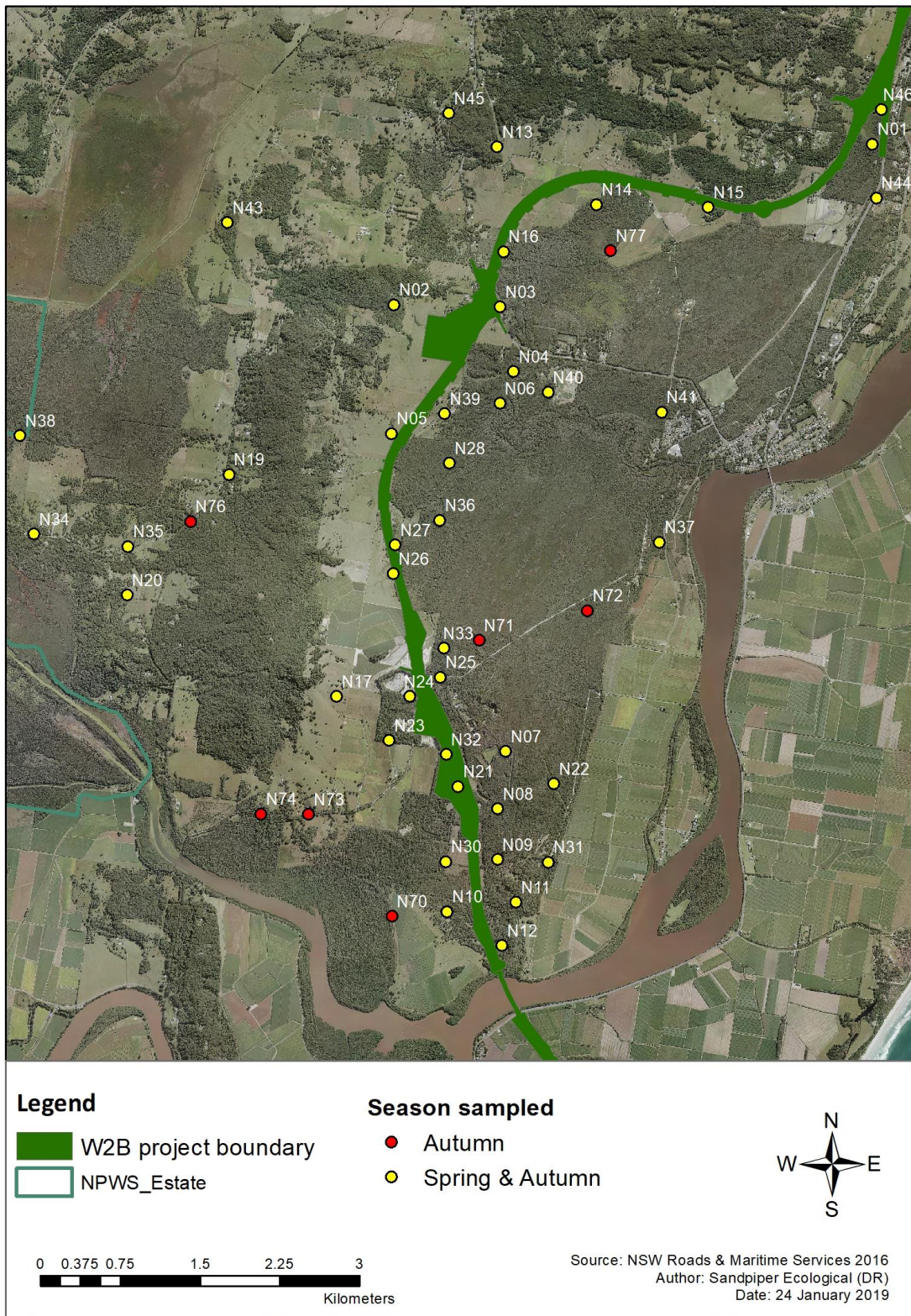


Figure 3: Bagotville (section 10) sample sites.

3.1.3 Field survey

Two teams of three ecologists, each with minimum 5 years' experience in koala surveys, completed the field surveys. Survey method followed that used during baseline surveys (see Ecosure 2015; Phillips *et al.* 2015). At each site, a 250m-long and 40m-wide (i.e. 1 ha) transect was marked up with the grid reference point (provided by Pacific Complete) serving as the center point. The cardinal points at 25m from the center point were also flagged. Where possible, based on configuration of habitat and property access, transects were oriented north-south on flat/undulating sites and along the contour of steeper sites. At some sites, transect length was shortened so as not to include cleared land. Transects were marked at regular intervals with flagging tape and reflective tape to aid night-time orientation. Two direct count methods were used:

1. Transect searches

Direct counts on 250m x 40m transects (approximately 1 ha) involved three observers walking 20m apart – one on the center line and one either side. Observers were equipped with binoculars and searched trees for koalas.

2. Radial searches

Direct counts within a radial area involved three observers slowly searching all trees within a 25m radius of the central point (approximately 0.196 ha) for koalas. Radial areas and transects were centered around the same central point and were conducted concurrently.

Diurnal followed by nocturnal surveys were conducted at each site. Handheld spotlights were used to assist with nocturnal surveys. All koala observations were recorded with a handheld GPS unit and data collected on tree species, diameter at breast height of tree and individual characteristics of each koala (e.g. sex, age class, health status, behaviour, identifying features).

3.2 Koala density and population size estimates

Koala density and population estimates for Broadwater and Bagotville were derived from a Bayesian model-averaging and statistical estimation exercise. The data for the models were the koala counts from baseline surveys (Phillips and Chang 2013; Ecosure 2015; Phillips *et al.* 2015) and spring and autumn Year 1 monitoring surveys at Broadwater and Bagotville. Counts were the "dependent" variable, according to a Negative Binomial error distribution, and the "independent variables" were various survey-design factors (such as year, season, nocturnal transect vs. day-time transect, radial-search transect vs. line-transect).

The Watanabe-Akaike Information Criterion was used to weight models according to their posterior support. The models provided posterior estimates (mean and 95% Confidence Interval (CI)) of the baseline koala densities at Bagotville and Broadwater. The estimates incorporated multiple sources of uncertainty (sampling uncertainty due to sub-sampling a population, over-dispersion of the count distribution, and multi-model uncertainty from not knowing exactly which independent effects were most important for driving koala densities).

Density values were then extrapolated across the total area of preferred koala habitat prior to clearing for baseline surveys (i.e. 1,624.7 ha in Broadwater; 2,152 ha in Bagotville) and post-clearing for year 1 surveys (i.e. 1,615.9 ha in Broadwater; 2,124 ha in Bagotville) to derive a population size estimate for each period. To enable interpretation of the population trend at Bagotville relative to the PVA scenario 6 predictions (Kavanagh 2016), the trend in density estimates between baseline and overall year one derived from the Bayesian modelling was applied to the PVA baseline population size estimate. Applying the trend derived from the modelling to the PVA baseline value controls for differences in the method used to derive the baseline population estimates.

3.3 Power analysis

The KMP (2016) includes background information on use of a Power Analysis (PA) to determine minimum survey effort to reliably detect a decline in focal koala populations. This was based on a determination that survey effort which achieved 70% power (or confidence) to detect a 30% decline in the Bagotville population was acceptable (KMP 2016). Using baseline data for each focal population and a diurnal search detection probability of 1.0/observer, the KMP PA determined that to achieve the 70%/30% target 50 survey sites within each focal area would need to be double-sampled (i.e. two surveys/session) every six months (J. Rhodes unpub. data).

A subsequent prospective PA, which included current density data, would then be completed at the end of each reporting period to determine the minimum survey effort required going forward. Whereas the PA used to inform the KMP (2016) was based on a frequentist/null hypothesis testing approach, the prospective PA used in the current reporting period was based on a Bayesian estimation analysis.

The Bayesian analysis provided posterior estimates of koala densities using survey data from 2015 (the baseline year) and 2017/2018 (Year 1 of the monitoring program) at Bagotville and Broadwater. The analyses also estimated which survey-design factors were most influential on the observed koala counts. The outputs of the Bayesian estimation exercise were inputted into a Monte-Carlo simulation exercise to project the population of koalas into 2031 (Year 15 of the monitoring program) and assess the ability of the monitoring program to detect significant trends.

The analysis was divided into two steps: a Bayesian estimation analysis using existing survey data, and a prospective power analysis based on inputs from the Bayesian estimation exercise.

3.3.1 Bayesian estimation

The estimation exercise provided empirical estimates of important features of the koala monitoring program for incorporation into the power analyses. The estimation exercise used a Bayesian framework. This was important for two reasons. First, the framework allowed the integration of multiple sources of information, including count information from the koala survey programs and prior estimates of baseline densities. Bayesian analyses are capable of integrating these two types of information through the use of *prior distributions* and *data*.

Secondly, the exercise was Bayesian in the sense that the outputs (i.e., the posterior probability distributions) were interpreted as characterising our *uncertainty* about the koala populations, given the existing count data and priors. By quantifying the uncertainty in this manner, we get a defensible and robust characterisation of the survey process and koala populations, including: the plausible range of values for the baseline koala densities at Broadwater and Bagotville; uncertainty about the difference between day-time surveys vs. night-time surveys; uncertainty about the effect of spring vs. autumn surveys; and the presence of count overdispersion. These were important for projecting the population forward in the power analysis.

3.3.2 Prospective power analysis

The goal of the monitoring project is to achieve a statistical power of 0.70 to detect a population trend, assuming a worse case decline of -30% from baseline koala densities in 2015 until Year 15 of the monitoring program. Statistical power is one minus the Type-II error rate (i.e. incorrectly concluding there is no difference when there is a difference), meaning that we desire a Type-II error rate of at most 0.30. A key assumption is the specification of the Type-I error α (i.e. incorrectly concluding there is a difference when there is no difference). Given that the target Type-II error rate was mandated to be 0.30, we decided upon a Type-I rate cap at $\alpha \leq 0.30$.

In such an error-control framework, there is a trade-off between the two error rates: a value-judgement is necessary in order to determine whether the Type-I or Type-II error rates should be lower or higher. In particular, we must consider their respective costs of being wrong. In conservation context, it is generally costlier when one *fails* to identify a steep population decline (i.e. Type-II error) which may result in species extirpation or extinction. In contrast, it is generally less costly to raise false alarms (i.e. Type-I error), especially when a monitoring program is already underway. Therefore, in this study, it was justifiable to set the Type-I error to be equal or higher than the Type-II error. This is a precautionary approach which places more value on detecting ecological trends. Given the mandated Type-II error rate of 0.30, this meant that we should set α to be at least 0.30, and perhaps higher if the data and analyses lacked sufficient power.

The analyses also considered other combinations of population declines and Type-I error rates, in order to see how the statistical power would behave under decreasing α levels and decreasing magnitudes of $|\beta_t|$.

Combining Analyses

Using the outputs of the Bayesian estimation exercise, the prospective power analyses integrated multiple sources of statistical variation. Most power analyses only include *sampling variation* (the distribution of the test statistic under random samples of data) but fail to include *estimation & model uncertainty* (uncertainty in parameter values and model selection uncertainty). The sampling variation was incorporated into the analysis through the use of Monte-Carlo simulations of koala counts. The estimation/model uncertainty was incorporated by using the posterior distributions from the Bayesian estimation exercise for the simulations' parameter values (e.g., uncertainty in baseline densities, role of survey methodology, seasonality, etc.). In total, this meant that the prospective power analysis made fewer parametric assumptions and better reflected the overall uncertainty about the koala populations.

Data

The data consisted of koala counts from two locations. There were baseline surveys at Bagotville in spring 2013 and 2015, as well as Year 1 surveys in spring 2017/autumn 2018. At Broadwater, there were baseline surveys in spring 2015, as well as Year 1 surveys in spring 2017/autumn 2018. The surveys occurred along line-transects and radial-search transects in teams of three observers. The detection probability per team was assumed to be 100% (or $p_d=1$ per team).

Full details of model development are provided in Appendix B.

3.4 Faecal pellet (scat) collection

Faecal pellets (i.e. scats) were collected from koalas observed during surveys in the Bagotville area for cortisol and DNA analysis. When a koala was observed, the base of its tree was searched for fresh scats. If fresh scats were found, they were collected in accordance with the Collection of Scats Protocol and the methods for collection and storage described by Piggott (2004) and Wedrowicz *et al.* (2013). After consultation with the technicians undertaking the cortisol and DNA extraction/analysis, the collection and storage method was refined to involve placement of scats into a paper bag, then refrigerating/freezing as soon as practicable. Scat collection data also included location, tree species, dbh, koala sex/health (if possible) and weather at time of collection. Only very fresh scats were suitable for cortisol analysis whereas scats for DNA analysis could be up to a few days old. Effort was made to collect 75-100 scats for DNA analysis during the reporting period. An equivalent number of scats were collected for cortisol analysis study from individuals at the Tucki Tucki control site (approx. 11km west of the alignment). The separate cortisol study being conducted by RMS will be analysed and reported on by Sydney University.

3.5 DNA extraction and analysis

Koala genetic material were isolated from all scats supplied using either the scraping method outlined in Shultz *et al.* (2018) or using the washing techniques described in Wedrowicz *et al* (2013). Method of DNA isolation was dependent on structure and age of scat.

Genomic DNA was isolated using the NucleoSpin® DNA Stool kit (Macherey-Nagel, Germany). Each DNA isolate was tested for quality and concentration using spectrophotometry (Nanodrop, ThermoFisher Scientific, VIC, Australia) and real time PCR for confirmation of presence of host DNA in the sample (*Phascolarctos cinereus* beta-actin mRNA mRNA).

Full details of DNA extraction and analysis are provided in Appendix C.

3.6 Road mortality surveys

Koala road mortality surveys along the fenced section of Wardell Road and the existing Pacific Highway were undertaken during spring 2017 and winter 2018. Surveys involved a walking traverse of both sides of the road edge on two occasions between August and November. The Wardell Road transect occurred from Lumleys Lane to Thurgates Lane (1.54 km) and the existing Pacific Highway from Carlyle Street, Wardell to the Coolgardie interchange (3.3 km). Surveys were conducted 30 November and 1 December 2017 (spring) and 10 August 2018 (winter).

Road mortality results were supplemented by other data sources including incidental observations by Sandpiper staff while traveling road in the focal area, RMS, construction personnel and road mortality reports from Lismore-based Friends of the Koala.

4. Results

4.1 Population survey effort

Within the Broadwater focal area, 52 sites were surveyed in spring 2017 and 50 sites surveyed in autumn 2018. Total area searched for spring 2017 was 49.56 ha for transect surveys and 10.2 ha for radial surveys. The autumn 2018 search area was 47.85 ha (transects) and 9.8 ha (radial).

At Bagotville, 43 sites were surveyed in spring 2017 and 50 sites in autumn 2018. Total area searched included 40.96 ha for transect surveys and 8.43 ha for radial searches in spring 2017 and 47.94 ha (transects) and 9.8 ha (radial) in autumn 2018.

4.2 Population survey koala observations

4.2.1 Broadwater focal area

During spring 2017 population surveys, one koala was observed on transects during the diurnal surveys and two individuals were observed during nocturnal surveys (Table 2; Figure 4). One individual was observed on the same transect during diurnal and nocturnal surveys. A further two koalas were observed incidentally off-transect while moving between sites. No individuals were observed within radial search areas (Table 2).

During autumn 2018 surveys, four koalas were observed on transects during diurnal and nocturnal surveys (Table 2). Two of these individuals were observed on the same transects during diurnal and nocturnal surveys.

One individual was observed on transect and within a radial search area during both the diurnal and nocturnal surveys (Table 2). A further eight koalas were observed incidentally off-transect while moving between sites.

Baseline surveys conducted during spring 2015 reported seven adult koalas on transect, one within the radial search areas and one incidental/off-transect (Table 2).

Full details of Broadwater koala observations are provided in Table A1, Appendix A.

Table 2: Broadwater focal area koala observations for spring 2017 and autumn 2018 population surveys and observations reported for spring 2015 baseline surveys (Ecosure 2015).

Survey session (no. of transects surveyed)	Diurnal transect	Nocturnal transect	Diurnal radial	Nocturnal radial	Diurnal off-transect	Nocturnal off-transect
Spring 2017 (52)	1	2	0	0	1	2
Autumn 2018 (50)	4	4	1*	1*	7	3
Baseline - spring 2015 (54)	7	NA	1*	NA	1	NA

* Individual observed on transect and radial search area.



Figure 4: Broadwater survey sites and location of koalas observed during spring 2017 and autumn 2018 surveys.

4.2.2 Bagotville focal area

During spring 2017 population surveys, two koalas were observed on transects during the diurnal surveys and three individuals were observed during nocturnal surveys (Table 3). One individual was observed on the same transect during diurnal and nocturnal surveys. A further five koalas were observed incidentally off-transect while moving between sites. No individuals were observed within radial search areas (Table 3). The location of Bagotville survey sites and koala observations are shown in Figure 5.

During autumn 2018 surveys, five koalas were observed on transects during diurnal and nocturnal surveys (Table 3). Four individuals were each observed on the same transect during diurnal and nocturnal surveys. A further eight koalas were observed incidentally off-transect while moving between sites. One individual was observed both on transect and within a radial search area during both the diurnal and nocturnal surveys (Table 3).

Baseline surveys conducted during autumn 2015 reported three adult koalas on transect and five off-transect/incidental (Table 3).

Full details of Bagotville koala observations are provided in Table A2, Appendix A.

Table 3: Bagotville focal area koala observations for spring 2017 and autumn 2018 surveys and observations reported for autumn 2015 baseline surveys (Phillips et al.2015).

Survey session (no. of transects surveyed)	Diurnal transect	Nocturnal transect	Diurnal radial	Nocturnal radial	Diurnal off-transect	Nocturnal off-transect
Spring 2017 (43)	2	3	0	0	2	4
Autumn 2018 (50)	5	5	1*	1*	5	4
Baseline - autumn 2015 (46)	3	NA	NA	NA	5	NA

* Individual observed on transect and radial search area.

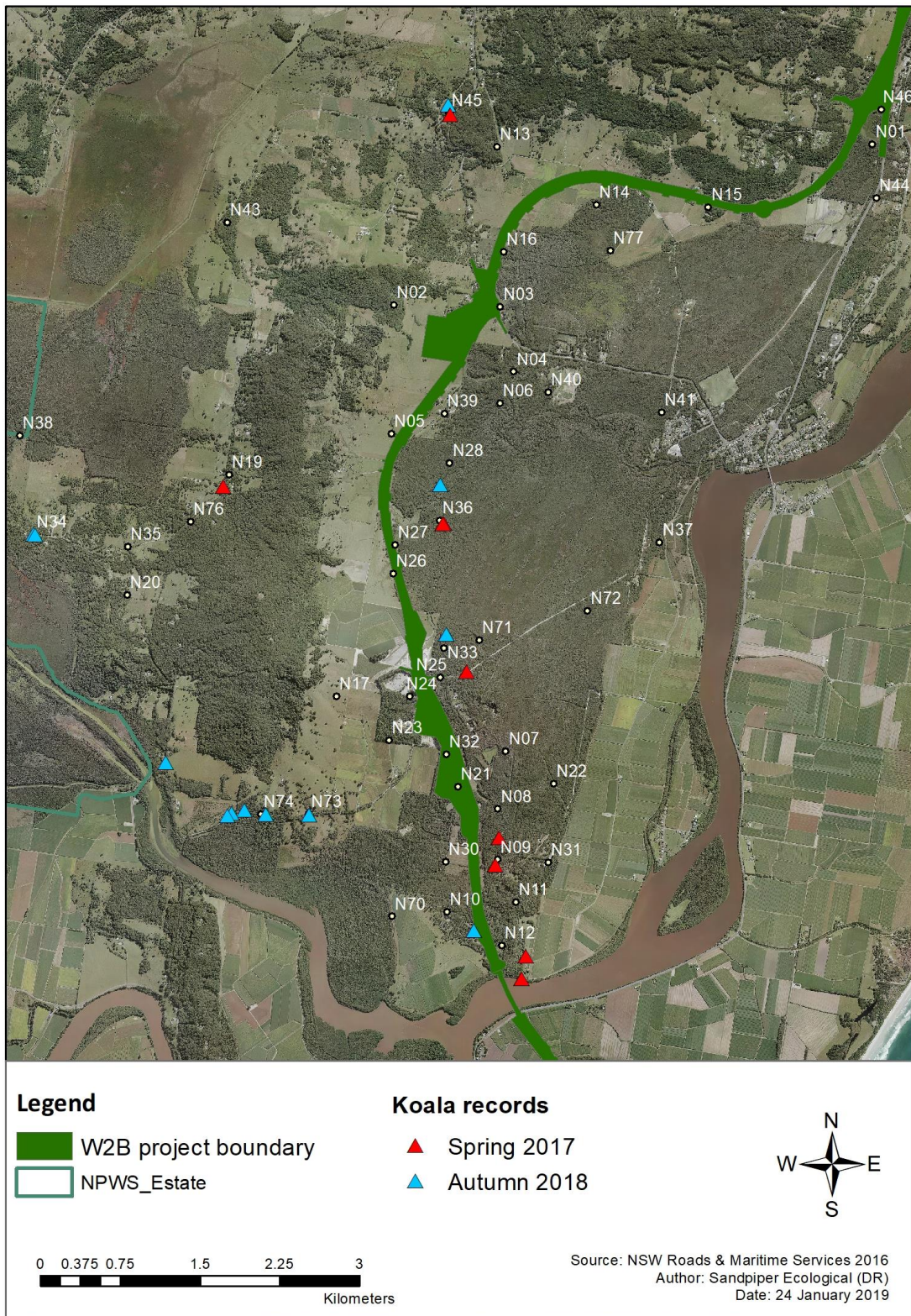


Figure 5: Bagotville survey sites and location of koalas observed during spring 2017 and autumn 2018 surveys.

4.3 Koala density and population size estimate

4.3.1 Broadwater population

Based on the Bayesian estimation analysis, the overall Year 1 density estimate for Broadwater was 0.089 koalas/ha (95%CI: 0.044-0.164). The density estimate for spring was 0.082 koalas/ha (95%CI: 0.037-0.157) and autumn was 0.097 koalas/ha (95%CI: 0.047-0.184). This compares to a refined baseline density estimate of 0.089 (95%CI: 0.039-0.177) koalas ha⁻¹ and represents a stable trend between baseline and overall year one.

Extrapolated population size estimate across 1,615.9 ha of preferred koala habitat for year 1 overall was 144 (95%CI: 71-265). The population estimate for spring was 133 (95%CI: 60-254) and autumn was 157 (95%CI: 76-297). This compares to an extrapolated baseline population estimate of 145 koalas (95%CI: 63-288) across 1,624.7 ha (Figure 6).

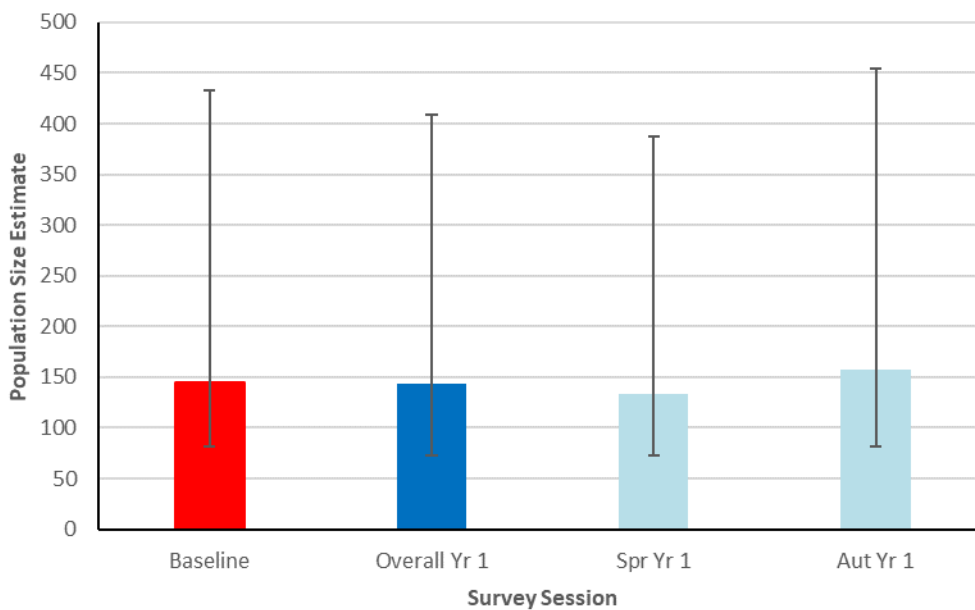


Figure 6: Comparison of Broadwater focal area population size estimates (\pm 95%CI) for baseline year (2015), monitoring year 1 overall, spring year 1 and autumn year 1.

4.3.2 Bagotville population

Based on the Bayesian estimation analysis, the overall Year 1 density estimate for Bagotville was 0.085 koalas/ha (95%CI: 0.038-0.170). The estimate for spring was 0.079 koalas/ha (95%CI: 0.034-0.160) and for autumn was 0.094 koalas/ha (95%CI: 0.039-0.2). This compares to a refined baseline density estimate of 0.092 (95%CI: 0.046-0.165) koalas ha⁻¹. This represents a 7.6% decline between baseline and overall year one. Despite the apparent decline, no population trend could be discerned given the high level of model uncertainty.

Extrapolated population size estimate for year 1 overall was 181 (95%CI: 81-361) across 2,124 ha of preferred koala habitat. The estimate for spring was 168 (95%CI: 72-340) and for autumn was 200 (95%CI: 83-425). This compares to an extrapolated baseline population estimate of 198 koalas (95%CI: 99-355) across 2,152 ha (Figure 7).

Applying the 7.6% decline in density estimates to the PVA predictions infers a decline in the Bagotville population from 236 to 218 koalas.

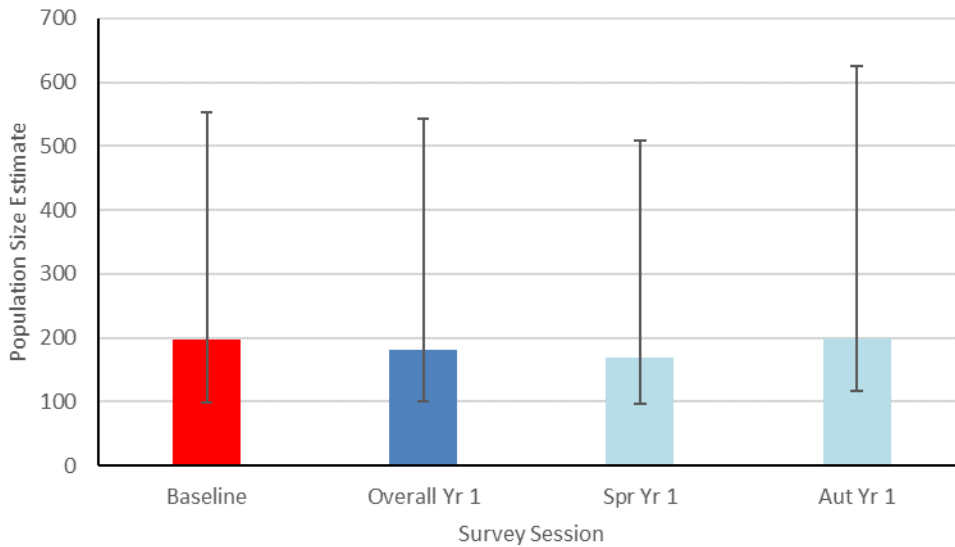


Figure 7: Comparison of Bagotville focal area population size estimates (\pm 95%CI) for baseline year (2015), monitoring year 1 overall, spring year 1 and autumn year 1.

4.4 Power analysis

4.4.1 Estimation exercise

The posterior distributions for the Bayesian estimation analysis of the koala counts at Broadwater and Bagotville are shown in Figure 8. The important results were as follows (refer to Appendix B for full report):

- the baseline koala density at Bagotville was estimated to be 0.092 koalas/ha (SE 0.031, 95%CI: 0.046-0.165), while the baseline density at Broadwater was 0.089 koalas/ha (SE 0.036, 95%CI: 0.039-0.177);
- for Year 1 at Bagotville, the overall koala density was estimated to be 0.085 koalas/ha (SE 0.035, 95%CI: 0.038-0.170), while for Spring it was 0.079 koalas/ha (SE 0.033, 95%CI: 0.034-0.160) and for Autumn the estimate was 0.094 koalas/ha (SE 0.041, 95%CI: 0.039-0.200);
- for Year 1 at Broadwater, the overall estimated density was 0.089 koalas/ha (SE 0.031, 95%CI: 0.044-0.164); in Spring, the estimate was 0.082 koalas/ha (SE 0.031, 95%CI: 0.037-0.157), while for Autumn the estimate was 0.097 koalas/ha (SE 0.036, 95%CI: 0.047-0.184);
- there was considerable model uncertainty, whereby the top 14 models only accounted for 50% of the posterior model probability, implying that the estimates were somewhat sensitive to the set of models and may change with different models;
- the top WAIC model included effects for season ($\beta_s \neq 0$) but not transect-type ($\beta_r = 0$) nor night-time ($\beta_n = 0$), and had very little estimable overdispersion ($\bar{\theta}$ effectively fixed at 5); all five top models had similar specifications;

- there seemed to be some evidence of a seasonal difference in koala counts, such that the counts in the autumn were approximately 23% higher than the counts in spring (although the variance was wide, SE 36.7%);
- the marginal effects of night-time vs. day-time surveys and radial vs. line-transects had marginal distributions that were sharply peaked at 0 (known as *shrinkage* due to the low model probabilities estimated for those models which included these effects), suggesting that, given the current amount of data, their effects were unimportant for predicting koala counts (see Report Appendix II for more exploration of these);
- no population trend could be discerned, given that the trend parameters had posteriors which were almost identical to their priors (i.e., no learning took place).

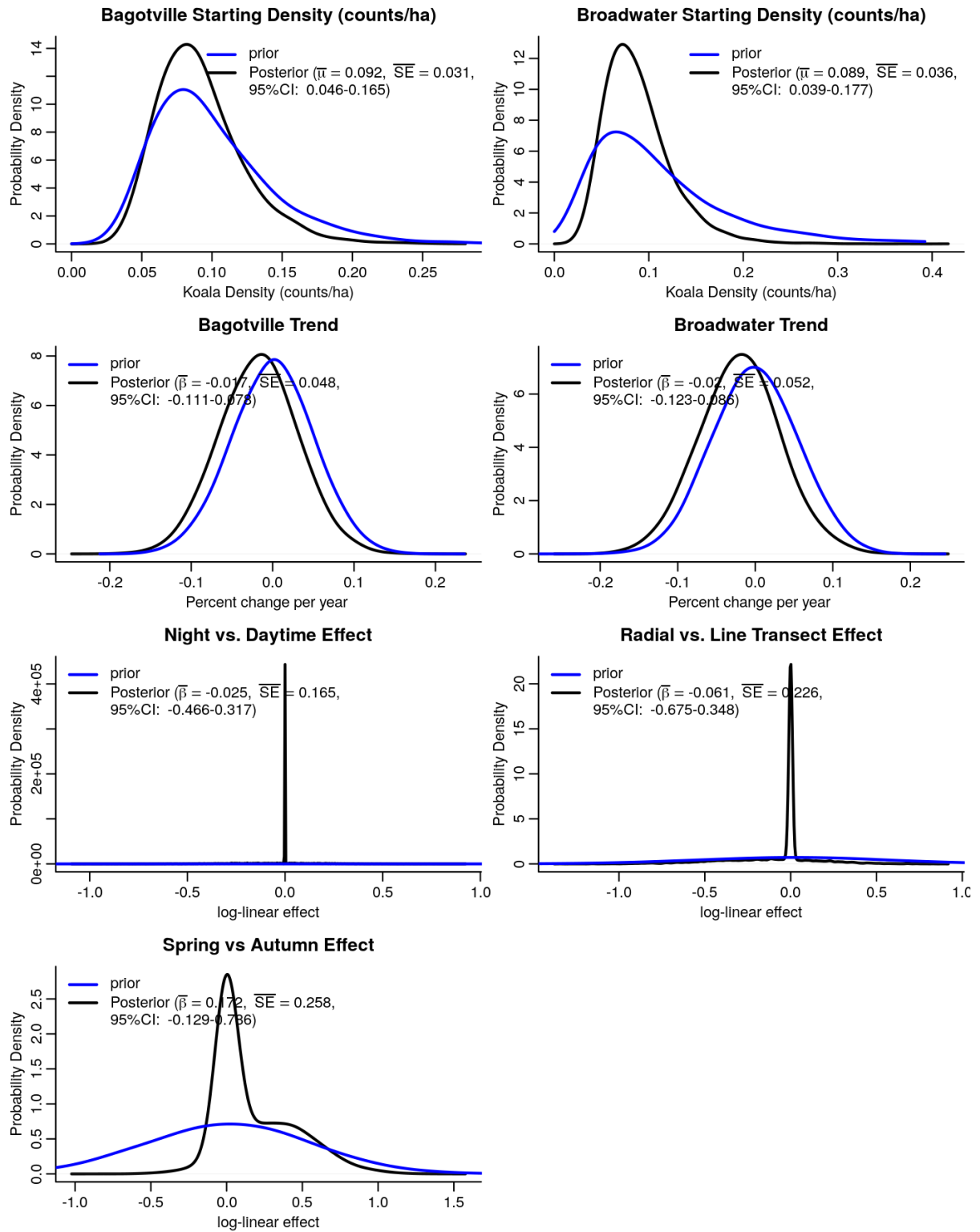


Figure 8: Bayesian model-averaged estimates of the covariates affecting koala surveys. Bayesian priors are in blue and posteriors are in black.

4.4.2 Power analysis

The estimated power curves for Broadwater and Bagotville using different combinations of trend and Type-I error rates are shown in Figure 9. With a decline of -30% from the 2015 baseline densities and an α value of 0.30, the estimated power for Bagotville and Broadwater were 0.752 and 0.741, respectively. These values achieved the desired power rates.

Using the full-model specification, the results were nearly identical. For comparisons, if we instead relax the Type-I error rate to a maximum of 0.35, then the estimated power rates were 0.787 and 0.773 at Bagotville and Broadwater respectively. The power curves (Figure 9) also suggest that the program may be able to maintain a tighter Type-I error control of $\alpha \leq 0.23$ and still achieve the desired power of 0.70.

Using the Bayesian preponderance of evidence framework, the probability of estimating a decline at Bagotville and Broadwater was 0.954 and 0.945 respectively (given a decline of -30%).

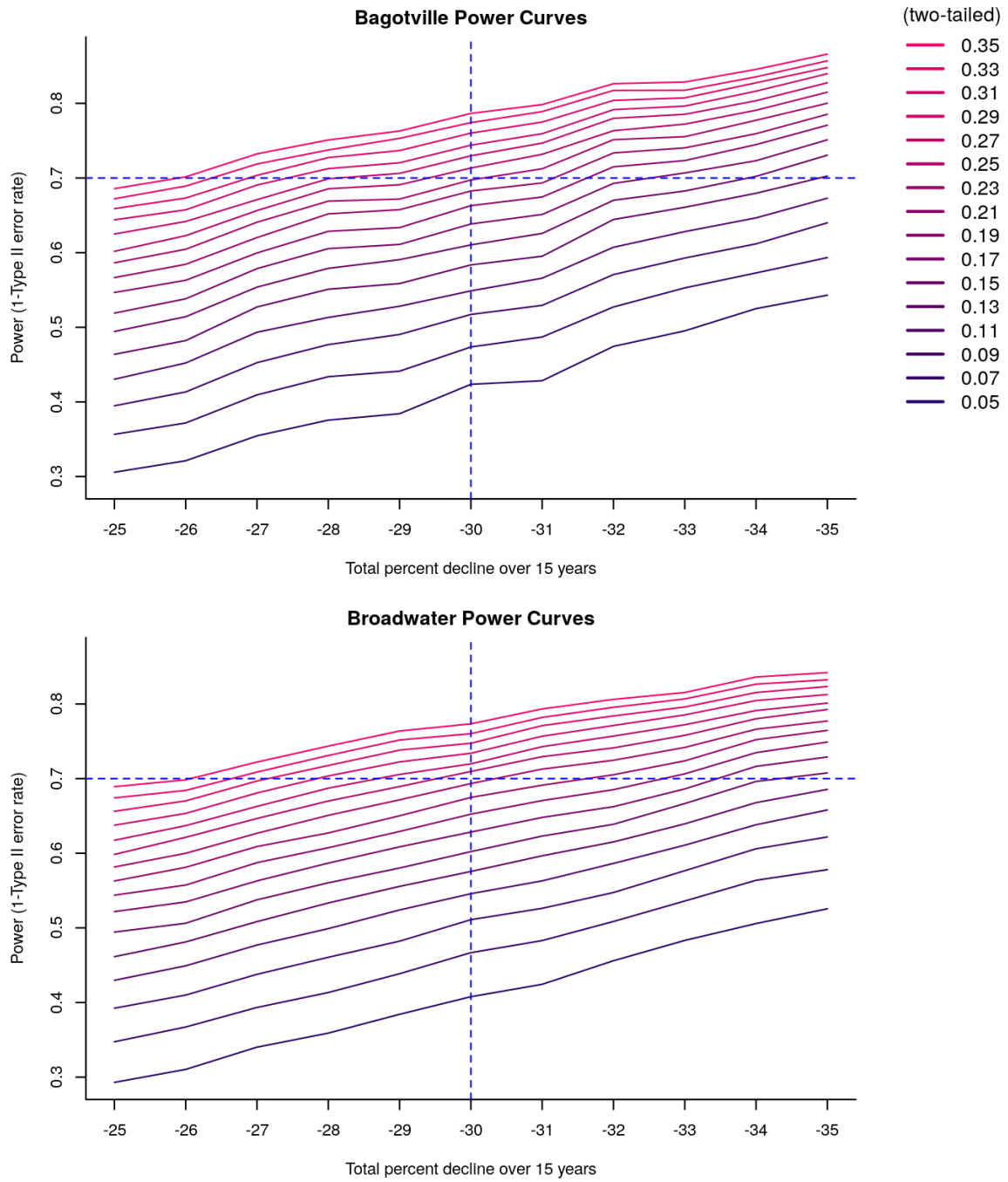


Figure 9: Statistical power to detect a -30% drop in baseline densities (2015) in Year 15 of the monitoring program for different maximum levels of Type-I errors (lines).

4.5 DNA extraction and analysis

4.5.1 Genotypes

Nineteen scat samples were collected from individuals observed during surveys and incidental observations across the Bagotville focal area between January and May 2018. From these samples, genotypes across 30 microsatellite loci for 19 samples (from herein will be referred to as the population) were generated.

There were no departures from Hardy Weinberg Equilibrium from the population, therefore a total of 30 loci were retained for analysis. All 19 koala scat samples collected had unique multi-locus genotypes. The probability of identity for the 30 loci was 2.4×10^{-31} (individuals) and 2.5×10^{-12} (siblings) which indicates that the microsatellite loci were sufficiently discriminating for population analysis. In addition, there was 19 individual genetic profiles identified indicating that 19 distinct individuals had been sampled.

4.5.2 Genetic diversity

Analysis of genetic diversity was performed using the software GENALEX version 6.5 (Peakall and Smouse, 2012) to calculate mean number of alleles and observed and expected heterozygosity. FSTAT (Goudet, 2001) was used to calculate inbreeding coefficient; whereby a positive value indicates that individuals in a population are more related than you would expect under a model of random mating, and a negative value indicating that individuals in a population are less related.

Genetic diversity values of the population are presented in Table 4. Analysis reveals that the population is exhibiting moderate to high diversity (A_{mean} and H_e) when compared to previous studies of NSW koala genetic diversity based on comparable microsatellite marker data (Table 5), despite the indication of an increased inbreeding coefficient value (Table 4).

Table 4: Summary of genetic diversity statistics for the Bagotville koala population. N = Number of individuals sampled; A_{mean} = Mean number of alleles; H_o = Observed heterozygosity; H_e = Expected heterozygosity; F_{IS} = Inbreeding coefficient (the proportion of variance in a population that is contained within an individual; $F_{IS} > 0.00$ suggests inbreeding).

Population	N	A_{mean}	F_{IS}	H_o	H_e
Bagotville-Coolgardie	19	8.2	0.214	0.571	0.726

Table 5: Summary and comparison of genetic diversity to NSW koala populations

Population	Reference	N	A_{mean}	H_e
Bagotville-Coolgardie	Current study	19	8.20	0.726
Lismore	Lee <i>et al.</i> (2012)	13	10.30	0.65
Blue Mountains	Lee <i>et al.</i> (2010)	9	6.83	0.743
Southern Tablelands	Lee <i>et al.</i> (2010)	15	5.08	0.586
Campbelltown	Lee <i>et al.</i> (2010)	14	3.17	0.542

4.5.3 Genetic relatedness

Genetic relatedness was estimated for every pair of individuals within the population to provide an indication of which pairs of individuals at each site are likely to be related (Figure 10).

Genetic relatedness of within-population individuals was calculated in GENALEX version 6.5 (Peakall and Smouse, 2012) using the Queller and Goodnight estimator of relatedness. This is an indicator of the proportion of shared ancestry in pairs of individuals. Expected values are ≤ 0 for unrelated individuals, 0.25 for half-sib pairs and 0.5 for parent-offspring or full-sib pairs. Relatedness values will form a distribution around these expected values.

	BB-k01-DNA	BT-k2	BT-k3	BT-k4	BT-k5	KH3-DNA	KH4-DNA	SF-k05-DNA	SF-k06-DNA	SF-k07-DNA	SF-k08-DNA	SF-k09-DNA	SF-k10-DNA	SF-k11-DNA	SF-k12-DNA	SF-k13-DNA	SF-k14-DNA	SF-k15-DNA	SF-k16-DNA
BB-k01-DNA	0.000																		
BT-k2	0.000	0.000																	
BT-k3	0.000	0.081	0.000																
BT-k4	0.012	0.140	0.000	0.000															
BT-k5	0.000	0.000	0.003	0.000	0.000														
KH3-DNA	0.000	0.000	0.000	0.000	0.000	0.000													
KH4-DNA	0.000	0.000	0.000	0.000	0.085	0.000	0.000												
SF-k05-DNA	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000											
SF-k06-DNA	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000										
SF-k07-DNA	0.046	0.134	0.000	0.247	0.000	0.000	0.000	0.000	0.000	0.000									
SF-k08-DNA	0.056	0.202	0.000	0.134	0.000	0.000	0.000	0.000	0.429	0.000									
SF-k09-DNA	0.000	0.335	0.000	0.208	0.000	0.000	0.000	0.000	0.276	0.415	0.000								
SF-k10-DNA	0.040	0.252	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.119	0.345	0.352	0.000						
SF-k11-DNA	0.146	0.083	0.000	0.110	0.000	0.000	0.000	0.000	0.000	0.163	0.118	0.339	0.174	0.000					
SF-k12-DNA	0.320	0.000	0.000	0.102	0.000	0.000	0.000	0.000	0.000	0.271	0.132	0.150	0.193	0.230	0.000				
SF-k13-DNA	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.086	0.000	0.000	0.019	0.000	0.093	0.039	0.000	0.000			
SF-k14-DNA	0.000	0.000	0.000	0.000	0.050	0.048	0.060	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
SF-k15-DNA	0.000	0.184	0.000	0.133	0.000	0.000	0.000	0.000	0.000	0.458	0.394	0.202	0.171	0.097	0.088	0.000	0.000	0.000	0.000
SF-k16-DNA	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.113	0.000	0.000	0.000	0.000	0.000

Figure 10: Genetic relatedness within the population. The identification number of each Individual sample is displayed on the X and Y axes. Significantly related individuals, full-sib pairs and parent-offspring are highlighted yellow; and half-sib pairs are highlighted green.

4.5.4 Population structure

Population structuring was determined using the Bayesian clustering program STRUCTURE version 2.3.4 (Pritchard *et al.* 2000). Analysis of koala population genotype data involved 5 replicates of K = 1 to K = 10 (K = genetic cluster) using 100,000 iterations with 100,000 iterations discarded as burn-in. The number of K clusters was determined using both the maximum likelihood and the deltaK method of Evanno *et al.* (2005).

The STRUCTURE analysis of the population suggests there is a division into two main genetic clusters (i.e. K = 2). This indicates there is the presence of two sub-populations within the focal area, with genetic segregation between the 2 clusters. However, the location of individuals within and between clusters is spatially mixed and does not display landscape segregation (Figure 11).

Full details of DNA extraction and analysis are provided in Appendix C.

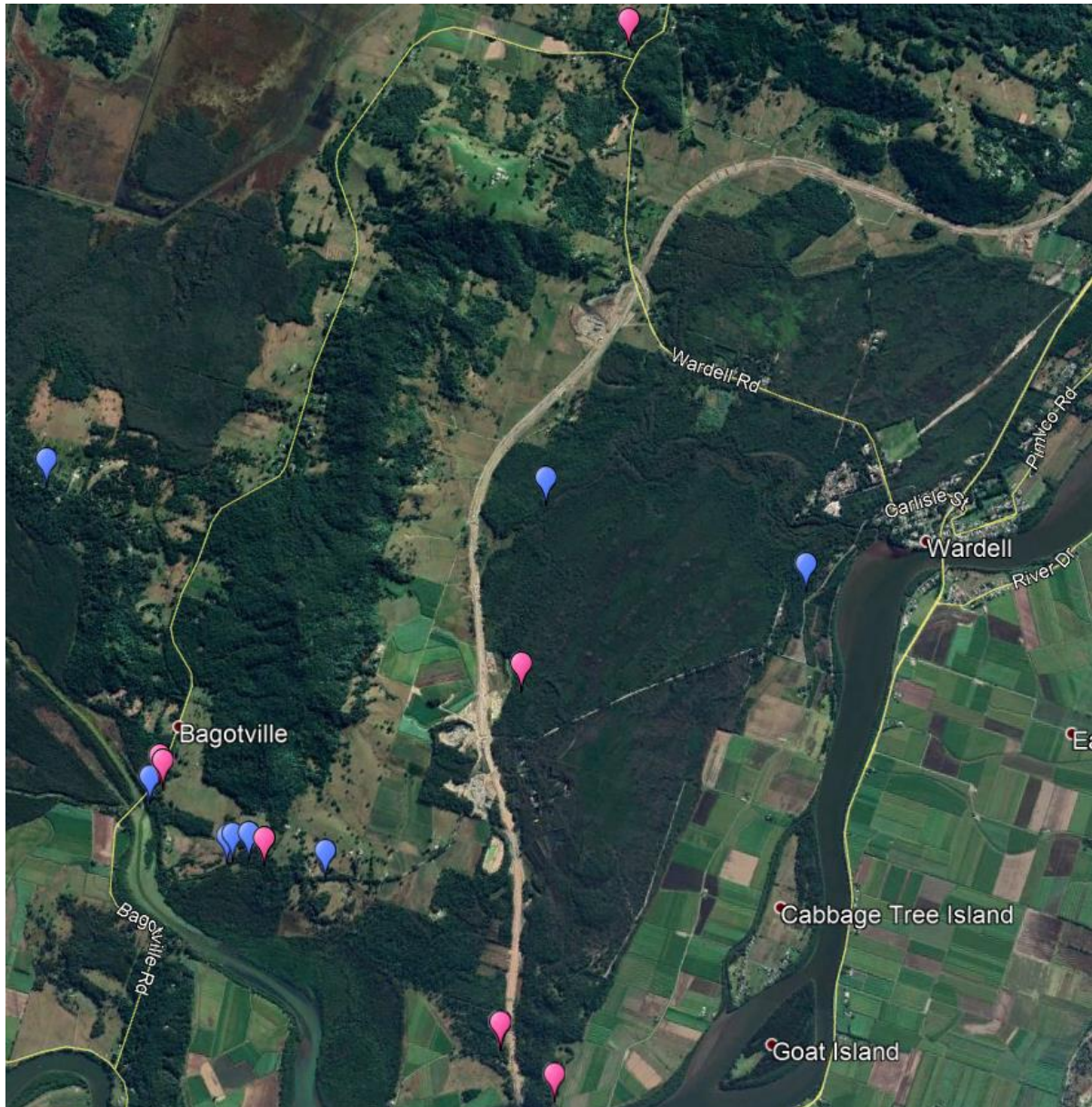


Figure 11: Genetic structure of the population according to location of scat samples. Blue and red pins represent individuals of each cluster.

4.6 Road mortality surveys

No koalas were detected during spring 2017 or winter 2018 road mortality surveys. No carcasses of threatened species were recorded although species of flying fox recorded may have been grey headed flying fox *Pteropus poliocephalis* which is listed as vulnerable under the NSW *Biodiversity Conservation Act 2016* and the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999* (Table 6).

Table 6: Details of fauna detected on road/roadside during walking road mortality surveys. Wardell Road = 1.54 km; Old Pacific Highway = 3.3 km); Po = possible; Pr = probable; D = definite

Season/Road	Date	Start time	End time	Species recorded	Confidence	Location	Easting	Northing
Spring 2017								
Wardell Road	30/11/2017	0800	0845	Bandicoot spp	Po	Carriageway	542797	6798386
				Green tree frog	Po	Carriageway	544060	6798011
Old Pacific Highway	1/12/2017	0730	0930	Nil				
Winter 2018								
Wardell Road	10/08/2018	0800	0900	Cane toad	D	Shoulder	543433	6798126
				Cane toad	D	Shoulder	543428	6798126
Old Pacific Highway	10/08/2018	0900	1100	Fox	Pr	Carriageway	546344	6799916
				Chicken	Po	Shoulder	546044	6799234
				Flying fox spp.	D	Shoulder	545918	6798998
				Flying fox spp.	D	Shoulder	545856	6798834
				Southern boobook	D	Grass	545753	6798512
				Tawny Frogmouth	D	Shoulder	545528	6797842
				Short-beaked Echidna	Pr	Shoulder	545378	6797553
				Little wattlebird	D	Grass	545742	6798428
				Flying fox spp.	D	Shoulder	545820	6798692
				UnID med animal	NA	Carriageway	545949	6799028
				Bandicoot spp.	Pr	Shoulder	546209	6799506

5. Discussion

5.1 Koala density and population size estimate

Based on the Bayesian estimation analysis, baseline and year 1 koala density estimates in the Broadwater focal area were identical (i.e. 0.089 koalas ha⁻¹). Extrapolated population estimates were 145 koalas (baseline) compared to 144 (year 1) although this is an artefact of rounding up and marginally less preferred habitat post-clearing (i.e. year one) compared to during baseline surveys. There was evidence of seasonal difference in koala counts such that autumn counts were approximately 23% higher than spring although the variance was quite high (i.e. SE 36.7%). Based on year 1 data, the population trend in Broadwater was stable.

For the Bagotville focal area, the Bayesian estimation analysis reported a baseline estimate of 0.092 (95%CI: 0.046-0.165) koalas ha⁻¹ and a year 1 estimate of 0.085 koalas ha⁻¹ (95%CI: 0.038-0.170), suggesting a decline of 7.6%. If we apply this 7.6% decline to the PVA baseline estimate of 236 koalas (Kavanagh 2016), it infers a population reduction by 18 koalas. Such a decline is well inside the 17% (lower bound of the 90% confidence

interval) within the first five years prescribed by the KMP. As such, no corrective actions have been triggered by the KMP.

Despite the apparent 7.6% decline between baseline and year one density values, it is important to note that no population trend could be discerned from the Bayesian estimation analysis given the high level of model uncertainty. This suggests that more survey data (i.e. subsequent monitoring years) are required to reduce model uncertainty. Moreover, wildlife populations can vary temporally for many reasons (Krebs 2009) and require longer time frames to detect meaningful trends. This is evident at Bagotville where baseline koala density estimates derived from 2013 and 2015 sampling varied by 42% (Phillips and Chang 2013; Phillips et al. 2015).

It should be noted that in applying Bayesian estimation analysis to both the baseline and year 1 data, the Bagotville population data differ from that cited in the KMP which was derived using a Frequentist approach. Further, a correction factor accounting for the unsampled 0-1 age cohort has not been applied to the data. Importantly, the same analysis method has been applied to both the baseline and year one data enabling direct and robust comparison and trend interpretation.

The current method of population surveys in Bagotville and Broadwater utilises both diurnal and nocturnal monitoring, whereas baseline surveys conducted diurnal monitoring only. The current method of diurnal followed by nocturnal surveys and concurrent transect and radial searches conducted by the same team may compromise independence. Further, current and baseline surveys assume zero detection error although a recent study reported higher detectability using spotlighting compared to day searches (Wilmott *et al.* 2018). Survey methodology and detectability will be considered in the end of year two review.

During the reporting period, a predator control program has been operating within lands surrounding section 10. The program instigated by RMS has resulted in removal of 21 wild dogs and foxes which should reduce the predation risk for koalas residing within and near section 10 (Australia Feral Pest Management Service, 2018). It should also be noted that the focal koala populations may be affected by other impacts outside the control of the project, such as local land development, clearing activities and the 18% below average rainfall experienced in the study area during the reporting period (BOM, Meerschaum Vale Station No. 58171). Moreover, the Bagotville population was subjected to a wildfire that burnt out 350 ha of forest on the east side of the alignment during September 2017.

5.2 Power analysis

The prospective power analysis demonstrated that the koala monitoring programs at Broadwater and Bagotville are likely to achieve their target levels of statistical power (>0.7) in order to detect a -30% population decline over 15 years of monitoring, while maintaining a Type-I error rate of $\alpha \leq 0.30$. These results are conditional on current levels of survey effort continuing into the future, such as 400 transects per year at either location. There is some weak evidence that the program could tweak the survey protocol (such as discontinuing the radial-search transects and night-time surveys) and still maintain the requisite power (see Appendix II).

The analyses revealed the challenge of sampling a population at very low densities and drawing conclusions from sparse counts. The koala counts were highly zero-inflated which made it difficult to reliably estimate which survey covariates had the most important influence on koala counts (such as differences between night-time vs. day-time surveys, seasonal effects, or substantive differences between Bagotville vs. Broadwater). Such covariates undoubtedly have *some* effect on koala counts, even if it is not possible to robustly identify which ones are most influential right now. This is important because the existence of unexplained heterogeneity decreases one's power to detect trends. In lieu of more data, this study tried to make the best

use of the available data via model-averaging, which provided a principled framework to shrink unimportant effects to zero, while properly accounting for the degree of estimation uncertainty about the system.

Subsequent power analyses should better resolve which factors are most important for surveying (such as seasonal effects) and could strengthen evidence for modifying the protocol (such as discontinuing the radial-search transects and/or night-time surveys). It would also strengthen the conclusions of the power analyses and remove/reduce the need for informative Bayesian priors.

5.3 DNA analysis

Genetic diversity serves as a way for populations to adapt to changing environments. With more variation, it is more likely that some individuals in a population will possess variations of alleles that are suited for the environment. Those individuals are more likely to survive to produce offspring bearing that allele. The population will continue for more generations because of the success of these individuals. Importantly, genetic diversity of the sampled population reveals a moderate-high level of diversity when compared to similar studies (e.g. Lee *et al.* 2010), despite the indication of an increased inbreeding coefficient value.

Two previous genetic studies within the focal area have similarly reported moderate levels of genetic diversity and negligible levels of inbreeding (Norman *et al.*, 2015; Neaves *et al.* 2015). Whereas the evidence from the current study suggests genetic sub-structuring into two genetic clusters within the population, these clusters are not spatially segregated but largely mixed across the focal area. Norman *et al.* (2015) also reported some evidence of genetic structuring but rather between the individuals in the north and south of the study area. Evidence from the current and previous studies suggest that gene flow was occurring across the focal area (Norman *et al.*, 2015; Neaves *et al.* 2015).

5.4 Road mortality

No koalas were detected during spring 2017 or winter 2018 road mortality surveys. Records retained by Pacific Complete on W2B Project Wide Koala Observations, which is supplemented by data from Friends of the Koala, reported one koala road mortality during the reporting period (Pacific Complete, unpub. data). The individual was struck on Old Bagotville Road, approximately 1km west of the alignment during a long-weekend outside of construction hours.

In endeavouring to achieve the key mitigation measure of the PVA (Kavanagh 2016) to reduce koala mortality by 4-8 individuals per year, RMS have installed six vehicle-activated signs at road mortality hot-spots across the broader section 10 study area and fenced Wardell Road and the existing Pacific Highway. Since installation of fencing, no road strikes have been reported on these two stretches of road compared to 10 in the previous year (FOK, unpublished data).

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Appendix A – Individual koala details

Table A1: Details of individual koalas observed during the Broadwater population monitoring.

Site	Survey type	Sex	Condition	Tree species	DBH (mm)	Additional information
Spring 2017						
S15	Transect (day)	Male	Good	<i>Eucalyptus robusta</i>	180	Clean rump, healthy looking, eyes ok.
S15	Transect (night)	Male	Good	<i>E. robusta</i>	220	Same individual as above
S15a	Incidental (day)	Female	Good	<i>Acacia disparrima</i>	300	Clean rump, healthy looking, eyes ok.
S15b	Incidental (night)	Female	Good	<i>E. robusta</i>	?	Likely same individual as above
S26	Transect (night)	Female	Moderate	<i>E. robusta</i>	?	Dirty rump
S32a	Incidental (night)	Male	Good	<i>E. racemosa</i>	390	Looked ok. Rump clean, left eye ok, right eye unsighted
Autumn 2018						
S02	Transect (day)	Female	Good	<i>Acacia melanoxylon</i>	120	Eyes ok. Rump clean. Sitting at about 3m in a small acacia in a small stand of swamp magonany
S02	Transect (night)	Female	Good	<i>E. robusta</i>	370	Same individual as above
S08	Transect (day)	Male	Good	<i>E. robusta</i>	160	Looked healthy. Eyes and rump ok.
S08	Transect (night)	Male	Good	<i>E. robusta</i>	390	Same individual as above
S08a	Incidental (night)	Female	Good	<i>E. robusta</i>	410	Looks good. Eyes reflecting well in the light. Rump looks clean.
S12a	Incidental (day)	Unknown	Good?	<i>E. robusta</i>	320	Looks like a male though not confirmed. Both eyes look ok. Rump not sighted
S25a	Incidental (day & night)	Male	Good	<i>E. robusta</i>	230 & 160	Both eyes clear and rump clean and dry. Looked generally very healthy. Same codominant tree during diurnal and nocturnal surveys, approx. 4m off transect
S32	Transect (day & night)	Unknown	Good	<i>E. robusta</i>	160	Unsure of sex, possibly male. Rump looked clean, left eye sighted, looked ok. Same tree during diurnal and nocturnal surveys
S38	Transect (day & night)	Female	Poor	<i>E. robusta</i>	370	Rump and right eye clear, left eye major infection and closed. Same tree during diurnal and nocturnal surveys
S38a	Incidental (day)	Male	Moderate	<i>E. robusta</i>	640	Looks skinny, might just be old. Good views - eyes and rump clear.
S41a	Incidental (day)	Male	Poor	<i>E. robusta</i>	530	Left eye and rump clear, right eye heavily infected
S43a	Incidental (day)	Male	Moderate/Good	<i>E. racemosa</i>	850	Eyes looked clear, rump slightly stained

Site	Survey type	Sex	Condition	Tree species	DBH (mm)	Additional information
S51a	Incidental (night)	Unknown	Good	<i>E. tereticornis</i>	1370	Clear eyes, rump clean
S51a	Incidental (night)	Unknown	Poor/ Moderate	<i>E. tereticornis</i>	1370	Clear eyes, stained rump. Possibly a diseased individual. In same tree with koala above

Table A2: Details of individual koalas observed during the Bagotville population monitoring.

Site	Survey type	Sex	Condition	Tree species	DBH (mm)	Additional information
Spring 2017						
N09	Transect (night)	Female	Good	<i>Corymbia intermedia</i>	120	Eyes and rump look clear
N09a	Incidental (night)	Female	Good	<i>E. pilularis</i>	720	Eyes and rump look clear
N12a	Incidental (day)	Female & male back young	Good	<i>E. tereticornis</i>	490	Breeding female with large male joey on back. Red ear tag in the female's right ear. Both joey and female looked healthy; eyes and rump look clear.
N12b	Incidental (night)	Male	Good	<i>E. tereticornis</i>	60	Young male. 2m up small tree. Eyes and rump look clear
N19	Transect (day)	Female	Moderate	<i>C. maculata</i>	540	Stained rump. Clear eyes. 13m up tree
N25a	Incidental (night)	Male	Good	On ground	N/A	Koala on the ground on road side of OBR fencing. Walking, moving, looked healthy; No evidence of injury or car strike; Active sternal gland. Eyes and rump look clear
N36	Transect (day & night)	Female	Good	<i>E. robusta</i>	?	Eyes and rump look clear. Same tree during diurnal and nocturnal surveys
N36	Transect (night)	Unknown	Good	<i>E. racemosa</i>	700	Small koala, sub-adult? Eyes and rump look clear
N45a	Incidental (night)	Male	Good	<i>E. microcorys</i>	550	Looked healthy, Eyes were ok. Rump ok.
Autumn 2018						
N10a	Incidental (day)	Male	Good	<i>E. patentinervis</i>	760	Eyes ok, no obvious concerns. Three koalas in same tree.
N10b	Incidental (day)	Female	Moderate/ Good	<i>E. patentinervis</i>	760	Mildly stained rump, eyes ok. In same tree as koala above.
N10c	Incidental (day & night)	Unknown	Good	<i>E. patentinervis</i>	760	Eyes and rump ok. In same tree as koalas above. Observed during diurnal and nocturnal surveys
N28	Transect (day & night)	Unknown	Good	<i>E. robusta</i>	360	No obvious concerns. Eyes and rump ok
N33	Transect (day & night)	Female	Good	<i>E. resinifera</i>	760	Left eye good. No tags, possible pouch budge. Rump ok. Same tree during diurnal and nocturnal surveys.
N34	Transect (day)	Female	Good	<i>E. tereticornis</i>	410	Red right ear tag. Eyes ok. Rump clean.
N34a	Incidental (night)	Female	Good	<i>E. tereticornis</i>	200	Same individual as above but has moved off transect

Site	Survey type	Sex	Condition	Tree species	DBH (mm)	Additional information
N45	Transect (day & night)	Male	Moderate/Good	<i>E. microcorys</i>	630	Small male, excellent coat, eyes clear, rump stained
N73	Transect (night)	Unknown	Moderate/Good	Unknown	720	Small koala. Clear eyes, rump mild staining
N74	Transect (day & night)	Female	Good	<i>E. tereticornis</i>	540	Clear eyes and rump; small female
N74a	Incidental (day)	Female	Good	<i>E. tereticornis</i>	590	Clear eyes and rump. Pouch young
N74b	Incidental (day)	Unknown	Good?	<i>E. tereticornis</i>	630	Clear rump, no view of eyes.
N74c	Incidental (night)	Unknown	Good	Unknown rainforest tree	170	Clear eyes and rump
N74d	Incidental (night)	Unknown	Good	<i>E. tereticornis</i>	430	Clear eyes and rump

Appendix B – Power analysis report

Prospective Power Analysis of Koala Monitoring Programs in Bagotville and Broadwater, NSW, Australia.

9th December 2018

By Robert W Rankin, Ph.D.

Georgetown University, Department of Biology, Washington DC, USA

Summary

This document presents a prospective power analysis of a koala monitoring program at two sites (Bagotville and Broadwater) in New South Wales, Australia. The objective of the monitoring program is to be able to detect a significant change in koala population density over 15 years of monitoring. Specifically, at Bagotville, the monitoring program wants to be able to detect a -30% change from baseline densities in 2015 while maintaining Type-I and Type-II error rates at 0.3 (i.e., a statistical power of 0.7 and $\alpha \leq .3$).

The analysis was divided into two steps: a Bayesian estimation analysis using existing survey data, and a prospective power analysis based on inputs from the Bayesian estimation exercise.

The Bayesian analysis provided posterior estimates of koala densities using survey data from 2015 (the baseline year) and 2017/2018 (Year 1 of the monitoring program) at Bagotville and Broadwater. The analyses also estimated which survey-design factors were most influential on the observed koala counts. The outputs of the Bayesian estimation exercise were inputted into a Monte-Carlo simulation exercise to project the population of koalas into 2031 (Year 15 of the monitoring program), and assess the ability of the monitoring program to detect significant trends.

The prospective analysis concluded that the monitoring programs are likely to meet their objective power of 0.7 at a Type-I error rate of $\alpha \leq .30$ at Broadwater and Bagotville. Further insights and recommendations were also provided about possible modifications of the survey-design (Appendix II).

Methods

This analysis consisted of two parts: *estimation* and *prospective power analysis*. The estimation exercise was a preparatory exercise for the prospective power analysis.

Bayesian Estimation

The estimation exercise provided empirical estimates of important features of the koala monitoring program for incorporation into the power analyses. The estimation exercise used a Bayesian framework. This was important for two reasons. First, the framework allowed the integration of multiple sources of information, including count information from the koala survey programs and prior estimates of baseline densities. Bayesian

analyses are capable of integrating these two types of information through the use of *prior distributions* and *data*.

Secondly, the exercise was Bayesian in the sense that the outputs (i.e., the posterior probability distributions) were interpreted as characterising our *uncertainty* about the koala populations, given the existing count data and priors. By quantifying the uncertainty in this manner, we get a defensible and robust characterisation of the survey process and koala populations, including: the plausible range of values for the baseline koala densities at Broadwater and Bagotville; uncertainty about the difference between day-time surveys vs. night-time surveys; uncertainty about the effect of spring vs. autumn surveys; and the presence of count overdispersion. These were important for projecting the population forward in the power analysis.

Prospective Power Analysis

The goal of the monitoring project is to achieve a statistical power of 0.70 to detect a population trend, assuming a worse case decline of -30% from baseline koala densities in 2015 until Year 15 of the monitoring program. Statistical power is one minus the Type-II error rate (the rate of falsely rejecting the hypothesis of a trend, $H_a: \beta_t \neq 0$), meaning that we desire a Type-II error rate of at most 0.30. A key assumption is the specification of the Type-I error α (the rate of false alarms, i.e., falsely rejecting a hypothesis of no trend, $H_0: \beta_t = 0$).

Setting Error Rates

Given that the target Type-II error rate was mandated to be 0.30, we decided upon a Type-I rate cap at $\alpha \leq 0.30$. In such an error-control framework, there is a trade-off between the two error rates: a value-judgement is necessary in order to motivate whether the Type-I or Type-II error rates should be lower or higher. In particular, we must consider their respective costs of being wrong. In conservation context, it is generally costlier when one *fails* to identify a steep population decline (which results in reactionary and expensive interventions). In contrast, it is generally less costly to raise false alarms, especially when a monitoring program is already underway. Therefore, in this study, it was justifiable to set the Type-I error to be equal or higher than the Type-II error. This is a precautionary approach which places more value on detecting ecological trends.¹ Given the mandated Type-II error rate of 0.30, this meant that we set should set α to be at least 0.30, and perhaps higher if the data and analyses lacked sufficient power.

The analyses also considered other combinations of population declines and Type-I error rates, in order to see how the statistical power would behave under decreasing α levels and decreasing magnitudes of $|\beta_t|$.

1 This contrasts with the “conventional” approach in the hard sciences whereby one sets the Type-I errors to be as low as possible (e.g., $\alpha = 0.01$). In such cases, one’s value-judgement is that one does not wish to reject an established theory (or ‘null’ hypothesis) unless there is overwhelming evidence against it. This reasoning does not apply to conservation issues.

Combining Analyses

Using the outputs of the Bayesian estimation exercise, the prospective power analyses integrated multiple sources of statistical variation. Most power analyses only include *sampling variation* (the distribution of the test statistic under random samples of data) but fail to include *estimation & model uncertainty* (uncertainty in parameter values and model selection uncertainty). The sampling variation was incorporated into the analysis through the use of Monte-Carlo simulations of koala counts. The estimation/model uncertainty was incorporated by using the posterior distributions from the Bayesian estimation exercise for the simulations' parameter values (e.g., uncertainty in baseline densities, role of survey methodology, seasonality, etc.). In total, this meant that the prospective power analysis made fewer parametric assumptions and better reflected the overall uncertainty about the koala populations.

Data

The data consisted of koala counts from two locations. There were baseline surveys at Bagotville in the Spring of 2013 and 2015, as well as "Year 1" surveys in Spring 2017/Autumn 2018. At Broadwater, there were baseline surveys in the Spring of 2015, as well as Year 1 surveys in Spring 2017/Autumn 2018. The surveys occurred along line-transects and radial-search transects in teams of three observers. The detection probability per team was assumed to be 100% (or $p_d=1$ per team).

Part 1: Estimation

Model

Bayesian hierarchical models were run using the JAGS sampler (Plummer 2007, 2014). The models were constructed from the following density formula:

$$\eta_{l,t,j} = \frac{N_{l,t,j}}{A_{l,t,j}} \iff N_{l,t,j} = \eta_{l,t,j} A_{l,t,j}$$

where η is the density of koalas at location l and time t and line-transect/radial-transect j ; N is the number of koalas; and A is the area at transect j . The location index l refers to either Broadwater or Bagotville.

The above expression can be re-written so that the survey data (i.e., the observed koala counts y) can be related to the quantity of inferential interest (i.e., the koala density η) through a statistical count distribution. The preferred count distribution is the Poisson or the Negative Binomial (NB, which is an overdispersed Poisson distribution with overdispersion parameter θ).

$$y_{l,t,j} \sim \text{Pois}(\mathbb{E}[y]_{l,t,j}) \text{ or } y_{l,t,j} \sim \text{NB}(\mathbb{E}[y]_{l,t,j}, \theta)$$

where $\mathbb{E}[y]$ is the expected koala counts at l,t,j . Notice the inequality $\mathbb{E}[y]_{l,t,j} \leq N_{l,t,j}$ because of factors such as imperfect detection ($p_d \leq 1$). Thus, we substitute in the correction factor for the density equation:

$$\mathbb{E}[y]_{l,t,j} = p_d \cdot \eta_{l,t,j} \cdot A_{l,t,j}$$

For the statistical analysis, we re-express this expectation on the log scale...

$$\log(\mathbb{E}[y]_{l,t,j}) = \log(\eta_{l,t,j} \cdot p_d \cdot A_{l,t,j})$$

$$\log(\mathbb{E}[y]_{l,t,j}) = \log(\eta_{l,t,j}) + \log(p_d \cdot A_{l,t,j})$$

... and then replace the koala density term (η) with a log-linear model:

$$\log(\mathbb{E}[y]_{l,t,j}) = \boldsymbol{\beta}^T \mathbf{x}_{l,t,j} + \log(p_d \cdot A_{l,t,j})$$

$$\therefore y_{l,t,j} \sim \text{NB}\left(e^{(\boldsymbol{\beta}^T \mathbf{x}_{l,t,j} + \log(p_d \cdot A_{l,t,j}))}, \theta\right)$$

Thus, we can estimate the change in koala density using count data (y), area of transects (A), and various covariates (\mathbf{x} , p_d). The right-hand-side of the model has two terms. The term $\log(p_d \cdot A_{l,t,j})$ is known as an “offset” which includes imperfect detection (p_d) and the area surveyed (A): these are assumed to be known and without uncertainty (for the remainder of the analysis, we assume that $p_d = 1$ but maintain the notation for future reference²). The 2nd term $\boldsymbol{\beta}^T \mathbf{x}_{l,t,j}$ includes the vector of parameters that we wish to estimate ($\boldsymbol{\beta}$), while \mathbf{x} is a vector of covariates that describes the observation at i,t,j . We can expand the term $\boldsymbol{\beta}^T \mathbf{x}_{l,t,j}$ to its individual regression constituents:

$$\begin{aligned} \log(\mathbb{E}[y]_{l,t,j}) &= \beta_0 + \beta_l \cdot \mathbb{I}[l = \text{Broadwater}]_{t,j} \\ &+ \beta_t t + \beta_{t,bw} \cdot t \cdot \mathbb{I}[l = \text{Broadwater}]_{t,j} \\ &+ \beta_r \cdot \mathbb{I}[r = \text{radial}]_{t,j} \\ &+ \beta_n \cdot \mathbb{I}[n = \text{night}]_{l,t,j} + \beta_s \cdot X_{l,t,j}^{(\text{season})} \\ &+ \log(p_d \cdot A_{l,t,j}) \end{aligned} \quad (\text{Eqn. 1})$$

where β_0 is the log-density intercept (Bagotville); β_l is the marginal difference in log-density between Broadwater and Bagotville; β_t is the annual change in koala density per year; $\beta_{t,bw}$ is the interaction term between the main trend and Broadwater’s trend; β_r is the marginal effect of surveying a radial-search vs. a line-transect; β_n is the marginal effect of surveying during the night-time vs. day-time; β_s is the marginal seasonal effect of surveying in the spring vs. autumn. The expression $\mathbb{I}[\cdot]$ is an “indicator function” which takes a characteristic of the observation at j (such as a night-time survey vs. day-time survey, and radial vs. line-transect survey) and converts it into a dichotomous covariate of 0’s and 1’s. Finally, $X^{(\text{season})}$ is a dichotomous covariate with values -0.5 and 0.5, which represents spring vs. autumn surveys. The latter covariates was scored as -0.5/0.5 so that the intercept (β_0) represented: i) the base-line density at Bagotville in 2015, and ii) a mid-point between the marginal effects of autumn vs. spring. Therefore, the baseline densities average out the seasonal effects.

One important detail about Eqn. 1 was the judicious use of *pooling* to better estimate marginal effects across both Broadwater and Bagotville in the face of severe data sparsity (such as transect/radial effects, day-time/night-time effects, seasonal effects, and overdispersion). The pooled marginal effects were shared over both Broadwater and Bagotville (although future analysts may wish to estimate them independently).

2 There were three observers per surveys, such that the probability of missing a koala by all three observers was assumed to be approximately zero, which therefore motivated 100% perfect detection probability.

The above model (Eqn 1) could be analysed with either a Poisson or Negative Binomial count distribution. It could also be analysed in either a Bayesian or frequentist framework. However, the Bayesian framework was used because the objective of the estimation exercise was to obtain *posterior distributions* about the baseline densities and marginal effects ($\beta_l, \beta_n, \beta_r$) for use in the prospective power analysis.

Bayesian Priors

Bayesian priors are a necessary component of posterior inference. While conservationists generally use uninformative priors, informative priors were useful in these analyses for two reasons.

First, Bayesian priors provide a coherent, unified framework to integrate other types of information alongside *data* within an analysis. Specifically, a baseline koala population density at Bagotville in 2015 was available as a plausible prior estimate to help construct a prior (estimated previously to be 0.091 koalas/ha, SE 0.03). Such information was not considered robust, but could nonetheless help guide the MCMC estimation program under sparse-data situations.

The second important use of Bayesian priors was to guard against extreme values and ensure that the analysis produced reasonable posteriors for the regression parameters ($\beta_0, \beta_t, \beta_l, \beta_{t,bw}, \beta_s, \beta_r, \beta_n$). This was important due to the extreme sparsity of count data. I therefore used the following Gaussian priors $\mathcal{N}(\mu_0, \sigma_0^2)$, which were motivated according to an overarching rationale of trying to assign low prior probability to extremely implausible values, while also being non-informative at intermediate parameter values.

Bagotville baseline (log) density. The prior on the log baseline density at Bagotville was given the following Gaussian distribution: $\pi(\beta_0) = \mathcal{N}(\log(0.091), 0.41^2)$. The mean (0.091 koalas/ha) was provided from a previous population viability analyses at Bagotville, while the variance (0.41^2) was set to be approximately one-half the *precision* of the previous estimated variance (0.307^2). By decreasing the precision from the previous estimate, the prior is declaring weak confidence in the previous estimate, while nonetheless borrowing some information about the mean. Note that the Bagotville baseline log-density in 2015 was arbitrarily chosen to be the regression intercept β_0 in Eqn. 1.

Broadwater. The prior on the marginal difference between the Broadwater log-density vs. Bagotville was $\pi(\beta_l) = \mathcal{N}(0, 0.54^2)$. This prior encoded the expectation that the koala densities at Broadwater were similar to the densities at Bagotville, while allowing a 20% chance that they could be double or half the density.

Trend. The prior on the main trend variable was $\pi(\beta_t) = \mathcal{N}(0, 0.05^2)$. This had a prior expectation of no trend, and an 80% expectation that the annual trend was between extreme values of -6.2% to 6.6%. These extreme values are equivalent to a 62% decrease over 15 year, and 260% increase over 15 years, respectively. The prior made it unlikely that the estimated trend was beyond such values.

Trend-Broadwater. The prior on the interaction term (marginal difference in the trend at Broadwater) was $\pi(\beta_{t,bw}) = \mathcal{N}(0, 0.02^2)$. This kept the expected difference between the trend at Broadwater and

Bagotville to be 0, while allowing an 80% chance that it could be between -2.5% to 2.5% (which compounds to differences of -30% to 140% over 15 years). More extreme values had only a 20% chance.

Transects, Night-time, Season. The prior on the marginal effect of the radial- vs. line-transects was $\pi(\beta_r) = \mathcal{N}(0, 0.54^2)$. This gave a prior expectation that there was no difference in counts of koalas between transect types, while allowing a 10% chance that the radial-transect had double the koalas counts as compared to line-transects, and a 10% chance that they had half the koala counts. The marginal effects of night-time vs. day-time, and autumn vs. spring, had the same prior mean and variance.

The priors are plotted in Figure 1, alongside their posteriors.

Overdispersion. For the Negative Binomial overdispersion parameter θ , a Gamma prior was used with a prior mean of 5 (such a value implied almost no overdispersion, and that the conditional counts of koalas was nearly Poisson). Unfortunately, the use of uninformative priors for θ led to radical values and an inestimable θ . I therefore used a quasi-Empirical Bayesian method of let the data decide upon a reasonable range of informative Gamma priors. Within this range, each possible Gamma had the same prior mean (5) but differed according to the prior variance. The set of priors had the following shape and rate parameters: $\{(5,1), (10,2), (20,4), (40,8), (500,100)\}$. Low values allowed the possibility of some overdispersion, while higher values effectively forced the Negative Binomial to behave like a Poisson distribution. The different values were used in separate models; the final overdispersion parameter was estimated by model-averaging over all these different priors, as detailed in the following section. In other words, I let the decision about the informativeness of the θ prior be a model-selection/model-averaging decision, which is known as “Empirical Bayes.”

Model-selection and Model-Averaging

In the full model of Eqn. 1, β is over-parameterised due to the sparsity of counts of koalas at Bagotville and Broadwater. In future analyses, more data should make parameter estimation more reliable (e.g., better estimates of marginal effects of season, night-time vs day-time, radial vs line transects). It is important to explore the influence of such covariates, otherwise their unexplained variance will decrease the power of future analysts to estimate the true biological trend in koala population counts. Inasmuch as we can reduce unexplained variance, we should.

In the current analysis, it was important to try to discriminate between important and spurious covariates, in order to minimise unexplained variance (and improve power), while not overfitting the data. This dilemma, between excess variance and overfitting is best handled via a model-selection and model-averaging framework: i.e., to find the best combination of parameters that yield the highest predictive accuracy, while minimising the influence of spurious covariates.

To this end, a variety of models were run and weighted by the Watanabe-Akaike Information Criterion (WAIC; Watanabe 2010, Gelman et al. 2014). The WAIC-weights (a.k.a. pseudo model probabilities) were used for model-averaging in order to get a justifiable amount of model-complexity and improve the accuracy of the estimates. When a parameter is unsupported by the WAIC, its posterior distribution shows strong *shrinkage* to

zero, which is crucial for predictive accuracy (Copas 1983, 1997, Longford 2006), i.e., its posterior distribution has a sharp spike at zero (signifying no effect), and a low posterior probability everywhere else.

The 8 candidate specifications for β were:

$$\begin{aligned}
 m_1: \beta &= [\beta_0, \beta_t, \beta_{t,bw}, \beta_l]^\top \\
 m_2: \beta &= [\beta_0, \beta_t, \beta_{t,bw}, \beta_l, \beta_n]^\top \\
 m_3: \beta &= [\beta_0, \beta_t, \beta_{t,bw}, \beta_l, \beta_r]^\top \\
 m_4: \beta &= [\beta_0, \beta_t, \beta_{t,bw}, \beta_l, \beta_s]^\top \\
 m_5: \beta &= [\beta_0, \beta_t, \beta_{t,bw}, \beta_l, \beta_n, \beta_r]^\top \\
 m_6: \beta &= [\beta_0, \beta_t, \beta_{t,bw}, \beta_l, \beta_n, \beta_s]^\top \\
 m_7: \beta &= [\beta_0, \beta_t, \beta_{t,bw}, \beta_l, \beta_r, \beta_s]^\top \\
 m_8: \beta &= [\beta_0, \beta_t, \beta_{t,bw}, \beta_l, \beta_n, \beta_r, \beta_s]^\top \text{(Eqn. 2)}
 \end{aligned}$$

In addition to the above 8 model specifications, each specification was combined with one of five different Gamma priors for the Negative Binomial overdispersion parameter θ (described in the section above), for a total of 40 different models. Each model was run in JAGS (Plummer 2007, 2014) with 80000 MCMC samples plus a 5000-sample burn-in period. Posteriors were inspected for adequate mixing and convergence.

The final posterior distributions were model-averaged according to each models' WAIC weight. This was achieved by subsampling each models' MCMC draws $(\beta^{(m)}, \theta^{(m)})$ according to its model weights:

$$p(m|\mathbf{y}) \approx \frac{e^{-0.5\text{WAIC}^{(m)}}}{\sum_m^M e^{-0.5\text{WAIC}^{(m)}}}$$

Power Analyses

The goal of the power analyses was to estimate the rate of Type-II errors (falsely rejecting the hypothesis of a trend, $H_a: \beta_t \neq 0$) while detecting a -30% decline from baseline levels at Broadwater and Bagotville between years 2015 and 2031. The estimate of statistical power, per location, is one minus the rate of Type-II errors. The error rates were conditional on:

1. a negative trend of -30% from baseline koala densities in 2015 to Year 15 of the monitoring program (2031);
2. a cap on the rate of Type-I errors at $\alpha \leq 0.3$ (falsely rejecting a null hypothesis $H_0: \beta_t = 0$);
3. a monitoring effort of 400 transects per year per location (Broadwater and Bagotville separately) for 15 Years between 2017 to 2031, including a uniform allocation of effort among survey conditions such as day-time/night-time, spring/autumn, and line-transect/radial-transects (see Appendix II for an alternative scenario);
4. marginal effects for survey-design factors (day-time/night-time, spring/autumn, and line-transect/radial-search transects) empirically derived from the Bayesian analysis;

5. baseline koala densities in 2015 derived from the Bayesian estimation analysis.

The power analysis used Monte Carlo (MC) integration to incorporate several sources of important uncertainty. First, there was the uncertainty in the baseline densities at Bagotville and Broadwater, as quantified by the posterior distributions of baseline densities from the previous Bayesian estimation exercise. Secondly, there was estimation uncertainty in the marginal effects of different survey conditions (such as day-time/night-time, spring/autumn, and line-transect/radial-search). This uncertainty was also incorporated by using the posterior distributions from the Bayesian estimation exercise. Third, there was the *multi-model* uncertainty due to multiple candidate models for estimating statistical power. Consider that the future analysts who are tasked with estimating the population trends will want to improve their statistical accuracy by including or excluding certain covariates, and will likely perform model-selection by AIC. The simulations included such post-doc model-selection.

These three sources of uncertainty made the calculation of Type-II errors non-trivial and best estimated through MC simulations.

The power analysis proceeded as follows:

1. set the annual percent decline to $-\delta$, and set parameters $\beta_t = \log(1 - \delta)$, $\beta_{t,bw} = 0$;
2. set the desired Type-I error rate to α ;
3. for i in 1 to 4000 Monte Carlo iterations, do:
 - I. get a sample parameter values from the Bayesian posteriors (e.g., baseline densities, overdispersion, marginal effects of day-time/night-time, spring/autumn, and line-transect/radial-search) $\beta_{-t}^{(i)} \sim \pi(\beta_{-t}|\mathbf{y})$, $\theta^{(i)} \sim \pi(\theta|\mathbf{y})$, and combine these samples with the specified trend: $\beta^{(i)} = (\beta_{-t}^{(i)}, \beta_t, \beta_{t,bw})^\top$;
 - II. simulate count data using the linear model in Eqn. 1 and parameters $\beta^{(i)}$

$$y_{l,t,j}^{(i)} \sim \text{NB}\left(e^{(\mathbf{x}_{l,t,j}\beta^{(i)} + \log(p_d \cdot A_{l,t,j}))}, \theta^{(i)}\right);$$
 - III. use the simulated data $\mathbf{Y}^{(i)}$ to get maximum-likelihood estimates of the trend and standard error $(\hat{\beta}_t^{(i)}, \hat{\text{se}}(\beta_t)^{(i)})$ for both Broadwater and Bagotville, including:
 - i. option 1: use the Poisson *full-model* (model m_8 in Eqn. 2), or
 - ii. option 2: use the best AIC Poisson model from models m_1 to m_8

(this analysis proceeded with option 2, but I also ran option 1 for comparison purposes)
 - IV. for each location l (Broadwater and Bagotville) compare the two-tailed Fisher p-value to α and calculate the score statistic l

$$I_l^{(i)} = 2 \left(1 - \text{PDF}_{\mathcal{N}} \left(\frac{|\hat{\beta}_t^{(i)}|}{\hat{\text{se}}(\beta_t)^{(i)}} \right) \right) \leq \alpha$$

4. over all 4000 iterations, the estimated Type-II error rate (per / location Broadwater and Bagotville) was

$$\hat{b}_{l,\alpha,\beta_t} \approx \frac{1}{4000} \sum_{i=1}^{4000} I_l^{(i)} \quad \text{and the power is } 1 - \hat{b}_{l,\alpha,\beta_t}$$

Useful Variants

For comparison purposes, some alternative specifications of the above MC power analysis were also considered.

Poisson vs. Negative-Binomial: In step 3(III), the data could have been analysed from according to a Poisson GLM or Negative-Binomial GLM. However, at very low densities, the two are difficult to distinguish, and trial analyses revealed that the NB models were prone to failure from data sparsity.

Full-model vs. AIC best-model: In step 3(III), the full-model approach represents a more conventional and tractable power analysis but using the AIC best-model is more in line with modern multi-model inference. Practitioners typically use model-selection to negotiate underfitting vs. overfitting and improve the accuracy of estimates. However, such model-selection means that the power estimates are slightly biased high (Leeb and Pötscher 2005). In contrast, the power estimates from the full-model are unbiased, but overly pessimistic due to increased estimation variance: practitioners would, in reality, use model-selection to improve estimation accuracy and reduce variance. This is known as the bias-variance trade-off (Wit et al. 2012). In this study, both alternatives were employed for comparison purposes.

One-tailed vs. two-tailed test-statistics: The test statistic in step 3(IV) was compared to a *two-tailed rejection region*, which means that we reject the null hypothesis (of no trend) if the test statistic is either extremely positive (population increase) or extremely negative (population decline). This is the conventional approach, but it leads to diminished power because we must guard against Type-I errors on both ends of the test statistic's distribution, whereas in this study we are only really interested in declines. Alternatively, we could focus only on the Type-I errors at the negative end of the distribution, which is in keeping with the principal concern of the koala conservation program to detect a negative population trend. This allows us to not worry about significant population increases. A one-tailed rejection region would lead to higher statistical power to detect population declines, at the cost of foregoing the ability to detect a population increase. For comparison purposes only, both one-tailed and two-tailed power analyses were performed. Among statisticians however, it is considered dishonest to switch between one-tailed vs. two-tailed error control for the purpose of increasing one's power, after the fact.

Bayesian Alternative Notion of Power

Whereas the frequentist power analysis described above is concerned with long-run error rates (Neyman and Pearson 1933), a Bayesian would ask: "given a negative trend, what is the probability that the estimate is correctly estimating a negative trend $\beta_t < 0$?" This can be approximated as:

$$p(\hat{\beta}_t < 0 | \beta_t^{\text{true}}) \approx \frac{1}{4000} \sum_{i=1}^{4000} \mathbb{I}[\hat{\beta}_t^{(i)} < 0]$$

This type of decision follows the notion of *preponderance of evidence*, whereby one declares that the koala population is declining if the probability of a decline is greater than 50%, i.e., if $\pi(\beta_t < 0) > 0.5$. This is fundamentally different from the Neyman-Pearson power-analysis decision rule which seeks to minimise two long-run error rates (Type-I and Type-II), i.e., one has a certain prescribed long-term *confidence* of avoiding Type-I errors (α).

Often, the distinction between Bayesian probabilities of a decline vs. the frequentist notions of a “significant” decline is explained according to a courtroom analogy: Bayesians are like civil judges, whereas frequentists are like criminal judges. In the civil court, judges ask what was more probable (was a decline *more probable* than no decline?), whereas in criminal courts, a judge seeks a high rate of prosecutions (*power*) conditional on capping the rate of long-run *false positives* (wrongly convicting innocent people, a Type-I error) at α .

For comparison purposes only, this alternative “Bayesian power analysis” was also computed.

Auxiliary Analyses

This study also conducted two additional analyses to provide insight into monitoring program’s survey design, such as whether or not to continue with night-time surveys and radial-search transects. These supplementary analyses have been placed in Appendix II.

Results

Bayesian Estimation Exercise

The posterior distributions from the Bayesian estimation analysis at Broadwater and Bagotville are shown in Figure 1. The important results were as follows:

- the baseline koala density at Bagotville was estimated to be 0.092 koalas/ha (SE 0.031, 95%CI: 0.046-0.165), while the baseline density at Broadwater was 0.089 koalas/ha (SE 0.036, 95%CI: 0.039-0.177);
- for Year 1 at Bagotville, the overall koala density was estimated to be 0.085 koalas/ha (SE 0.035, 95%CI: 0.038-0.170), while for Spring it was 0.079 koalas/ha (SE 0.033, 95%CI: 0.034-0.160) and for Autumn the estimate was 0.094 koalas/ha (SE 0.041, 95%CI: 0.039-0.200);
- for Year 1 at Broadwater, the overall estimated density was 0.089 koalas/ha (SE 0.031, 95%CI: 0.044-0.164); in Spring, the estimate was 0.082 koalas/ha (SE 0.031, 95%CI: 0.037-0.157), while for Autumn the estimate was 0.097 koalas/ha (SE 0.036, 95%CI: 0.047-0.184);
- there was considerable model uncertainty, whereby the top 14 models only accounted for 50% of the posterior model probability, implying that the estimates were somewhat sensitive to the set of models and may change with different models;

- the top WAIC model included effects for season ($\beta_s \neq 0$) but not transect-type ($\beta_r = 0$) nor night-time ($\beta_n = 0$), and had very little estimable overdispersion ($\bar{\theta}$ effectively fixed at 5); all five top models had similar specifications;
- there seemed to be some evidence of a seasonal difference in koala counts, such that the counts in the Autumn were approximately 23% higher than the counts in Spring (although the variance was wide, SE 36.7%);
- the marginal effects of night-time vs. day-time surveys and radial vs. line-transects had marginal distributions that were sharply peaked at 0 (known as *shrinkage* due to the low model probabilities estimated for those models which included these effects), suggesting that, given the current amount of data, their effects were unimportant for predicting koala counts (see Appendix II for more exploration of these);
- no population trend could be discerned, given that the trend parameters had posteriors which were almost identical to their priors (i.e., no learning took place).

Power Analysis

Figure 2 shows the estimated power curves for Broadwater and Bagotville using different combinations of trend and Type-I error rates. With a decline of -30% from the 2015 baseline densities and an α value of 0.30, the estimated power for Bagotville and Broadwater were 0.752 and 0.741, respectively. These achieved the desired power rates. Using the full-model specification, the results were nearly identical. For comparisons, if we instead relax the Type-I error rate to a maximum of 0.35, then the estimated power rates were 0.787 and 0.773 at Bagotville and Broadwater respectively. The power curves (Figure 2) also suggest that the program may be able to maintain a tighter Type-I error control of $\alpha \leq 0.23$ and still achieve the desired power of 0.70. Using the Bayesian preponderance of evidence framework, the probability of estimating a decline at Bagotville and Broadwater were 0.954 and 0.945 respectively (given a decline of -30%).

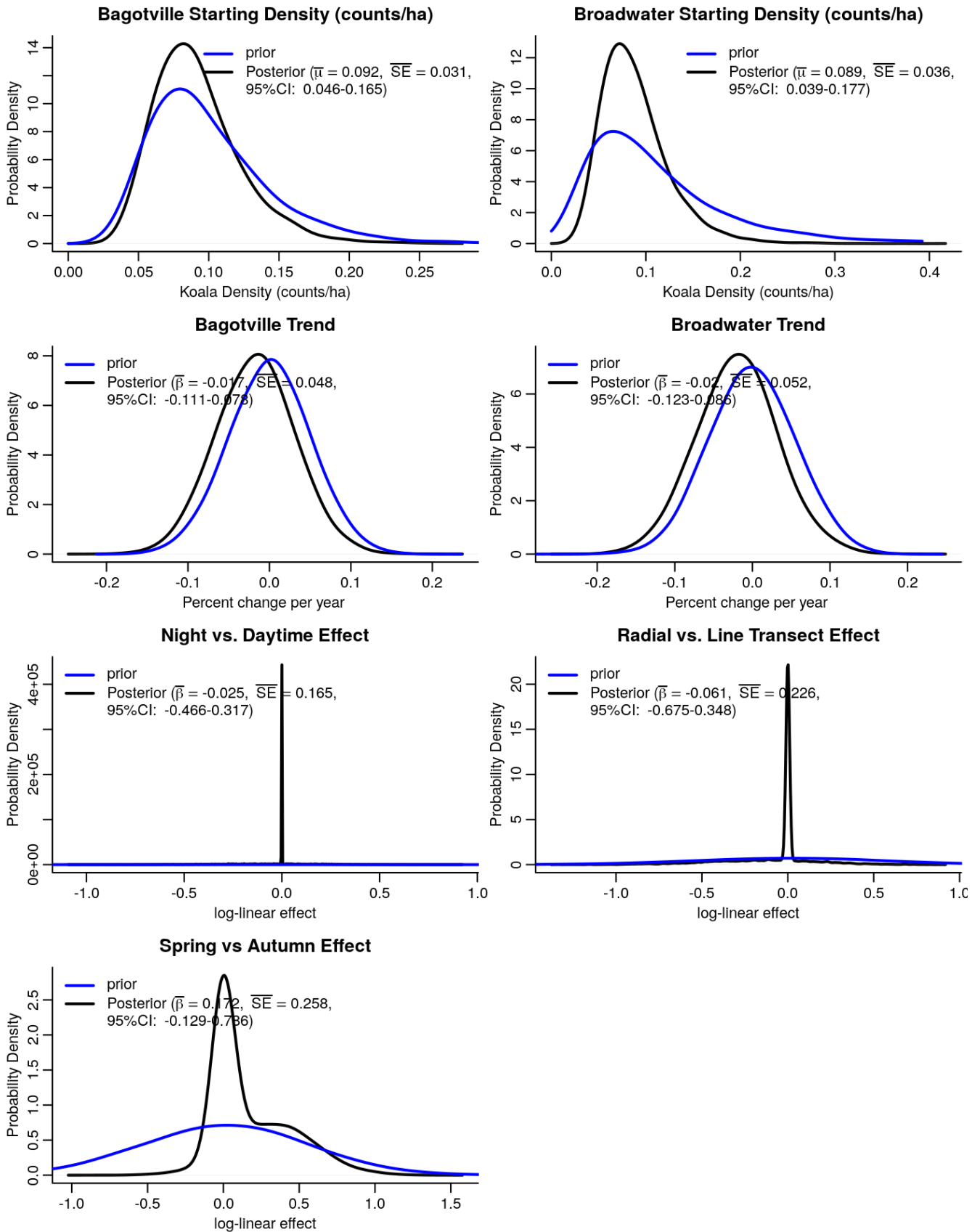


Figure 1: Bayesian model-averaged estimates of the covariates affecting koala surveys. Bayesian priors are in blue and posteriors are in black.

Conclusions

The prospective power analysis demonstrated that the koala monitoring programs at Broadwater and Bagotville are likely to achieve their target levels of statistical power (>0.7) in order to detect a -30% population decline over 15 years of monitoring, while maintaining a Type-I error rate of $\alpha \leq 0.30$. These results are conditional on current levels of survey effort continuing into the future, such as 400 transects per year at either location. There is some weak evidence that the program could tweak the survey protocol (such as discontinuing the radial-search transects and night-time surveys) and still maintain the requisite power (see Appendix II).

The analyses revealed the challenge of sampling a population at very low densities and drawing conclusions from sparse counts. The koala counts were highly zero-inflated which made it difficult to reliably estimate which survey covariates had the most important influence on koala counts (such as differences between night-time vs. day-time surveys, seasonal effects, or substantive differences between Bagotville vs. Broadwater). Such covariates undoubtedly have *some* effect on koala counts, even if it is not possible to robustly identify which ones are most influential right now. This is important because the existence of unexplained heterogeneity decreases one's power to detect trends. In lieu of more data, this study tried to make the best use of the available data via model-averaging, which provided a principled framework to shrink unimportant effects to zero, while properly accounting for the degree of estimation uncertainty about the system.

The project managers may wish to re-do the prospective power analysis after more data has been sampled, in order to revisit these results. First, more data could better resolve which factors are most important for surveying (such as seasonal effects) and could strengthen evidence for modifying the protocol (such as discontinuing the radial-search transects and/or night-time surveys). It would also strengthen the conclusions of the power analyses, and possibly allow for better certainty about the initial baseline conditions at Broadwater and remove/reduce the need for informative Bayesian priors.

Nevertheless, it is a positive result that, despite the high uncertainty about baseline conditions at Broadwater, the current monitoring regime looks to be on-track to meet its target statistical power.

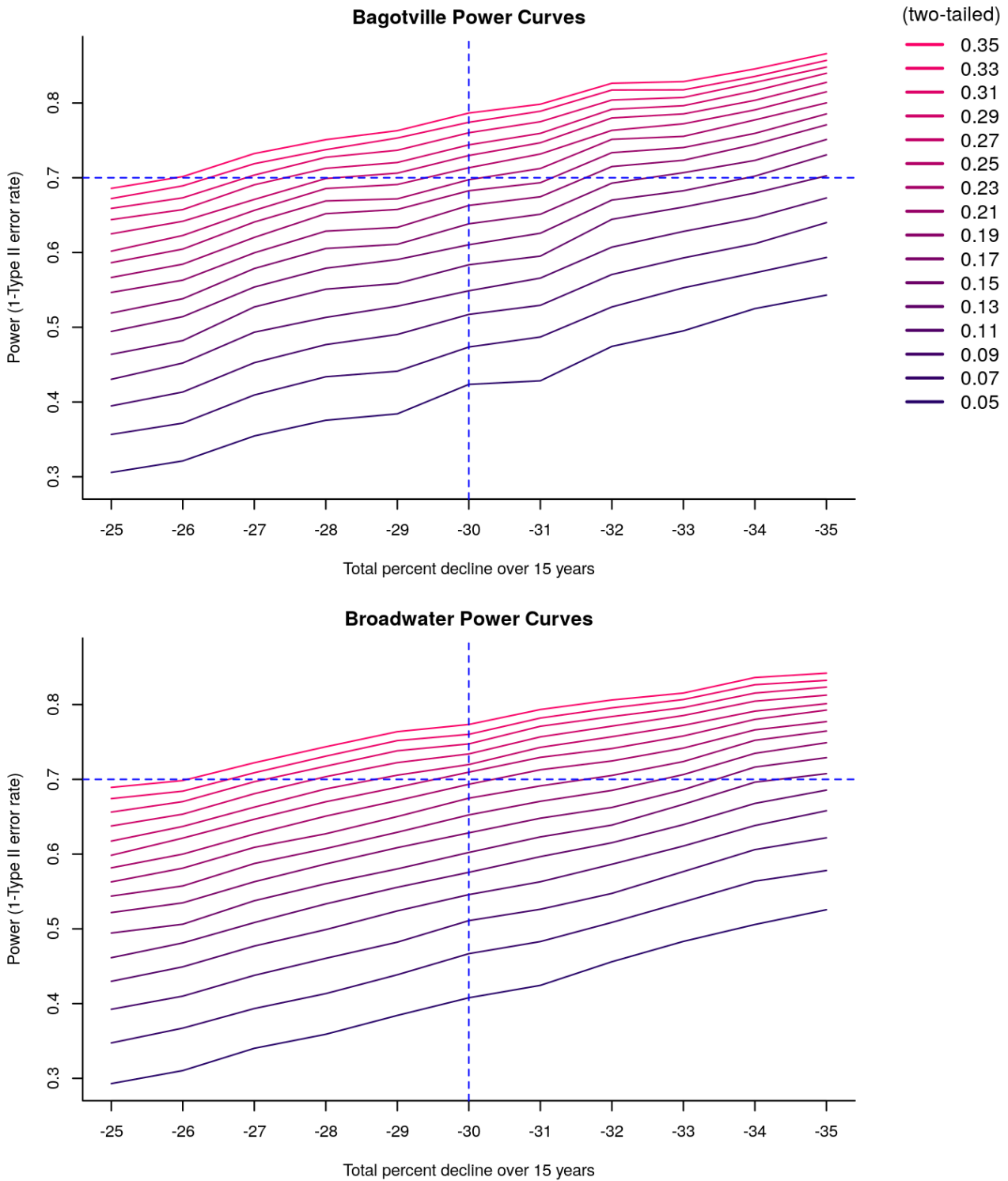


Figure 2: Statistical power to detect a -30% drop in baseline densities (in 2015) in Year 15 of the monitoring program, for different maximum levels of Type-I errors (lines)

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APPENDIX I: Auxiliary Analyses to Inform Koala Survey Design Considerations in Bagotville and Broadwater, NSW, Australia.

By Robert W Rankin, Ph.D.

This Appendix provides two supplementary analyses to help inform possible modifications of the survey design protocol for monitoring koala populations at Bagotville and Broadwater. Specifically, I present a quantitative analysis of the importance of night-time surveys and radial-search transects, using the count data at Bagotville and Broadwater, as presented in the main analyses.

The two questions were:

1. Was there an important difference between night-time vs. day-time surveys?
2. Will there be a significant drop in statistical power if radial-search transects are discontinued in the future?

Night-time Surveys

According to the managers who oversee the surveys, the night-time surveys are sometimes disturbing to local land-owners and they would prefer not to do them. This analysis sought quantitative evidence about whether or not there was any meaningful difference between the counts during night-time surveys vs. day-time surveys.

The Bayesian model-selection exercise can inform this management decision by calculating the probability that there was a marginal effect on counts of day-time vs. night-time transects (regardless of the direction and magnitude of the effect). In a Bayesian framework, this importance indicator is known as the "Bayesian posterior inclusion probability", which can be interpreted as: the probability that the "correct model" includes a marginal effect due to night-time surveys. As a probability, the indicator varies between 0 and 1.

If the inclusion probability of the night-time effect is near 0, then there is strong quantitative evidence that night-time surveys are no different from the day-time surveys (and can be dropped). If the inclusion probability is near 1, then there is strong quantitative evidence that night-time surveys are different and should be continued. If the inclusion probability is approximately 0.5, then it means there is no evidence either for or against keeping the night-time transects (i.e., no "power" to detect a difference).

Posterior inclusion probabilities can also be communicated as a posterior odds-ratio (a.k.a. Bayes Factors), which have conventional thresholds to interpret the strength of evidence. For instance, Kass and Raftery (1995) consider an odds-ratio of $>3.2:1$ to be strong evidence in favour of an effect (i.e., night-time surveys are different), while its reciprocal $<1:0.3125$ would be considered strong evidence in favour of the opposite (i.e., night-time surveys are *not* different from day-time surveys) (Kass and Raftery 1995). A resulting odds-ratio in the range of 0.3125 to 3.2 would be considered as lacking strong evidence to inform a robust decision.

Results: Night-time Surveys

The posterior inclusion probability for the night-time effect was 0.356, which is a posterior odds-ratio of 1:0.552. This means that there was slight evidence *against* the hypothesis that night-time surveys were meaningfully different from day-time surveys. Re-written as the reciprocal odds, it means that there was an approximate 2:1 odd that the night-time surveys were *not different* from day-time surveys in terms of counts.

The calculated odds ratio was between the 3.2:1 and 1:0.3125 “strong evidence” thresholds from Kass and Raftery (1995). Therefore, while there is some weak evidence that there is no meaningful difference between the night-time and day-time surveys, given the sparsity of the data, it would seem prudent to wait for one or two more surveys and revisit this analysis.

Radial-Search Transect Surveys

The second supplementary analysis pertained to the usefulness of the radial-search transects, and whether discontinuing the radial transects would result in an appreciable decline in power to detect koala population declines.

On the one hand, the loss of information from discarding the radial surveys would presumably reduce the statistical power, as would be expected from any reduction in data. On the other hand, the radial surveys contribute much less information to the analysis as compared to the line-transects (due to their smaller search area), and so discontinuing the radial-search transects may not lead to an important degradation in power. Thirdly, the radial surveys are not entirely independent of the line-transects, as so they may distort the statistical power estimates by providing the illusion of independent information.

To answer this question, I re-ran the prospective power analyses in the main document (Appendix I), but modified the scenario such that radial surveys were discontinued after Year 1. All statistics were recalculated. These were compared to the prospective error rates from the default scenario, which maintained the radial surveys for all 15 years of surveys.

Results: Radial-Search Transects and Power

Assuming that the radial-search transects are discontinued after Year 1, the estimated power for Bagotville and Broadwater were 0.721 and 0.716 respectively, using a Type-I error rate of 0.30. These power estimates were slightly lower than the results obtained in the main analysis which kept the radial-search transects in all years. Nonetheless, for this scenario, the power achieves the target rate of 0.70. For a maximum Type-I error rate of 0.35, the estimated power for Bagotville and Broadwater were 0.758 and 0.751, respectively.

Therefore, there is evidence that dropping the radial-search transects will lead to a slight decrease in power (of 0.02-0.03 points), but that, overall, the program will still achieve its desired statistical at Bagotville.

Given that, under this modified scenario, there is a reduction in statistical power at Broadwater close to the target value of 0.70, it may be prudent to maintain the radial-search transects into Year 2, but at a decreased intensity (e.g., run radial-search transects at only 30% or 50% of the line-transects), and then reanalysis the data to confirm these results.

Appendix C – DNA extraction and analysis report

Final Report

Koala Genetic Analysis:

NSW

Prepared for Sandpiper Ecological Surveys Pty Ltd

By Lyndal Hulse BAppSc MScAg



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EXECUTIVE SUMMARY

This report presents the findings of a genetic analysis undertaken for 19 koala scats collected from northern New South Wales, indicating koala activity within the survey site. Genetic analysis consisted of genotyping samples across 30 microsatellite marker loci.

Genetic diversity of the sampled population reveal a moderate-high level of diversity when compared to similar studies, although there is an indication of increased inbreeding within the population sampled which is reflected in the relatedness analysis. There is genetic sub-structuring into two genetic clusters within the population which indicates some gene flow occurring within the population.

From data obtained here, management actions should be directed towards protecting and conserving the integrity of the current habitats, and increasing the connectivity between patches to minimise roadkill, support animal dispersal and increase gene flow.

INTRODUCTION

BACKGROUND

Sandpiper Ecological requested genetic analysis to determine the level of genetic diversity and population structure whereby koala (*Phascolarctos cinereus*) genetic material was non-invasively sampled and analysed. Koala scat samples were retrieved from transects of the survey site.

This report presents the findings of a genetic analysis on a sub-set of a koala population located in Northern New South Wales.

PURPOSE

The purpose of this study is to gain an understanding of current koala presence/absence across the survey site assessed as well as to gain an initial understanding of population structure, genetic diversity and health of a sub-sample of northern NSW koalas. This study aims to provide data that can be used to inform effective measures and strategies to conserve or recover koala populations in northern NSW.

STUDY AREA

Study area is located adjacent to the Pacific Highway, between Wardell and Broadwater, northern NSW. Figure 1 depicts survey site and locations of koala scat retrieval between the months of January – May, 2018.

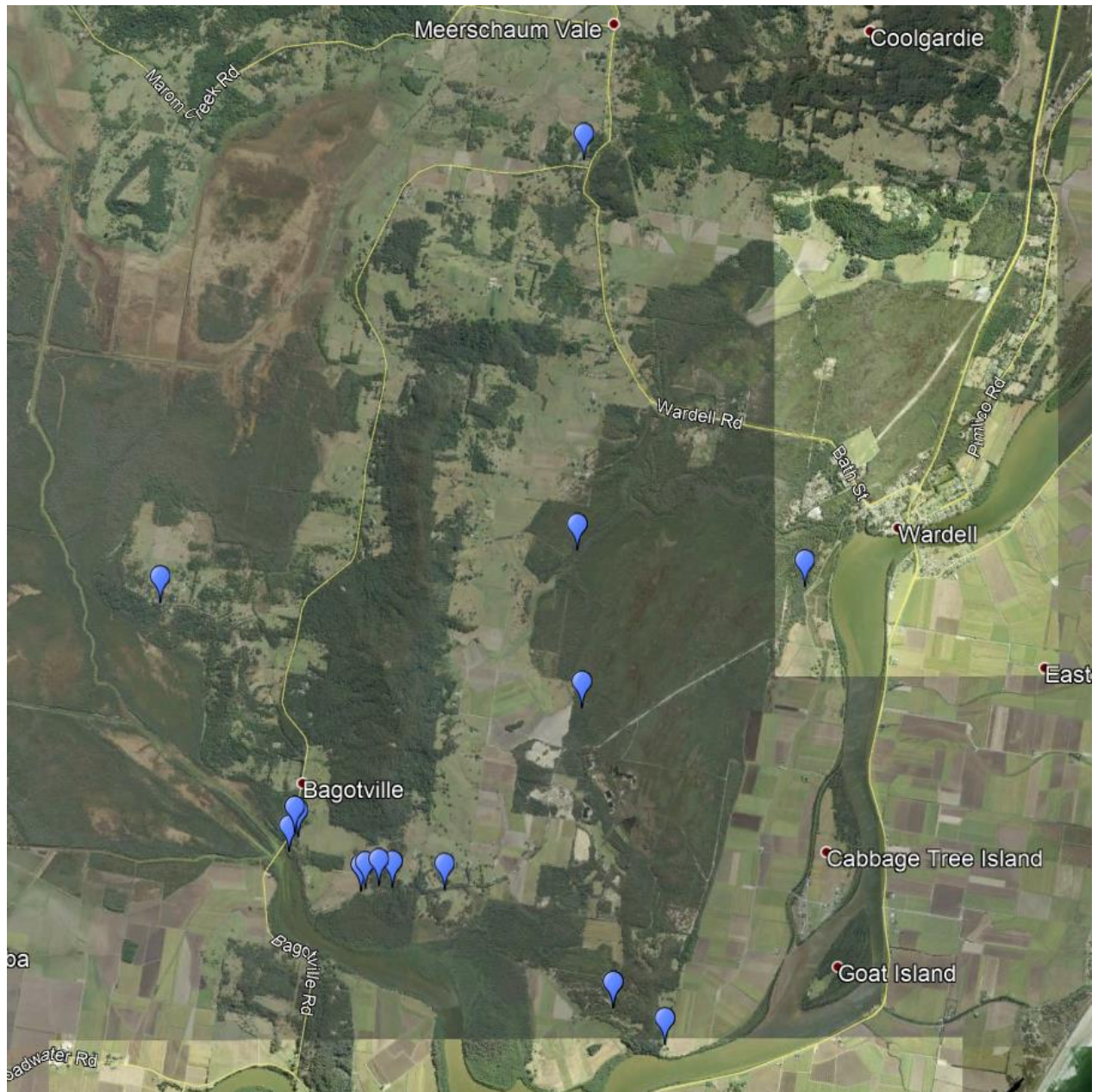


Figure 12. Northern NSW 2018 koala survey site (blue marker = scat collection)

SCAT ANALYSIS METHODOLOGY

SCAT ANALYSIS PROTOCOL

Scats received via mail from Sandpiper Ecological were processed upon arrival. Koala genetic material were isolated from all scats supplied using either the scraping method outlined in Shultz et al (2018) or using the washing techniques described in Wedrowicz et al (2013). Method of DNA isolation was dependent on structure and age of scat.

DNA ISOLATION

Genomic DNA was isolated using the NucleoSpin® DNA Stool kit (Macherey-Nagel, Germany). Each DNA isolate was tested for quality and concentration using spectrophotometry (Nanodrop, ThermoFisher Scientific, VIC, Australia) and real time PCR for confirmation of presence of host DNA in the sample (*Phascolarctos cinereus* beta-actin mRNA mRNA).

GENETIC ANALYSIS

GENOTYPES

Genotypes across 30 microsatellite loci for 19 samples (from herein will be referred to as the population) were generated from scats received via collection by Azure Ecology at a survey site located in northern NSW (Table 1).

Table 1. Scat sample identification and collection details

Scat Record No.#	Collection Date	Easting	Northing
BT-K2	11/01/2018	539597	6794365
BT-K3	05/01/2018	539597	6794365
BT-K4	11/01/2018	539544	6794192
BT-K5	11/01/2018	542993	6792424
SF-K05-DNA	13/04/2018	538383	6796473
SF-K06-DNA	12/04/2018	544293	6796600
BB-K01-DNA	16/05/2018	542241	6795499
SF-K14-DNA	17/05/2018	542202	6796939
SF-K13-DNA	17/05/2018	542518	6792754
SF-K12-DNA	16/05/2018	542278	6800500
SF-K16-DNA	17/05/2018	542518	6792754
SF-K10-DNA	14/05/2018	539627	6794330
SF-K15-DNA	14/05/2018	540494	6793863
SF-K07-DNA	14/05/2018	540368	6793884
SF-K08-DNA	14/05/2018	540244	6793856
SF-K09-DNA	14/05/2018	540204	6793832
SF-K11-DNA	14/05/2018	540973	6793840
KH3-DNA	23/03/2018		
KH4-DNA	28/03/2018		

Identification as per sample bag labelling

There were no departures from Hardy Weinberg Equilibrium from the population, therefore a total of 30 loci were retained for analysis. All 19 koala scat samples collected had unique multi-locus genotypes. The probability of identity for the 30 loci was 2.4×10^{-31} (individuals) and 2.5×10^{-12} (siblings) which indicates that the microsatellite loci were sufficiently discriminating for population analysis. In addition, there was 19 individual genetic profiles identified indicating that 19 distinct individuals had been sampled.

GENETIC DIVERSITY

Genetic diversity is the variability of genes in a species and determines the potential fitness of a population and ultimately its long-term persistence. In population genetics, the concept of heterozygosity is commonly extended to refer to the population as a whole, i.e., the fraction of individuals in a population that are heterozygous for a particular locus. It can also refer to the fraction of loci within an individual that are heterozygous. High heterozygosity (close to 1.0) means a lot of genetic variability, whereas, low heterozygosity (close to 0.0) means little genetic variability.

Gene diversity is composed of two elements; 1) the number of alleles and 2) the abundance (or evenness) of the alleles within loci. Both of these would increase the expected heterozygosity. If a population consists of an excess of homozygotes for different alleles this leads to a low observed heterozygosity but does not affect the expected heterozygosity calculated from Hardy-Weinberg Equilibrium.

Genetic diversity serves as a way for populations to adapt to changing environments. With more variation, it is more likely that some individuals in a population will possess variations of alleles that are suited for the environment. Those individuals are more likely to survive to produce offspring bearing that allele. The population will continue for more generations because of the success of these individuals.

Analysis of genetic diversity was performed using the software GENALEX version 6.5 (Peakall and Smouse, 2012) to calculate mean number of alleles and observed and expected heterozygosity. FSTAT (Goudet, 2001) was used to calculate inbreeding coefficient; whereby a positive value indicates that individuals in a population are more related than you would expect under a model of random mating, and a negative value indicating that individuals in a population are less related.

Genetic diversity values of the population are presented in Table 2 and 4. Individual animal heterozygosity is presented in Figure 2. Analysis reveals that the population is exhibiting moderate to high diversity (A_{mean} and H_e), when compared to previous studies of NSW koala genetic diversity analysis, based on comparable microsatellite marker data (Table 3), despite the indication of an increased inbreeding coefficient value.

Table 2. Summary of genetic diversity statistics for the koala population

Population	N	A_{mean}	F_{IS}	H_o	H_e
NSW	19	8.2	0.214	0.571	0.726

N: Number of individuals sampled **A_{mean} :** Mean number of alleles

H_o : Observed heterozygosity **H_e :** Expected heterozygosity

F_{IS} : Inbreeding coefficient - the proportion of variance in a population that is contained within an individual; $F_{\text{IS}} > 0.00$ suggests inbreeding.

Table 3. Summary and comparison of genetic diversity to NSW koala populations

Population	Reference	N	A_{mean}	H_e
Northern NSW	Current study	19	8.20	0.726
Lismore	Lee et al. (2012)	13	10.30	0.65
Blue Mountains	Lee et al. (2010)	9	6.83	0.743
Southern Tablelands	Lee et al. (2010)	15	5.08	0.586
Campbelltown	Lee et al. (2010)	14	3.17	0.542

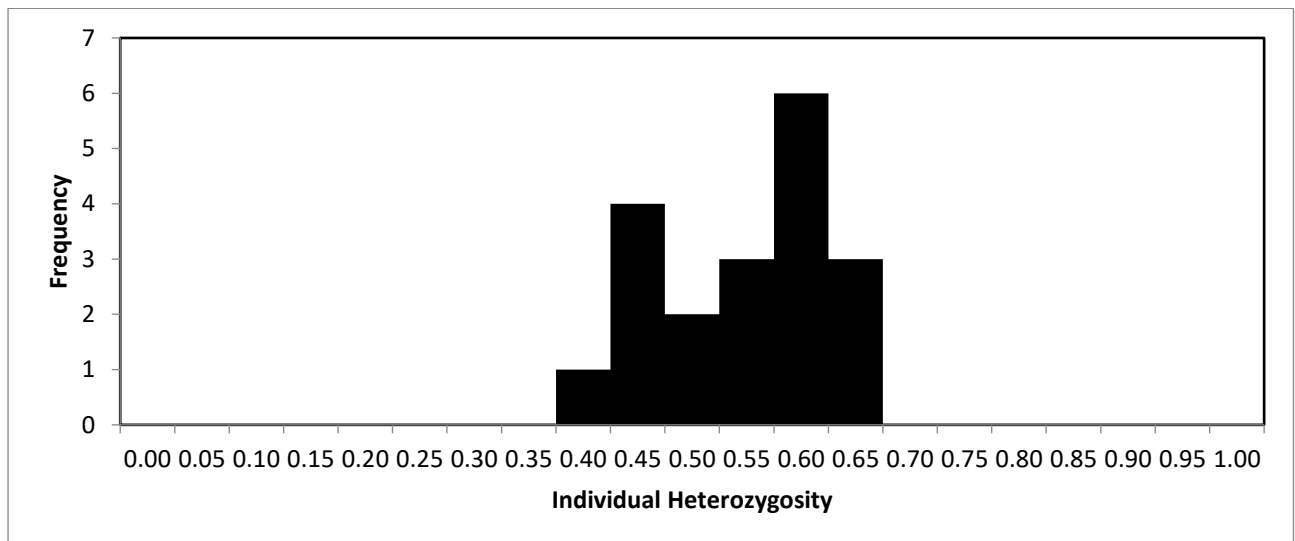
**Figure 2.** Frequency Distribution of Heterozygosity of Total Population Individual Animals

Table 4. Within Loci Genetic Diversity

Locus	Number of Alleles (Na)	Observed Heterozygosity (Ho)	Expected Heterozygosity (He)
AF31	7	0.778	0.745
AF35	9	0.412	0.628
AF33	5	0.474	0.555
AF34	10	0.789	0.805
AF36	11	0.611	0.823
AF32	11	0.500	0.875
Phci2	10	0.895	0.774
Phci5	7	0.474	0.749
Phci9	5	0.263	0.614
Phci10	17	0.684	0.859
Phci27	6	0.800	0.778
Phci31	8	0.474	0.794
Pcin22	11	0.474	0.839
Phci15	10	0.765	0.777
Phci19	7	0.316	0.573
Phci22	7	0.316	0.697
Pcin03	4	0.368	0.500
Pcin6	8	0.267	0.691
Pcin7	7	0.526	0.675
Pcin15	8	0.842	0.785
Pcin19	8	0.632	0.755
Pcin20	4	0.526	0.625
Pcin23	6	0.579	0.485
Phci18	11	0.556	0.849
Pcin5	10	0.632	0.801
Pcin8	10	0.556	0.806
Pcin10	8	0.579	0.720
Pcin11	8	0.824	0.820
Pcin14	7	0.684	0.759
Pcin21	6	0.526	0.623
Mean	8.2	0.571	0.726
SE	0.485	0.032	0.020

GENETIC RELATEDNESS

Genetic relatedness was estimated for every pair of individuals within the population to provide an indication of which pairs of individuals at each site are likely to be related.

Genetic relatedness of within-population individuals was calculated in GENALEX version 6.5 (Peakall and Smouse, 2012) using the Queller and Goodnight estimator of relatedness. This is an indicator of the proportion of shared ancestry in pairs of individuals. Expected values are ≤ 0 for unrelated individuals, 0.25 for half-sib pairs and 0.5 for parent-offspring or full-sib pairs. Relatedness values will form a distribution around these expected values.

Table 5 presents relatedness values between each individual sample. Significantly related individuals, full-sib pairs and parent-offspring, are highlighted yellow; and half-sib pairs are highlighted green.

	BB-k01-DNA	BT-K2	BT-K3	BT-K4	BT-K5	KH3-DNA	KH4-DNA	SF-K05-DNA	SF-K06-DNA	SF-K07-DNA	SF-K08-DNA	SF-K09-DNA	SF-K10-DNA	SF-K11-DNA	SF-K12-DNA	SF-K13-DNA	SF-K14-DNA	SF-K15-DNA	SF-K16-DNA
BB-k01-DNA	0.000																		
BT-K2	0.000	0.000																	
BT-K3	0.000	0.081	0.000																
BT-K4	0.012	0.140	0.000	0.000															
BT-K5	0.000	0.000	0.003	0.000	0.000														
KH3-DNA	0.000	0.000	0.000	0.000	0.000	0.000													
KH4-DNA	0.000	0.000	0.000	0.000	0.085	0.000	0.000												
SF-K05-DNA	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000											
SF-K06-DNA	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000										
SF-K07-DNA	0.046	0.134	0.000	0.247	0.000	0.000	0.000	0.000	0.000	0.000									
SF-K08-DNA	0.056	0.202	0.000	0.134	0.000	0.000	0.000	0.000	0.000	0.429	0.000								
SF-K09-DNA	0.000	0.335	0.000	0.208	0.000	0.000	0.000	0.000	0.000	0.276	0.415	0.000							
SF-K10-DNA	0.040	0.252	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.119	0.345	0.352	0.000						
SF-K11-DNA	0.146	0.083	0.000	0.110	0.000	0.000	0.000	0.000	0.000	0.163	0.118	0.339	0.174	0.000					
SF-K12-DNA	0.320	0.000	0.000	0.102	0.000	0.000	0.000	0.000	0.000	0.271	0.132	0.150	0.193	0.230	0.000				
SF-K13-DNA	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.086	0.000	0.000	0.019	0.000	0.093	0.039	0.000	0.000			
SF-K14-DNA	0.000	0.000	0.000	0.000	0.050	0.048	0.060	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
SF-K15-DNA	0.000	0.184	0.000	0.133	0.000	0.000	0.000	0.000	0.000	0.458	0.394	0.202	0.171	0.097	0.088	0.000	0.000	0.000	
SF-K16-DNA	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.113	0.000	0.000	0.000	0.000	0.000

Table 5. Genetic relatedness within the population

POPULATION STRUCTURE

A population is a group of individuals existing within sufficiently close proximity that any two individuals within the population may reproduce (i.e. random mating). A population may exist as a single large population (where mating is random) or multiple subpopulations with varying levels of connectivity. Genetic diversity is best conserved in a single large population and is more at risk of being lost where a population is fragmented, existing as multiple smaller subpopulations.

Population structuring was determined using the Bayesian clustering program STRUCTURE version 2.3.4 (Pritchard et al. 2000). Analysis of koala population genotype data involved 5 replicates of $K = 1$ to $K = 10$ ($K =$ genetic cluster) using 100,000 iterations with 100,000 iterations discarded as burn-in. The number of K clusters was determined using both the maximum likelihood and the delta K method of Evanno et al. 2005.

The STRUCTURE analysis of the population suggests there is a division into two main genetic clusters (Figure 3 and 4). This indicates there is the presence of two sub-populations within the survey site, with genetic segregation between to the 2 clusters. However, there does not appear to be landscape features within the survey site that would potentially serve as a gene-flow barrier.

K = 2



Figure 3. Population substructure based on 30 loci. Each bar represents an individual koala and colours indicate the proportion of the population cluster to which an individual was assigned.

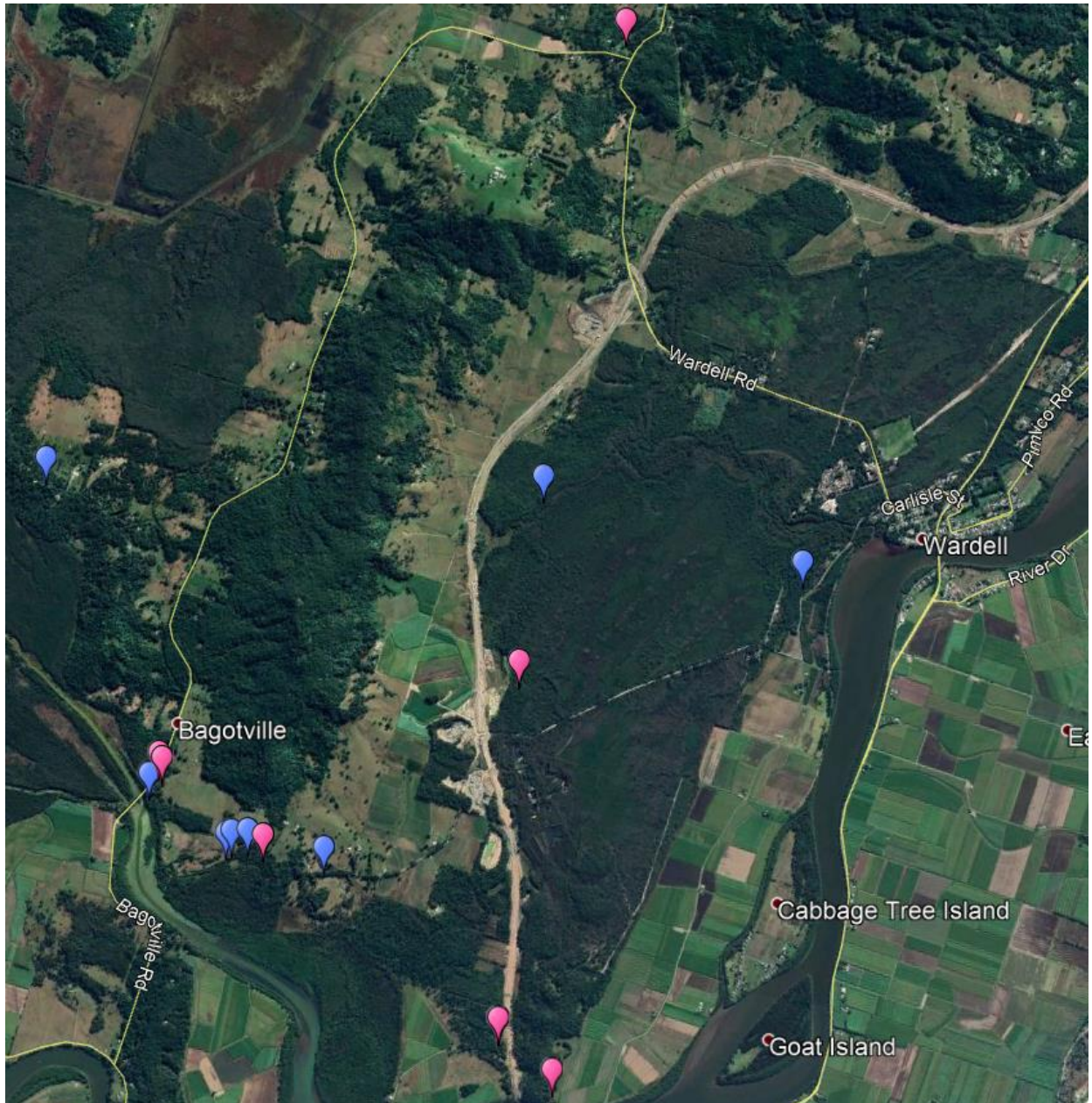


Figure 4. Genetic structure of the population, whereby clusters are shown by colour.

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